# JOURNAL OF FORENSIC CHEMISTRY AND TOXICOLOGY

**(A PEER-REVIEWED AND REFEREED JOURNAL)**

**VOLUME 10 NUMBER 1 JANUARY - JUNE 2024**


**RED FLOWER PUBLICATIONS PVT LTD**
**New Delhi - 110091**

| *Revised Rates for 2024 (Institutional)*<br>**Title of the Journal** | **Frequency** | **India(INR) Print Only** | **India(INR) Online Only** | **Outside India(USD) Print Only** | **Outside India(USD) Online Only** |
|---|---|---|---|---|---|
| Community and Public Health Nursing | Triannual | 6500 | 6000 | 507.81 | 468.75 |
| Indian Journal of Agriculture Business | Semiannual | 6500 | 6000 | 507.81 | 468.75 |
| Indian Journal of Anatomy | Quarterly | 9500 | 9000 | 742.19 | 703.13 |
| Indian Journal of Ancient Medicine and Yoga | Quarterly | 9000 | 8500 | 703.13 | 664.06 |
| Indian Journal of Anesthesia and Analgesia | Bi-monthly | 8500 | 8000 | 664.06 | 625 |
| Indian Journal of Biology | Semiannual | 6500 | 6000 | 507.81 | 468.75 |
| Indian Journal of Cancer Education and Research | Semiannual | 10000 | 9500 | 781.25 | 742.19 |
| Indian Journal of Communicable Diseases | Semiannual | 9500 | 9000 | 742.19 | 703.13 |
| Indian Journal of Dental Education | Quarterly | 6500 | 6000 | 507.81 | 468.75 |
| Indian Journal of Diabetes and Endocrinology | Semiannual | 9000 | 8500 | 703.13 | 664.06 |
| Indian Journal of Emergency Medicine | Quarterly | 13500 | 13000 | 1054.69 | 1015.63 |
| Indian Journal of Forensic Medicine and Pathology | Quarterly | 17000 | 16500 | 1328.13 | 1289.06 |
| Indian Journal of Forensic Odontology | Semiannual | 6500 | 6000 | 507.81 | 468.75 |
| Indian Journal of Genetics and Molecular Research | Semiannual | 8000 | 7500 | 625 | 585.94 |
| Indian Journal of Law and Human Behavior | Semiannual | 7000 | 6500 | 546.88 | 507.81 |
| Indian Journal of Legal Medicine | Semiannual | 9500 | 9000 | 742.19 | 703.13 |
| Indian Journal of Library and Information Science | Triannual | 10500 | 10000 | 820.31 | 781.25 |
| Indian Journal of Maternal-Fetal & Neonatal Medicine | Semiannual | 10500 | 10000 | 820.31 | 781.25 |
| Indian Journal of Medical and Health Sciences | Semiannual | 8000 | 7500 | 625 | 585.94 |
| Indian Journal of Obstetrics and Gynecology | Quarterly | 10500 | 10000 | 820.31 | 781.25 |
| Indian Journal of Pathology: Research and Practice | Triannual | 13000 | 12500 | 1015.63 | 976.56 |
| Indian Journal of Plant and Soil | Semiannual | 7500 | 7000 | 585.94 | 546.88 |
| Indian Journal of Preventive Medicine | Semiannual | 8000 | 7500 | 625 | 585.94 |
| Indian Journal of Research in Anthropology | Semiannual | 13500 | 13000 | 1054.69 | 1015.63 |
| Indian Journal of Surgical Nursing | Triannual | 6500 | 6000 | 507.81 | 468.75 |
| Indian Journal of Trauma and Emergency Pediatrics | Quarterly | 10500 | 10000 | 820.31 | 781.25 |
| Indian Journal of Waste Management | Semiannual | 10500 | 10000 | 820.31 | 781.25 |
| International Journal of Food, Nutrition & Dietetics | Triannual | 6500 | 6000 | 507.81 | 468.75 |
| International Journal of Forensic Science | Semiannual | 11000 | 10500 | 859.38 | 820.31 |
| International Journal of Neurology and Neurosurgery | Quarterly | 11500 | 11000 | 898.44 | 859.68 |
| International Journal of Pediatric Nursing | Triannual | 6500 | 6000 | 507.81 | 468.75 |
| International Journal of Political Science | Semiannual | 7000 | 6500 | 546.88 | 507.81 |
| International Journal of Practical Nursing | Triannual | 6500 | 6000 | 507.81 | 468.75 |
| International Physiology | Triannual | 8500 | 8000 | 664.06 | 625 |
| Journal of Aeronautical Dentistry | Quarterly | 8000 | 7500 | 625 | 585.94 |
| Journal of Animal Feed Science and Technology | Semiannual | 9000 | 8500 | 703.13 | 664.06 |
| Journal of Cardiovascular Medicine and Surgery | Quarterly | 11000 | 10500 | 859.38 | 820.31 |
| Journal of Emergency and Trauma Nursing | Semiannual | 6500 | 6000 | 507.81 | 468.75 |
| Journal of Food Additives and Contaminants | Semiannual | 6500 | 6000 | 507.81 | 468.75 |
| Journal of Food Technology and Engineering | Semiannual | 6000 | 5500 | 468.75 | 429.69 |
| Journal of Forensic Chemistry and Toxicology | Semiannual | 10500 | 10000 | 820.31 | 781.25 |
| Journal of Global Medical Education and Research | Semiannual | 7000 | 6500 | 546.88 | 507.81 |
| Journal of Global Public Health | Semiannual | 13000 | 12500 | 1015.63 | 976.56 |
| Journal of Microbiology and Related Research | Semiannual | 9500 | 9000 | 742.19 | 703.13 |
| Journal of Nurse Midwifery and Maternal Health | Triannual | 6500 | 6000 | 507.81 | 468.75 |
| Journal of Orthopedic Education | Triannual | 6500 | 6000 | 507.81 | 468.75 |
| Journal of Pharmaceutical and Medicinal Chemistry | Semiannual | 17500 | 17000 | 1367.19 | 1328.13 |
| Journal of Plastic Surgery and Transplantation | Semiannual | 27500 | 27000 | 2148.44 | 2109.38 |
| Journal of Psychiatric Nursing | Triannual | 6500 | 6000 | 507.81 | 468.75 |
| Journal of Radiology | Semiannual | 9000 | 8500 | 703.13 | 664.06 |
| Journal of Social Welfare and Management | Quarterly | 8500 | 8000 | 664.06 | 625 |
| New Indian Journal of Surgery | Quarterly | 9000 | 8500 | 703.13 | 664.06 |
| Ophthalmology and Allied Sciences | Triannual | 7000 | 6500 | 546.88 | 507.81 |
| Pediatrics Education and Research | Quarterly | 8500 | 8000 | 664.06 | 625 |
| Physiotherapy and Occupational Therapy Journal | Quarterly | 10000 | 9500 | 781.25 | 742.19 |
| RFP Gastroenterology International | Semiannual | 7000 | 6500 | 546.88 | 507.81 |
| RFP Indian Journal of Hospital Infection | Semiannual | 13500 | 13000 | 1054.69 | 1015.63 |
| RFP Indian Journal of Medical Psychiatry | Semiannual | 9000 | 8500 | 703.13 | 664.06 |
| RFP Journal of Biochemistry and Biophysics | Semiannual | 8000 | 7500 | 625 | 585.94 |
| RFP Journal of Dermatology | Semiannual | 6500 | 6000 | 507.81 | 468.75 |
| RFP Journal of ENT and Allied Sciences | Semiannual | 6500 | 6000 | 507.81 | 468.75 |
| RFP Journal of Gerontology and Geriatric Nursing | Semiannual | 6500 | 6000 | 507.81 | 468.75 |
| RFP Journal of Hospital Administration | Semiannual | 8000 | 7500 | 625 | 585.94 |
| Urology, Nephrology and Andrology International | Semiannual | 8500 | 8000 | 664.06 | 625 |

**Terms of Supply:**

1. Agency discount 12.5%. Issues will be sent directly to the end user, otherwise foreign rates will be charged.
2. All back volumes of all journals are available at current rates.
3. All journals are available free online with print order within the subscription period.
4. All legal disputes subject to Delhi jurisdiction.
5. Cancellations are not accepted orders once processed.
6. Demand draft/cheque should be issued in favour of "**Red Flower Publication Pvt. Ltd.**" payable at **Delhi.**
7. Full pre-payment is required. It can be done through online (http://rfppl.co.in/subscribe.php?mid=7).
8. No claims will be entertained if not reported within 6 months of the publishing date.
9. Orders and payments are to be sent to our office address as given below.
10. Postage & Handling is included in the subscription rates.
11. Subscription period is accepted on calendar year basis (i.e. Jan to Dec). However orders may be placed any time throughout the year.

**Order from**

Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi - 110 091 (India)
Mobile: 8130750089, Phone: 91-11-79695648 E-mail: sales@rfppl.co.in, Website: www.rfppl.co.in

# Journal of Forensic Chemistry and Toxicology






Corresponding address
**Red Flower Publication Pvt. Ltd.**
48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I
Delhi - 110 091(India)
Phone: 91-11-79695648,
E-mail: info@rfppl.co.in, Web:www.rfppl.co.in

**Journal of Forensic Chemistry and Toxicology (JFCT) (pISSN: 2454-9363, eISSN: 2455-8311)** is a peer-reviewed scholarly journal and aims to publish the most complete and reliable source of information on the discoveries and current developments in the mode of original articles, review articles, case reports, short communications, etc. in all areas of **Forensic Chemistry and Toxicology** and making them available online freely without any restrictions or any other subscriptions to researchers worldwide. Forensic chemistry is unique among chemical sciences in that its research, practice, and presentation must meet the needs of both the scientific and the legal communities. As such, forensic chemistry research is applied and derivative by nature and design, and it emphasizes metrology and validation. Forensic chemistry encompasses organic and inorganic analysis, toxicology, arson investigation, and serology. JFCT deals with Forensic Medicine, Forensic Science, Forensic Chemistry, Analytical Toxicology, Analytical Chemistry, DNA Fingerprinting, Sexual Medicine, Environment Medicine etc.

---


**Subscription Information**

**Institutional** (1 year) INR 10500/USD 820.31

**Payment method**

**By cheque/Demand Draft:**

Cheque should be in the name of **Red Flower Publication Pvt. Ltd.** payable at Delhi.

**By Bank Transfer/TT:**

**Bank name:** Bank of India

**Swift Code:** BKIDINBBDOS

**Account Name:** Red Flower Publication Pvt. Ltd.

**Account Number:** 604320110000467

**Branch:** Mayur Vihar Phase-I

Delhi – 110 091 (India)

Send all Orders to: **Red Flower Publication Pvt. Ltd.,** 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091, India, Phone: 91-11-79695648, Fax: 91-11-22754205, E-mail: sales@rfppl.co.in, Website: www.rfppl.co.in.

# Journal of Forensic Chemistry and Toxicology

**January - June 2024,**
**Volume 10 Number 1**

## Contents

### *Editorial*

**Editorial** **07**
*A K Jaiswal*

### *Original Articles*

**Stature Estimation from Postmortem Multidetector Computed Tomography (PMCT) Foramen Magnum Measurement in North Indian Population** **11**
*Karthi Vignesh Raj K, Abhishek Yadav, Sudhir K Gupta, Abilash S, Swati Tyagi, Gokul G, Manju Kumari*

**Comparative Analysis of DNA Extraction Techniques and their Analysis from Blood Stained Clothes** **21**
*Vaishali Vardiya, Anita Yadav, Tanushree Dheer, Rajesh Kumar*

**$^{1}$H-NMR based Metabolic Fingerprinting in Forensic Investigations** **27**
*Pradeep Kumar, AK Jaiswal, Sujeet Kumar Mewar*

**The Estimation of Genome Potential for STR Analysis in bloodstain collected at different temperatures** **35**
*Samruddhi Payas, Anita Yadav, Tanushree Dheer, Rajesh Kumar*

### *Review Articles*

**Flavored Hookah abuse: An Alarming Toxicity of Concern among Youths** **41**
*Neha Jain*

### *Case Reports*

**Natural death turned Unnatural** **47**
*Dheeraj Abhaykumar Binodkumar, Kamalkant Sahu*

**Don't Cry Over Spilled Milk: A Case Report on Intoxicating Mystery for Forensic Chemistry** **51**
*Vivekanshu Verma*

***Guidelines for Authors*** **57**

**Red Flower Publication (P) Ltd.**

*Presents its Book Publications for sale*

1. **Beyond Medicine: A to E for Medical Professionals) (2020)**
   *Kalidas Chavan*
   **INR390/USD31**
2. **Biostatistical Methods For Medical Research (2019)**
   *Sanjeev Sarmukaddam* **INR549/USD44**
3. **Breast Cancer: Biology, Prevention And Treatment (2015)**
   *Dr. A. Ramesh Rao* **INR 395/USD31**
4. **Chhotanagpur A Hinterland of Tribes (2020)**
   *Ambrish Gautam* **INR250/ USD20**
5. **Child Intelligence (2004)**
   *Dr. Rajesh Shukla, Md, Dch.* **INR100/ USD50**
6. **Clinical Applied Physiology and Solutions (2020)**
   *Varun Malhotra* **INR263/USD21**
7. **Comprehensive Medical Pharmacology (2019)**
   *Dr. Ahmad Najmi* **INR599/USD47**
8. **Critical Care Nursing in Emergency Toxicology (2019)**
   *Vivekanshu Verma* **INR460/USD34**
9. **Digital Payment (Blue Print For Shining India) (2020)**
   *Dr. Bishnu Prasad Patro* **INR329/USD26**
10. **Drugs in Anesthesia (2020)**
    *R. Varaprasad* **INR449/USD35**
11. **Drugs In Anesthesia and Critical Care (2020)**
    *Dr. Bhavna Gupta* **INR595/USD46**
12. **MCQs in Medical Physiology (2019)**
    *Dr. Bharati Mehta* **INR300/ USD29**
13. **MCQs in Microbiology, Biotechnology and Genetics (2020)**
    *Biswajit Batabyal* **INR285/USD22**
14. **MCQs In Minimal Access and Bariatric Surgery (2nd Edition) (2020)**
    *Anshuman Kaushal* **INR545/USD42**
15. **Patient Care Management (2019)**
    *A.K. Mohiuddin* **INR999/USD78**
16. **Pediatrics Companion (2001)**
    *Rajesh Shukla* **INR 250/USD50**
17. **Pharmaceutics-1 (A Comprehensive Hand Book) (2021)**
    *V. Sandhiya* **INR525/ USD50**
18. **Poultry Eggs of India (2020)**
    *Prafulla K. Mohanty* **INR390/USD30**
19. **Practical Emergency Trauma Toxicology Cases Workbook (2019)**
    *Dr. Vivekanshu Verma, Dr. Shiv Rattan Kochar, Dr. Devendra Richhariya* **INR395/USD31**
20. **Practical Record Book of Forensic Medicine & Toxicology (2019)**
    *Dr. Akhilesh K. Pathak* **INR299/USD23**
21. **Recent Advances in Neonatology (2020)**
    *Dr. T.M. Ananda Kesavan* **INR 845/USD66**
22. **Shipping Economics (2018)**
    *Dr. D. Amutha* **INR347/USD45**
23. **Skeletal and Structural Organizations of Human Body (2019)**
    *Dr. D.R. Singh* **INR659/USD51**
24. **Statistics In Genetic Data Analysis (2020)**
    *S.Venkatasubramanian* **INR299/USD23**
25. **Synopsis of Anesthesia (2019)**
    *Dr. Lalit Gupta* **INR1195/USD75**
26. **A Handbook of Outline of Plastic Surgery Exit Examination (2022)**
    *Prof Ravi Kumar Chittoria & Dr. Saurabh Gupta* **INR 498/USD 38**
27. **An Introductory Approach to Human Physiology (2021)**
    *Satyajit Tripathy, Barsha Dasssarma, Motlalpula Gibert Matsabisa* **INR 599/USD 46**
28. **Biochemical and Pharmacological Variations in Venomous Secretion of Toad (Bufo melanostictus)(2021)**
    *Dr. Thirupathi Koila & Dr. Venkaiah Yanamala* **INR 325/USD26**
29. **Climate, Prey & Predator Insect Poupulation in Bt Cotton and Non-Bt Cotton Agriculture Feilds of Warangal District (2022)**
    *Dr. Peesari Laxman,Ch. Sammaiah* **INR 325/USD26**
30. **Community Health Nursing Record Book Volume – I & II (2022)**
    *Ritika Rocque* **INR 999/USD 79**
31. **Handbook of Forest Terminologies (Volume I & II) (2022)**
    *Dr. C.N.Hari Prasath, Dr. A. Balasubramanian, Dr. M. Sivaprakash, V. Manimaran, Dr. G. Swathiga* **INR 1325/USD 104**
32. **MCQs of Biochemistry(2022)**
    *Sachin C. Narwadiya, Dr. Irfana Begum* **INR 399/USD 49**
33. **Newborn Care in the State of Uttar Pradesh(2022)**
    *Dr. Tridibesh Tripathy* **INR 545/USD 42**
34. **Osteoporosis: Weak Bone Disease(2022)**
    *Dr. Dondeti Uday Kumar & Dr. R. B. Uppin* **INR 399/USD49**
35. **Quick Updates in Anesthesia(2022)**
    *Dr. Rupinder Kaur Kaiche, Dr. Vidhyadhar Modak, Dr. Shilpa Sannakki & Dr. Vivek Gupta* **INR 599/USD 44**
36. **Textbook of Practice of Medicine with Homoeopathic Therapeutics(2022)**
    *Dr. Pramod Kumar* **INR 1325/USD104**
37. **Trends in Anthropological Research(2022)**
    *Dr. Jyoti Ratan Ghosh,Dr. Rangya Gachui* **INR 399/USD 49**

**Order from: Red Flower Publication Pvt. Ltd.,** 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi - 110 091(India), Mobile: 8130750089, Phone: 91-11-79695648, E-mail: info@rfppl.co.in, Website: www.rfppl.co.in

Journal of Forensic Chemistry and Toxicology
Volume 10 Number 1, January-June 2024
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.10124.1

# Editorial

**Dr. A K Jaiswal**

In the ever-evolving landscape of forensic science, the disciplines of forensic chemistry and toxicology stand out for their critical contributions to criminal justice, public health, and safety. As we navigate through the complexities of modern forensic investigations, it becomes imperative to acknowledge both the remarkable advancements and the persistent challenges that define our field. As we publish this issue of the Journal of Forensic Chemistry and Toxicology, we invite our readers to explore the diverse array of research articles, reviews, and case studies that reflect the current state and future directions of our field. Together, let us continue to push the boundaries of forensic science and strive for excellence in our pursuit of truth and justice.

In this edition through the article **"Stature Estimation from Foramen Magnum Measurements in North Indian Population using Postmortem Multidetector Computed Tomography,"** the author presents a novel approach to stature estimation using the dimensions of the foramen magnum in the North Indian population. By employing postmortem multidetector computed tomography (MDCT), the authors provide a non-invasive and precise method for forensic anthropologists and pathologists. This research not only enhances the accuracy of biological profiling in forensic cases but also underscores the importance of population-specific standards in forensic analysis.

Through the article **"Forensic Examination of Paint Samples using Thin Layer Chromatography, Fourier Transform Infrared Spectroscopy, and Atomic Absorption Spectroscopy"**, the writer explores the combined use of thin-layer chromatography (TLC), Fourier transform infrared spectroscopy (FTIR), and atomic absorption spectroscopy (AAS) to characterize paint samples. The integration of these techniques offers a comprehensive analytical framework, improving the reliability and discriminatory power of forensic paint analysis.

Through the article **"Comparative Analysis of DNA Extraction Techniques and Their Analysis from Blood-Stained Clothes"**, the author compares various DNA extraction techniques to determine their efficacy in retrieving genetic material from blood-stained clothes. By evaluating the yield, purity, and quality of DNA obtained, the authors provide valuable insights that can guide forensic practitioners in selecting the most effective methods for processing biological evidence in criminal cases.

Through the article **"$^{1}$H-NMR based Metabolic Fingerprinting in Forensic Investigations"**, the author highlights the potential of proton nuclear magnetic resonance ($^{1}$H-NMR) spectroscopy in metabolic fingerprinting for forensic purposes. The authors demonstrate how $^{1}$H-NMR can be used to identify and quantify metabolites in biological samples, providing a powerful tool for forensic toxicologists. The study emphasizes the role of metabolic profiling in detecting substance abuse, poisoning, and other forensic toxicology applications.

In the review article **"Flavored Hookah Abuse: An Alarming Toxicity of Concern among Youths"**, the increasing popularity of flavored hookah among youths poses significant public health concerns. This review article examines the toxicological implications of hookah smoking, focusing on the harmful effects of flavoring agents and the associated risks of addiction and disease. The author calls for heightened awareness and regulatory measures to address this emerging threat to youth health.

The Case Report **"Natural Death Turned Unnatural"**, details a seemingly natural death that, upon further forensic investigation, revealed elements of foul play. The authors discuss the investigative process, the forensic evidence gathered, and the conclusions drawn, highlighting the importance of thorough forensic examinations in uncovering hidden truths.

In the case report **"Don't Cry Over Spilled Milk: A Case Report on Intoxicating Mystery for Forensic Chemistry"**, the authors unravel a perplexing case of intoxication involving a seemingly innocuous substance-milk. Through meticulous chemical analysis, the forensic team identifies the presence of an intoxicant, demonstrating the critical role of forensic chemistry in solving unusual poisoning cases.

We hope these contributions will inspire further research and collaboration, ultimately enhancing our ability to serve justice and public safety.

# Instructions to Authors

Submission to the journal must comply with the Guidelines for Authors.
Non-compliant submission will be returned to the author for correction.

To access the online submission system and for the most up-to-date version of the Guide for Authors please visit:

http://www.rfppl.co.in

Technical problems or general questions on publishing with **JFCT** are supported by Red Flower Publication Pvt. Ltd.'s Author Support team (http://rfppl.co.in/article_submission_system.php?mid=5#)

Alternatively, please contact the Journal's Editorial Office for further assistance.

**Editorial Manager**
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi - 110 091(India)
Mobile: 9821671871, Phone: 91-11-79695648
E-mail: info@rfppl.co.in

## SUBSCRIPTION FORM

I want to renew/subscribe international class journal "**Journal of Forensic Chemistry and Toxicology**" of Red Flower Publication Pvt. Ltd.

**Subscription Rates (2024):**
- Institutional: **INR 10500/USD 820.31**

Name and complete address (in capitals):_____

_____

***Payment detail:***
***Online payment link:*** http://rfppl.co.in/payment.php?mid=15

Cheque/DD: Please send the US dollar check from outside India and INR check from India made payable to 'Red Flower Publication Private Limited'. Drawn on Delhi branch.

***Wire transfer/NEFT/RTGS:***
Complete Bank Account No. 604320110000467
Beneficiary Name: Red Flower Publication Pvt. Ltd.
Bank & Branch Name: Bank of India; Mayur *Vihar*
MICR Code: 110013045
Branch Code: 6043
IFSC Code: BKID0006043 (used for RTGS and NEFT transactions)
Swift Code: BKIDINBBDOS

***Term and condition for supply of journals***
1. Advance payment required by Demand Draft payable to **Red Flower Publication Pvt. Ltd**. payable at **Delhi.**
2. Cancellation not allowed except for duplicate payment.
3. Agents allowed 12.5% discount.
4. Claim must be made within six months from issue date.

SCAN HERE TO PAY
WITH ANY BHIM UPI APP

RED FLOWER PUBLICATIONS PRIVATE LIMITED
boism-9718168299@boi

***Mail all orders to***
Subscription and Marketing *Mana*ger
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur *Vihar* Phase-I
Delhi - 110 091(India)
Phone: 91-11-79695648
Cell: +91-9821671871
E-mail: sales@rfppl.co.in

*Original Article*

Journal of Forensic Chemistry and Toxicology
Volume 10 Number 1, January-June 2024
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.10124.2

# Stature Estimation from Postmortem Multidetector Computed Tomography (PMCT) Foramen Magnum Measurements in North Indian Population

**Karthi Vignesh Raj K[1], Abhishek Yadav[2], Sudhir K Gupta[3], Abilash S[4], Swati Tyagi[5], Gokul G[6], Manju Kumari[7]**

**How to cite this article:**

Karthi Vignesh Raj K, Abhishek Yadav, Sudhir K Gupta *et al*. Stature Estimation from Postmortem Multidetector Computed Tomography (PMCT) Foramen Magnum Measurements in North Indian Population. J Forensic Chemistry Toxicol. 2024;10(1):11–19.

**Abstract**

***Background:*** The present study is an attempt to estimate stature from the measurements of Foramen Magnum using postmortem multidetector computed tomography (PMCT) in the North Indian population. Foramen Magnum Length (FML) and Foramen Magnum Breadth (FMB) measurements for every skull were taken using the PMCT of 100 North Indian people.

***Materials and Method:*** PMCT examination was performed using a 16-slice MSCT spiral scanner, Cannon Medical Systems, results were evaluated with the Vitrea software v.6.9.1. The images were viewed using Multiplanar Reconstruction and Volume Rendering technique (MPR-VRT). STAT, FML, andFMB were measured and analyzed

***Results:*** The study population comprised 57 males and 43 females with a mean age of 38.3 years, respectively. Regression analysis was used in stature estimation from FML and FMB. The standard error of estimate (SEE) of the FML was 6.18 and FMB was 6.64 for the combined population while SEE ranged from 4.77 to 5.21 cm for the male population and 6.18 to 6.30 cm for the female population respectively.

***Conclusions:*** This study is the first to provide a metric and statistical analysis of the Foramen Magnum measurements in Northern India using PMCT and concluded that stature estimation is highly possible with minimum SEE from the Foramen Magnum measurements. Furthermore, the equations derived to estimate stature in this study can be considered as an alternative to solve forensic cases, in scenarios where only an isolated skull is retrieved and no long bones are available, which are considered better predictors to date.

**Keywords:** Stature estimation; Postmortem computed tomography; Foramen magnum length; Foramen magnum breath; Identification; Forensic anthropology.

**Author Affiliation:** [1]Assistant Professor, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, Guwahati 781101, Assam, [2]Additional Professor, [3]Professor & Head, [4]Assistant Professor, [5]Assistant Professor, [6]Senior Resident, [7]Junior Resident, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, New Delhi 110029, India.

**Corresponding Author: Abhishek Yadav,** Additional Professor, Department of Forensic Medicine & Toxicology, All India Institute of Medical Sciences, New Delhi 110029, India.

**E-mail:** drayad_in@yahoo.com

**Received on:** 05.03.2024 **Accepted on:** 27.06.2024

## INTRODUCTION

Forensic medicine is "the application of the principles and knowledge of medical science along with circumstantial evidence, to legal investigators and proceedings in courts of law to help in the administration of justice".[1] Forensic anthropology is the application of anthropological methods and theoretical knowledge in matters of legal concern.[2] Forensic anthropology is mainly concerned with creating a biological profile of the retrieved unknown skeletal remains. This biological profile is formed from the "BIG FOUR" i.e., Stature, Race, Sex, and Age of an individual. In other words, BIG FOUR helps the legal authorities in the circumstantial identification of an unknown individual. Identification is defined as "the determination of the individuality of a person".[3] Absolute identification is highly dependent on DNA profiling and Fingerprinting while Partial identification is the "ascertainment of only some features like stature, race, sex, age.[4]

The previous studies had concluded that long bones are the better predictor of stature[5-7], however, newer studies are being conducted to prove the efficiency of other smaller bones of the body.[8-14] The probability of retrieving the intact long bone when compared to other parts, particularly in scenarios involving mass disasters, burns, or mutilated skeletal remains is less common demanding the researchers to conduct newer studies on the smaller bones which helps in better prediction of stature and thus increasingthe chances of identification of the unknown individuals. In comparing the methodologies used to conduct the study, earlier researchers performed the traditional methods of using measuring tapes and vernier caliper to measure the bones either in a percutaneous manner in the case of living subjects or by measuring from the bony landmarks after dissection in case of deceased subjects. However, the application of evolving Postmortem Multidetector Computed Tomography (PMCT) has helped researchers to a great extent that any part of the body can be studiedin both living and dead subjects without mutilation in the case of dead subjects and maintaining the dignity of the dead.

The foramen magnum is the most vital structure, observed to have various shapes situated in the occipital bone of the skull base surrounded by many soft tissues. It helps in holding the face and ensures communication between the brain and other parts of the body.[15,16] The shape and size of the Foramen Magnum (FM) are one of the criteria in clinical assessment for various surgeries related to several pathological conditions. Even though the skull has various other orifices and foramen, FM has a significant role both for clinicians for treatment, and diagnosis purposes, as well as for the forensic anthropologist for identification purposes.[17] The literature available to estimate stature from FM is very less, however, very few authors had studied the sex determination from FM based on morphometry. Hence, the author's main objective was to provide a metric and statistical analysis of the FM measurements in Northern India using PMCT which is the latest advancement in Indian Forensic Medicine.

## METHODS

### 1. Sample collection

This cross-sectional study was conducted under the project of the Center for Advanced Research and Excellence in Virtual Autopsy at the Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences (AIIMS), New Delhi. This is a collaboration established by the two-premier institutes of the country i.e. Indian Council of Medical Research, New Delhi, and AIIMS, New Delhi. The PMCT functioning commenced on the 10th of August 2021 and by the end of the year, almost 597 PMCT examinations were completed. The data utilized in the present study were obtained from the deceased who underwent routine PMCT examination before conducting of autopsy examination. There was a variety of cases like road traffic accidents, falls from height, gunshots, electrocution, railway accidents, poisoning, hanging, strangulation, sudden death cases, etc. Northern India has the following states: Delhi, Haryana, Himachal Pradesh, Jammu and Kashmir, Uttar Pradesh, and Uttarakhand.Almost all the bones altering the stature fuses by 21 years, the deceased who were more than and equal to 21 years of age were included in the study. The study participants who had sustained a fracture of any bones of the body modifying the stature, had any deformity of spine altering the stature, with notable gross congenital cranial defects or skull fracture altering the measurements of the foramen magnum were excluded from the study. A total of 100 study participants were recruited for the study who were born and brought up in different states of Northern India abiding by the inclusion and exclusion criteria. A temporary identification consisting of details like serial number, postmortem no, age, sex, and locality was created in a data collection proforma before collecting the data.

### 2. Anthropometric measurements

PMCT examination was performed using a 16-slice MSCT spiral scanner, Cannon America Medical Systems, Inc Aquilion Lightning TSX-035A CT. Scanning parameters were 120kV and 70 mAs. 16 x 1 mm collimation was used for all the cases for data acquisition. All the raw data was processed into slices of 1mm thickness. The results of the study were evaluated with the Vitrea software v.6.9.1. The images were viewed using Multiplanar Reconstruction and Volume Rendering technique (MPR-VRT). Three variables were measured from the reconstructed imagesas shown in Fig. 1(a): Stature (STAT), Fig. 1(b) Foramen Magnum Length (FML), and Foramen Magnum Breadth (FMB). All the variables were measured using the electronic cursor (distance tool) available in the software and data were entered directly into a data collection proforma initially followed which was updated in Microsoft Excel 2016 Spreadsheet.

**STAT**

The starting point on the head end was from the most distal part of the vertex and the ending part in the lower limb was the distal-most part of the calcaneum.

**FML**

It is measured as the distance between opisthion and endobasion.

**FMB**

It is measured as the maximum width of the foramen magnum. Measured perpendicular to FML (Fig. 1).

### 3. Statistical Analysis

All these measurements were assessed twice by the investigator to ensure the intra-rater reliability of the measurement. Inter-rater reliability was assessed by measuring the parameter of randomly selected 20 cases by an independent person. All lengths were measured in centimeters to the nearest 0.1mm. The obtained data were tabulated and analyzed with the Kruskal-Wallis H test. The data on STAT, FML & FMB obtained from PMCT images was expressed as mean with SD and range. The linear relationship between the STAT and FML, STAT and FMB had been explored by using correlation analysis. Regression analysis both linear and multiple was used to establish the relationship between STAT and FML, STAT and FMB. All statistical analyses were carried out at a 5% level of significance using IBM Statistical Package for Social Sciences (SPSS)(v.23.0).

## RESULTS

### 1. Intra-observer error and Inter-observer

The intra-observer reliability (consistency) was assessed by using the test-retest method by measuring the length 2 times by the observer and the mean values were considered for analysis. Inter-observer reliability was carried out by measuring the length by two independent observers. The reliability was explored by using Cohen's kappa. It was observed that both intra and inter-observer reliability on the measurements were found to be 98% for STAT, 97% for FML, and 98% for FMB.

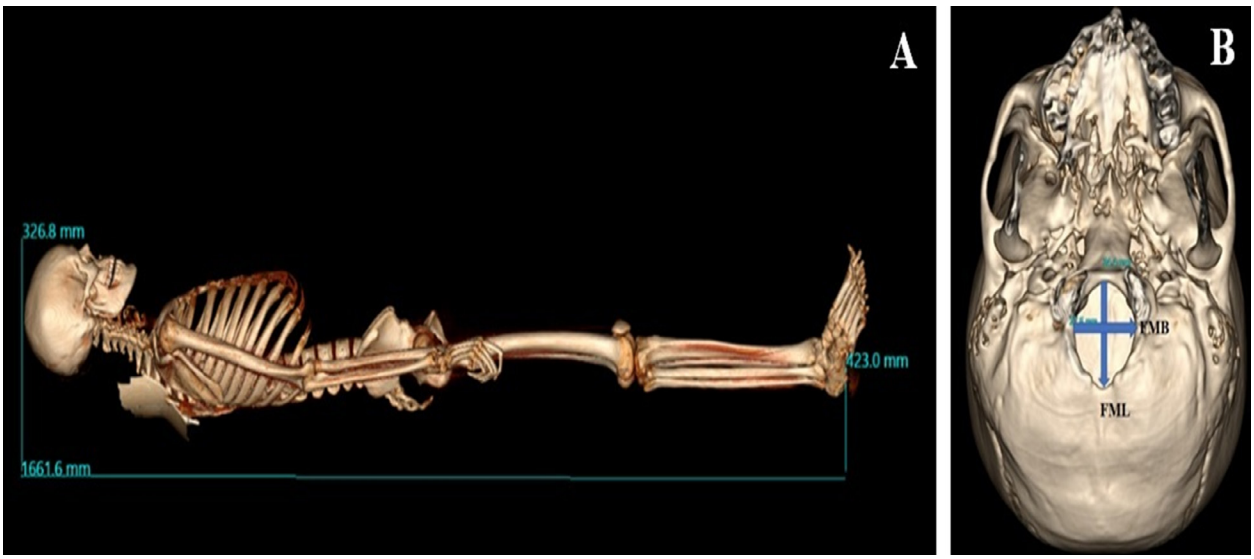


**Fig. 1(a):** Stature estimation using PMCT, **Fig. 1(b):** Measurements: FML & FMB

## 2. Sexual Dimorphism

The Kolmogorov-Smirnov test and Shapiro-Wilk test were conducted on FML and FMB to check for the normality of the population. The results reported that both were not normally distributed. Hence, the Kruskal-Wallis H test and Mann-Whitney test were performed. It was concluded that there was a significant difference in FML and FMB between male and female populations ($p<0.05$).

## 3. Descriptive statistics

100 subjects participated in this study, among which 57 (57%) were males and 43 (43%) were females. The randomly collected 100 population subjects, were further segregated according to age and gender. The descriptive statistics for the entire population, males and females are presented in Table 1. The independent variable age was mentioned in years, all the other outcome variables were measured in cm. The mean FML (anteroposterior diameter) of the study population was higher than the mean FMB (transverse diameter). Similarly, the mean anteroposterior diameter was greater than the mean transverse diameter in both the male and female populations. The male population had a higher mean FML and mean FMB than the female population. However, the maximum transverse diameter was the same in both male and female populations while the females showed a lesser value of minimum FML, maximum FML, and minimum FMB.

**Table 1:** Descriptive Statistics [* age in years, all measurements are in cms]

| Sex/ Parameters | Combined | | Male | | Female | |
|---|---|---|---|---|---|---|
| | Mean (SD) | Range | Mean (SD) | Range | Mean (SD) | Range |
| Age | 37.3(14.94) | 21-77 | 37.14(13.88) | 21-70 | 39.97(16.28) | 21-77 |
| STAT | 159.25(8.85) | 138.5-179.4 | 163.9.25(6.49) | 150.3-179.1 | 153.1(7.74) | 138.5-179.4 |
| FML | 3.71(0.65) | 2.21-5.97 | 3.95(0.65) | 2.21-5.97 | 3.40(0.52) | 2.24-4.92 |
| FMB | 3.17(0.60) | 1.86-4.97 | 3.36(0.58) | 2.49-4.97 | 2.91(0.53) | 1.86-4.97 |

STAT - Stature, FML - Foramen Magnum Length, FMB - Foramen Magnum Breath, SD - Standard Deviation

## 4. Correlation analysis

The correlation coefficient determines the strength and the direction of a linear relationship between two variables. Pearson correlation was used to determine the association between FML and STAT. There was a significantly higher correlation ($p<0.01$) observed between STAT and FML, STAT and FMB for both combined and gender-wise populations. There was a significant positive correlation with a higher correlation coefficient for the anteroposterior diameter than the transverse diameter. After clustering the sample population based on gender, a significant positive correlation was observed between STAT and FML measurements ($p<0.05$). Pearson correlation coefficients of the male population were greater than the female population. Similarly, the Pearson correlation between STAT and FMB showed a significant positive correlation ($p<0.05$). The male population had a better correlation than the female population. (Table 2).

**Table 2:** Pearson Correlation Coefficient and Linear Regression Equations (LRE) for estimation of stature (cm)

| Sex | LRE | SEE | $R^2$ | R | P |
|---|---|---|---|---|---|
| Combined (n=100) | S = 123.15 + 9.71* (FML) | 6.18 | 0.52 | 0.72 | P<0.001 |
| | S = 128.32 + 9.77* (FMB) | 6.64 | 0.44 | 0.67 | P<0.001 |
| Males (n=57) | S = 136.92 + 6.82* (FML) | 4.77 | 0.47 | 0.69 | P<0.001 |
| | S = 141.34 + 6.71* (FMB) | 5.21 | 0.37 | 0.61 | P<0.001 |
| Females (n=43) | S = 121.62 + 9.25* (FML) | 6.18 | 0.38 | 0.62 | P<0.001 |
| | S = 127.73 + 8.71* (FMB) | 6.30 | 0.36 | 0.60 | P<0.001 |

### 5. Multiple regression analysis

The accuracy of estimating the stature can be increased by the application of numerous regression equations.[18] Thus, the authors formulated multiple regression equations to examine the human height estimation accuracy presented in Table 3. This study achieved a higher stature estimation accuracy in multiple regression equations in all cases due to higher values of R (R: 0.75 for the combined dataset, R: 0.71 for males, and R: 0.67 for females). In addition, lower SEE values (± 5.93 cm for the combined data set, ± 4.66 cm for males, and ± 5.92 cm for females).

**Table 3:** Multiple Regression Equations (MRE) for estimation of stature(cm)

| Sex | R | $R^2$ | SEE | MRE | P |
|---|---|---|---|---|---|
| Combined (n=100) | 0.75 | 0.56 | 5.93 | 120.07 + 6.74(FML) +4.46 (FMB) | P<0.001 |
| Male (n=57) | 0.71 | 0.50 | 4.66 | 134.32 + 5.09 (FML)+2.82 (FMB) | P<0.001 |
| Female (n=43) | 0.67 | 0.44 | 5.92 | 118.54 + 5.92(FML) + 4.93 (FMB) | P<0.001 |

## DISCUSSION

The sole purpose of performing metric analysis of the bones of the human body is to find the stature of an individual from an unknown bone. This would be helpful in scenarios where a skeletonized body is received for identification purposes. However, the stature estimation will be a million-dollar question particularly if the sex of the bone is not known. Hence, the researchers suggested that stature estimation should succeed the sex determination.[19] The stature is estimated purely based on regression equations derived from the metric analysis of the various bones. The present study confirms that the foramen magnum can be utilized for the estimation of the stature of an abandoned skull from the North Indian population. The stature can be correlated even though gender is unknown, with the help of the regression equation specific to the population with a minimal SEE (6.18 for FML and 6.64 for FMB) and higher coefficient of determination (0.52 for FML and 0.44 for FMB). Yaming Cui et al in their study on metric analysis of the foramen magnum region on the Chinese population observed a SEE of 7.03(FMB) for the regression equation specific to their population. They observed a correlation coefficient of 0.31(FMB), $p<0.01$ and 0.71(FML), $p>0.01$ while the authors of this study observed 0.67(FMB), $p<0.01$ and 0.72(FML), $p<0.01$. The wide variation in the results could be due to the variation in the procedures undertaken in the study. The present study performed the measurements with the help of the PMCT software without any treatment of the bones and the electric cursor was used to measure the most exact dimensions. However, the authors of the Chinese study used vernier calipers to measure the measurements of the foramen magnum. The other difference could be the reduced sample size in the present study as the authors of the Chinese study analyzed 276 subjects.[14]

The comparative analysis of the correlation coefficient and SEE of the present study with a few other studies showed that the current study had a higher correlation, better coefficient of determination and lesser SEE in both gender with a P value <0.001 [Table 4].

**Table 4:** Comparison of the present study with other studies – Males & Females

| Author, Place (year) | Variables (Sample size) | Correlation Coefficient | P Value | SEE | $R^2$ |
|---|---|---|---|---|---|
| | | **Males** | | | |
| Babu *et al.*, South India (2014)[20] | FML (51) | 0.063 | 0.471 | - | - |
| | FMB (51) | 0.344 | 0.001 | - | - |
| Villarreal M, American White (2015)[21] | FML (117) | 0.18 (0.05) | 0.059 | - | - |
| | FMB(117) | 0.30 | 0.001 | 5.82 | - |
| Zhan M J *et al.*, Chinese (2019)[14] | FML (200) | 0.15 | 0.035 | 6.28 | - |
| | FMB (200) | 0.34 | <0.001 | 5.97 | - |

***Table Cont...***

| | | | | | |
|---|---|---|---|---|---|
| Gilbe P S et al., Western India (2020)[23] | FML (81) | 0.69 | - | 5.6 | 0.48 |
| | FMB (81) | 0.75 | - | 5.1 | 0.56 |
| Present Study (2021) | FML (57) | 0.69 | <0.001 | 4.77 | 0.47 |
| | FMB (57) | 0.61 | <0.001 | 5.21 | 0.37 |
| **Females** | | | | | |
| Villarreal M, American White (2015)[21] | FML(115) | 0.14 | 0.14 | - | - |
| | FMB(115) | 0.15 | 0.11 | - | - |
| Zhan *et al*., Chinese (2019)[14] | FML (200) | 0.12 | 0.120 | 5.79 | - |
| | FMB (200) | 0.26 | 0.261 | 5.63 | - |
| Gilbe P S *et al*., Western India (2020)[23] | FML (55) | 0.45 | - | 6.9 | 0.20 |
| | FMB (55) | 0.68 | - | 5.6 | 0.47 |
| Present Study (2021) | FML (43) | 0.62 | <0.001 | 6.18 | 0.38 |
| Present study (2021) | FMB (43) | 0.60 | <0.001 | 6.30 | 0.36 |

However, these differences in results observed among the studies listed could be due to differences in region, race, occupation, socioeconomic and nutritional factors as stated by the previous researchers[20-26] in addition to differences in sample size between the present study and other studies compared. The correlation coefficient of FMB with STAT was better among Nagpur males and females when compared with the North Indian males and females of the present study.[23] The comparison of the morphological pattern of FM observed in the current study when compared with other studies[27,28] confirmed the presence of variation in the anteroposterior diameter and the transverse diameter. The morphological results of the present study showed that the anteroposterior diameter is more than the transverse diameter (Table 5).

**Table 5:** Comparison of FML and FMB various studies with present study-Population Specific, Male Population and Female Population.

| **Study (year)** | **FML** | | | **FMB** | | |
|---|---|---|---|---|---|---|
| | **Min** | **Mean** | **Max** | **Min** | **Mean** | **Max** |
| **Population Specific** | | | | | | |
| Cui Y *et al*., Chinese (2013)[22] | 2.32 | 3.57 | 4.51 | 2.51 | 3.03 | 4.03 |
| Cui Y *et al*., South Chinese (2013)[22] | 2.32 | 3.56 | 4.51 | 2.62 | 3.04 | 3.68 |
| Cui *et al*., North Chinese (2013)[22] | 3.08 | 3.57 | 4.22 | 2.57 | 3.06 | 4.03 |
| Moodley M *et al*., South Africa (2019)[27] | 2.63 | 3.52 | 4.15 | 2.36 | 2.87 | 3.71 |
| Kumar A *et al*., Northern India (2018)[28] | 3.22 | 3.41 | 3.62 | 2.58 | 2.82 | 3.08 |
| Present study (2021) | 2.21 | 3.71 | 5.97 | 1.86 | 3.16 | 4.97 |
| **Male Population** | | | | | | |
| Kanchan T *et al*., Mangalore (2013)[29] | 2.70 | 3.45 | 4.10 | 2.30 | 2.73 | 3.2 |
| Babu *et al*., South India (2014)[20] | 3.3 | 3.89 | 4.9 | 2.9 | 3.32 | 4.6 |
| Villarreal M, American White (2015)[21] | 2.87 | 3.77 | 4.58 | 2.76 | 3.21 | 3.92 |

***Table Cont...***

| | | | | | | |
|---|---|---|---|---|---|---|
| Zhan *et al*., Chinese (2019)[14] | 2.83 | 3.45 | 4.23 | 2.40 | 2.93 | 3.49 |
| Moodley M *et al*., South Africa (2019)[27] | 2.84 | 3.57 | 4.16 | 2.47 | 2.94 | 3.51 |
| Gilbe P S *et al*., Western India (2020)[23] | 1.9 | 3.1 | 3.8 | 2.3 | 2.9 | 3.5 |
| Present study (2021) | 2.21 | 3.95 | 5.97 | 2.49 | 3.36 | 4.97 |
| **Female Population** | | | | | | |
| Kanchan T *et al*., Mangalore (2013)[29] | 2.70 | 3.36 | 3.90 | 2.2 | 2.67 | 3.1 |
| Villarreal M, American White (2015)[21] | 2.57 | 3.65 | 4.58 | 2.39 | 3.13 | 3.92 |
| Zhan *et al*., Chinese (2019)[14] | 2.89 | 3.35 | 4.07 | 2.39 | 2.89 | 3.51 |
| Moodley M *et al*., South Africa (2019)[27] | 2.67 | 3.43 | 3.87 | 2.38 | 2.79 | 3.71 |
| Gilbe P S *et al*., Western India (2020)[23] | 1.5 | 2.7 | 3.3 | 2.1 | 2.6 | 3.1 |
| Present study (2021) | 2.24 | 3.40 | 4.92 | 1.86 | 2.91 | 4.97 |

However, several researchers had studied various shapes of the foramen magnum and estimated sex[29], which the authors of the present study would explore in the future. An age-wise analysis concluded that the anteroposterior diameter continued to be greater than the transverse diameter as aging happened. However, the mean FML and mean FMB in the second decade were lesser when compared to the mean FML and mean FMB in the third decade. The mean values of both the FML and FMB dropped down from the fourth decade onwards (Table 6). This finding was contradictory to the observations of Hoyte[30] and Scheuer and Black[31] that bone resorption in the older stages of life causes an increase in the dimension of the foramen magnum region as the present study reports a decrease in the diameter as the aging happens. However further studies on the Indian population should validate our findings for a better understanding.

**Table 6:** Comparison of FML and FMB age-wise; Males and females separately

| | | FML | | | | FMB | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Years** | **N** | **Min** | **Max** | **Mean** | **SD** | **Min** | **Max** | **Mean** | **SD** |
| 21-30 | 41 | 2.67 | 5.49 | 3.73 | 0.60 | 2.39 | 4.97 | 3.24 | 0.65 |
| 31-40 | 22 | 2.82 | 5.97 | 3.89 | 0.79 | 1.94 | 4.87 | 3.61 | 0.72 |
| 41-50 | 15 | 2.21 | 4.47 | 3.46 | 0.59 | 2.39 | 4.05 | 3.28 | 0.39 |
| 51-60 | 12 | 2.86 | 4.35 | 3.38 | 0.68 | 1.86 | 3.78 | 3.15 | 0.59 |
| >60 | 10 | 2.79 | 4.14 | 3.15 | 0.40 | 2.68 | 3.71 | 3.04 | 0.34 |

The derived regression equation was validated by applying it to 10 randomly selected males and females respectively from the study population. The calculated stature observed on the application of the FML equation showed less difference in values than the FMB equation. The magnitude of the difference between observed and calculated stature is less than 2cm when FML equations were applied and less than 4cm when FMB equations were used. On applying the FML equation, 40% of males showed a difference of less than 0.5cm, 20% showed a difference in the range of 0.5-1cm, and 40% showed a difference of 1-1.5cm. The female population using the FML equation showed a similar result however 10% of the females showed a difference of 2.2cm. On the other hand, when the FMB equation was used, 80% of both males and females showed a difference of less than 3cm and 20% of both males and females respectively portrayed a difference in the range of 3cm to 4cm. Hence, the inference from the validation of the equations suggested that four proposed equations

for estimating stature based on the FML and FMB are of practical value when the gender of the skull was known.

## CONCLUSION

Linear regression equations for stature estimation from FML and FMB were established in this study. The correlation coefficients between STAT and FML, and STAT and FMB were highly significant and strongly correlated. The authors conclude that foramen magnum measurements in the North Indian population can be considered a reliable predictor of stature. Thus, in scenarios where an isolated skull is retrieved, the linear regression equations can still assist in measuring the possible stature.

## REFERENCES


1. P.V. Guhuraj, S.K. Gupta, Introduction and History, in: P.V. Guhuraj, S.K. Gupta (Ed.), Forensic Medicine and Toxicology, Third ed., Hyderabad, India, 2019.
2. S. Adebisi, Forensic Anthropology In Perspective: The Current Trend, Internet. J. Forensic. Sci. 4(2008)1.
3. R.R. Pickering, D.C. Bachman, Introduction, in: R.R. Pickering (Ed.), The use of forensic anthropology, Second ed. New York: CRC Press Group, 2009, pp. 4-30.
4. P.C. Dikshit, Identification. in: P.C. Dikshit (Ed,), Textbook of forensic medicine and toxicology, Second ed. New Delhi: Pee Pee publishers, 2011, pp86-90.
5. A.A. Ahmed, Estimation of stature using lower limb measurements in Sudanese Arabs, J. Forensic. Leg. Med. 20(2013)483–8. DOI: 10.1016/j.jflm.2013.03.019.
6. N.A. Ismail, N.H. Abd Khupur, K. Osman, A.H. Mansar, M.S. Shafie, F. N. Mohd, Stature estimation in Malaysian population from radiographic measurements of upper limbs, Egypt. J. Forensic. Sci. 8(2018)22. DOI: https://doi.org/10.1186/s41935-018-0055-9.
7. G. Saco-Ledo, J. Porta, I. Duyar, A. Mateos, Stature estimation based on tibial length in different stature groups of Spanish males, Forensic. Sci. Int. (2019)304. DOI: https://doi.org/10.1016/j.forsciint.2019.109973.
8. T.D. Holland, Estimation of adult stature from the calcaneus and talus, Am. J. Phys. Anthropol. 96(1995)315–20. DOI: https://doi.org/10.1002/ajpa.1330960308.
9. N.I. Ishak, N. Hemy, D. Franklin, Estimation of stature from hand and handprint dimensions in a Western Australian population, Forensic. Sci. Int. 216(2012)1-3. DOI: 10.1016/j.forsciint.2011.09.010.
10. G.N. Yonguc, A. Kurtulus, O. Bayazit, E. Adiguzel, I. Unal, S. Demir, Estimation of stature and sex from sternal lengths: an autopsy study. Anat. Sci. Int. 90(2015)89–96. DOI: 10.1007/s12565-014-0235-0
11. F. Giurazza, R. Del Vescovo, E. Schena, R.L. Cazzato, F. D'Agostino, R.F. Grasso, Stature estimation from scapular measurements by CT scan evaluation in an Italian population, Leg. Med. 15(2013)202–8. DOI: 10.1016/j.legalmed.2013.01.002.
12. C.Z. Li, W. Wu, B. Zhu, X.F. Liu, P. Huang, Y.Z. Wang, Multiple regression analysis of the craniofacial region of Chinese Han people using linear and angular measurements based on MRI, Forensic. Sci. Res. 2(2017)34-9. DOI: 10.1080/20961790.2016.1276120.
13. W. Kim, Y.M. Kim, M.H. Yun, Estimation of stature from hand and foot dimensions in a Korean population, J. Forensic. Leg. Med.55(2018)87-92. DOI: 10.1016/j.jflm.2018.02.011.
14. M.J. Zhan, J.H. Cui, K. Zhang, Y.J. Chen, Z.H. Deng, Estimation of stature and sex from skull measurements by multidetector computed tomography in Chinese, Leg. Med. 41(2019)101625. DOI: 10.1016/j.legalmed.2019.101625.
15. C. Manoel, F.B. Prado, P.H.F. Caria, F.C. Groppo, Morphometric analysis of the foramen magnum in human skulls of Brazilian individuals: its relation to gender, Braz. J. Morphol. Sci. 26(2009)104-8. DOI: 131801091.
16. P. M. Shepur, M. Magi, B. Nanjundappa, P. P. Havaldar, G. Premalathai, S.H. Saheb, Morphometric analysis of foramen magnum, Int. J. Anat. Res. 2(2014)249-55. ISSN 2321-428.
17. G. Kanodia, V. Parihar, Y.R. Yadav, P.R. Bhatele, D. Sharma, Morphometric analysis of posterior fossa and foramen magnum. J. Neurosci. Rural. Pract. 3(2012)261–6. DOI: 10.4103/0976-3147.102602.
18. M. Asadujjaman, M.H.O. Rashid, M.S. Rana, Stature estimation from footprint measurements in Bangladeshi adults, Forensic Sci. Res. (2020)1–8. DOI: https://doi.org/10.1080/20961790.2020.1776469.
19. O. Celbis, H. Agritmis, Estimation of stature and determination of sex from radial and ulnar bone lengths in a Turkish corpse sample, Forensic. Sci. Int. 158(2006)135–139.DOI: 10.1016/j.forsciint.2.
20. Y.P.R. Babu, S.M. Bakkannavar, S. Manjunath, Foramen magnum as a tool of estimating stature in a male population, J. South. Ind. Med. Leg. Assoc. 6(2014)46-48.
21. M. Villarreal, Academia. https://www.academia.edu/35905604/Estimation_of_Stature_from_the_Foramen_Magnum_Region_in_an_American_Population_A_Validation_Study, 2015 (accessed on 8 June 2022).
22. Y. Cui, J. Zhang, Stature estimation from foramen magnum region in Chinese population, J.

Forensic. Sci. 58(2013)1127-1135. https://doi.org/10.1111/1556-4029.12192.

23. P.S. Gilbe, S.B. Parchake, N.K. Tumram, P.G. Dixit, Estimation of height from the foramen magnum in the adult population–a preliminary study, A Forensic Med Criminol. 70(2020)124-35.DOI: https://doi.org/10.5114/amsik.2020.104172.
24. S. Kondo, M. Eto, Physical growth studies on Japanese-American children in comparision with native Japanese, in: S.M. Horvath, S. Kondo, H. Matsui, H.Y. Oshimura (Eds.), Human Adaptability: Comparative studies on human adaptability of Japanese, Caucasians and Japanese Americans, Tokyo Press, Tokyo, 1975. DOI: 10.1007/s12565-014-0235-0.
25. A. Telkka, On the prediction of human stature from the long bones, Acta Anat (Basel). 9(1950)103-117. PMID: 15403864.
26. I. Meadows, R.L. Jantz, Allometric secular change in the long bones from the 1800's to the present, J. Forensic. Sci. 40(1995)762-767. PMID: 7595319.
27. M. Moodley, C. Rennie, L. Lazarus, K. Satyapal, The Morphometry and Morphology of the Foramen Magnum In Age And Sex Determination Within The South African Black Population Utilizing Computer Tomography (CT) Scans, Int. J. Morphol. 37(2019). DOI: 10.4067/S0717-95022019000100251.
28. A. Kumar, P. Potdar, K. Singh, J.S. Dhakar, A study of foramen magnum and its clinical relevance, Santosh. Univ. J. Health. Sci. 5(2019)72-77. DOI: https://10.18231/j.sujhs.2019.015.
29. T. Kanchan, A. Gupta, K. Krishan. Craniometric analysis of foramen magnum for estimation of sex, Int. J. Med. Health. Biomed. Pharm. Eng. 7(2013)111–13.
30. D. Hoyte, The cranial base in normal and abnormal skull growth, Neurosurg. Clin. N. Am. 2(1991)515–37. PMID: 1821300.
31. L. Scheuer, S. Black, Developmental juvenile osteology, London, UK, Academic Press, 2000.

# Instructions to Authors

Submission to the journal must comply with the Guidelines for Authors.
Non-compliant submission will be returned to the author for correction.

To access the online submission system and for the most up-to-date version of the Guide for Authors please visit:

http://www.rfppl.co.in

Technical problems or general questions on publishing with **JFCT** are supported by Red Flower Publication Pvt. Ltd.'s Author Support team (http://rfppl.co.in/article_submission_system.php?mid=5#)

Alternatively, please contact the Journal's Editorial Office for further assistance.

**Editorial Manager**
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi - 110 091(India)
Mobile: 9821671871, Phone: 91-11-79695648
E-mail: info@rfppl.co.in

*Original Article*

Journal of Forensic Chemistry and Toxicology
Volume 10 Number 1, January-June 2024
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.10124.3

# Comparative Analysis of DNA Extraction Techniques and their Analysis from Blood Stained Clothes

**Vaishali Vardiya[1], Anita Yadav[2], Tanushree Dheer[3], Rajesh Kumar[4]**

**How to cite this article:**

Vaishali Vardiya, Anita Yadav, Tanushree Dheer *et al*. Comparative Analysis of DNA Extraction Techniques and their Analysis from Blood Stained Clothes. J Forensic Chemistry Toxicol. 2024;10(1):21–25.

## Abstract

The advancement of forensic DNA analysis has revolutionized criminal investigations, providing crucial evidence for identifying suspects and establishing their involvement in various crimes. The extraction of DNA from blood stains is a fundamental step in forensic investigations, enabling the profiling of individuals for identification and genetic analysis. The objective of this research is to evaluate and compare the DNA extraction methods on different fabric types, which is cotton, and silk. Blood stains were spotted in both the cloths and incubated for a certain period of time. After which, DNA extraction was done using phenol chloroform method. The quality and quantity of the extracted DNA are assessed using established techniques such as Gel electrophoresis and qPCR amplification. The results of the experiments indicate variations in the efficiency of DNA extraction methods depending on the fabric type. Ideally both the fabrics have shown similar pattern of DNA recovery but Cotton fabrics demonstrate higher DNA recovery rates, of DNA recovery but Cotton fabrics demonstrate higher DNA recovery rates, compared to silk fabric but this is non-significant. In conclusion, provides valuable insights into the challenges and opportunities associated with DNA extraction from blood stains on different fabrics for forensic investigation profiling. The research findings contribute to the advancement of forensic DNA analysis and offer practical guidance for forensic scientists to optimize their methods when handling blood-stained fabric evidence. Further research and development in this field will undoubtedly strengthen the forensic community's ability to solve crimes and administer justice with greater accuracy and reliability.

**Keywords:** DNA Extraction; Human DNA; Forensic DNA.

**Author Affiliation:** [1]M.Sc Student, [2]Associate Professor, [3]Assistant Professor, Department of Forensic Science, School of Sciences, Sanjeev Agrawal Global Educational University, Bhopal 462022, Madhya Pradesh, India, [4]Associate Professor, Department of Forensic Medicine & Toxicology, All India Institute of Medical Sciences, Deoghar 814152, Jharkhand, India.

**Corresponding Author: Rajesh Kumar**, Associate Professor, Department of Forensic Medicine & Toxicology, All India Institute of Medical Sciences, Deoghar 814152, Jharkhand, India.

**E-mail:** rajesh.fmt@aiimsdeoghar.edu.in

**Received on:** 04.03.2024 **Accepted on:** 27.06.2024



## INTRODUCTION

As forensic science works along the human body and attempts to establish to identify humans is a main theme to investigate and study.[1] In this way, forensic analysis is a specialty that studies psychological, physical, chemical, and biological phenomena that can affect humans including aspects of human identification, criminal, civil, labor, and administrative image examinations, forensic investigation etc.[2] Watson and Crick, identified the double helix structure of DNA. DNA can be found on clothes even by brief skin contact, clothing articles from crime scenes are frequently

submitted for trace DNA analysis to identify someone other than the wearer.[3] Numerous research has examined the intricacy of DNA transfer in recent years,[4] beginning with the active transfer of DNA from a human to an object. To the best of our knowledge, exposure to bodies of still or moving water was not one of these circumstances. Only tests using a washing machine have been used to evaluate whether epithelial or blood DNA remains after coming into contact with water.[7-10] For instance, it is necessary for DNA isolation from scientific crime scene materials to be efficient in eliminating a varied range of environmental impurities/inhibitors that impede subsequent PCR operations, such as denim dyes, humic acid, and tannins. The DNA extraction procedure is less complicated and may even be bypassed entirely for eg blood or buccal samples in order to save time.[11,12] Although, usefulness of a sample in criminological background is ultimately defined by the capacity to construct a DNA profile, DNA quantification is typically performed to control the efficacy of the extraction stage. To avoid overloading the PCR master mix with too much DNA, the information on the quantity of DNA is utilized to estimate the right amount of DNA to be added. Contrary to particular DNA-based hybridization tests, common quantification approaches employ photometric/ fluorometric assessment of the DNA amount, which are indifferent to the source of DNA.[13-15] The experiment is carried out using a device that can measure fluorescence at each PCR cycle straight through the cover of the reaction tube, allowing for online detection. In the annealing phase, the probe precisely binds to the product as the amplicon builds up during the reaction. Taq polymerase uses its 5'-exonuclease activity to cleave the probe during the extension phase, separating the quencher and fluorochrome molecules in the process.[16-19] Fluorescent signal is now being detected and released. By computing the actual template copy number from the calculated Ct value, this line can be used to determine the assay's active range and to quantify the amount of starting specified DNA from an unidentified sample.

## METHODOLOGY

### *Requirement*

*Reagents:* Solution B, 20% SDS, Proteinase K enzyme, Phenol chloroform Isoamyl alcohol mixture (25:24:1), Sodium acetate (5M, pH 5.2), Chilled Isopropanol (IPA), 70% ethanol, Nuclease free water, Eppendorf tubes 2 ml, Micropipette. Agarose 1% agarose, 10X TAE buffer, Loading dye - 0.25% bromophenol blue, 0.4 M tris, 48.5g per L, Ethidium bromide (10mg/ml).

*Equipment:* AgaroseGel Electrophoresis, Real-Time Polymerase Chain Reaction.

### *Standard preparation*

i. Solution B (pH 8.0): Dissolved in 15ml, 1M NaCl in 40ml, 1M trisHCl (pH 8), and added 10ml of 0.5 M EDTA in 95ml distilled water. Autoclaved the content and cooled it at room temperature. Then, 5ml of 20% SDS solution were added to this mixture and mixed it well.

ii. 20% SDS: Added 20g SDS in 100 ml of distilled water. Mixed it well and gently.

iii. Proteinase K: 10mg of Proteinase K was added in 1 ml autoclaved distilled water.

iv. Phenol–Chloroform Isoamyl Alcohol Mixture (PCI): 25 ml saturated phenol with 24 ml of chloroform and 1 ml of isoamyl alcohol.

v. 5M Sodium Acetate: 41g sodium acetate in 100 ml distilled water (pH 5.2).

vi. Isopropanol: Kept the IPA in -200C before use.

vii. 70% Ethanol: 70 ml absolute ethanol dissolved in 30 ml distilled water.

**Sample collection:** blood stains of 150 µl each were taken on silk and cotton cloth, collected from a single person. a total of 30 bloodstains were collected. The samples were stored at Room temperature (RT). Spots from silk cloth were coded VS1-VS15 and spots cotton were coded as VC1 – VC15. The samples were incubated at respective temperatures for 15 days before further processing.

***Sample Pretreatment:*** 6 samples were processed every alternate day for DNA extraction.

### *Procedure*

DNA extraction from blood stain on cloths:

i. Weighed the cloth and cut it in pieces.

ii. 1.5 ml Solution B, 50 µl 20% SDS and 5 µl Proteinase K was added to the cut cloth. prepared mixture incubated at RT overnight. After 24 hours again incubate the mixture 56°C for 45 mins.

iii. Transfer a fresh tube and 250 µl of Sodium acetate and 500 µl of PCI mixture were added

in the tube, and mixed it well.

iv. Centrifuged the mixture at 10,000 rpm for 15 minutes.

v. Three layers were formed. Carefully the upper layer which contains DNA was transferred in a fresh tube.

vi. 500 µl of chilled IPA was added to precipitate the DNA. Incubate the mixture at -20°C overnight.

vii. Centrifuged the mixture at 10000 RPM for 10 min. Discarded the supernatant.

viii. Pellet was washed with 500ul 70% ethanol by centrifuging the content at 10000 RPM for 5 mins. Supernatant was discarded and the pellet was air dried.

ix. After air dry the DNA pellet was dissolved in 15 µl of Nuclease free water.

x. The dissolved DNA was visualized under UV in agarose gel electrophoresis.

### *Agarose Gel Electrophoresis*

i. Diluted 10X TAE buffer to 1X for gel formation. (Mixed 3 ml TAE with 27 ml d/w to make it 1X). Mixed 300 mg agarose with 30 ml 1X TAE buffer in a flask.

ii. Heated the flask and dissolved the agarose. Heat protecting gloves were used when heated the agarose.

iii. The agarose when dissolved, but not in a boil. Once fully melted and allowed it to cool without setting and the edges of a gel-casting tray was sealed with tape.

iv. 0.5 µl of ethidium bromide added to the agarose solution at about 60°C and gently mixed then a fine comb inserted into the casting tray.

v. Poured the agarose to a depth of about 1 cm and allowed it to solidify and removed the tape and 10µl of a 500µl DNA sample were taken 2.5 µl loading dye as added.

vi. 1µl of 1kb ladder (in refrigerator), 9µl water, and 2.5 µl loading dye was used as a marker.

vii. Filled the tank to adjust above the gel bed using 1X TAE buffer and gel was placed in the tank ensured that the gel was submerged.

viii. Samples wells were filled with samples and run at 80V for about an hour until the front dye reached the bottom of the gel.

ix. electrodes are bubbling indicated that the circuit was complete.

x. After the electrophoresis photographed the gel under UV trans-illumination.

### *Real-Time Polymerase Chain Reaction*

q-PCR is a method for the quantitative detection of specific DNA or complementary DNA (cDNA) regions by selective amplification.

*Preparation of master mix for q-PCR and running the qPCR:* Reaction mixture was prepared for the total volume of 10µl.The amplification cycle was carried out for 45 cycles leading to the last stage of PCR which was melting.

**Table 1:** Reaction mixture for q-PCR

| Content | Volume (µl) |
|---|---|
| 2X qPCR Mix | 5 |
| Fw Primer (n65) | 0.5 |
| Rv Primer (n65) | 0.5 |
| RNAse free water | 3.8 |
| Sample (DNA) | 0.2 |

**Table 2:** Steps in q-PCR

| Contents | Temp($^{O}$C) | Duration |
|---|---|---|
| Pre-denaturation | 95 | 3 mint |
| Denaturation | 95 | 10 sec |
| Annealing & Extension | 60 | 20 sec |
| Melting | 72 | 3 min |

## RESULTS AND DISCUSSIONS

From the results obtained after gel electrophoresis it can be clearly seen that the blood samples from both the cloths have yielded similar intensity of DNA. So, it can be inferred that DNA can be retrieved from the both types of cloths.

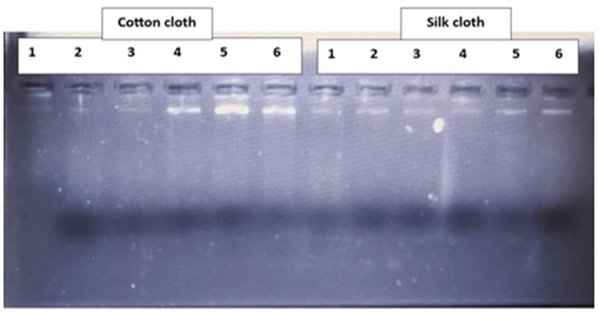


**Fig. 1.** Gel image of DNA bands under UV

**qPCR Analysis:** The technique most frequently used for DNA quantification and quality analysis in the past was spectrophotometry, which allows for the assessment of the amount and purity of DNA in a sample in respect to possible impurities like proteins.[20] The appropriate n65 primer was used to amplify the gDNA.

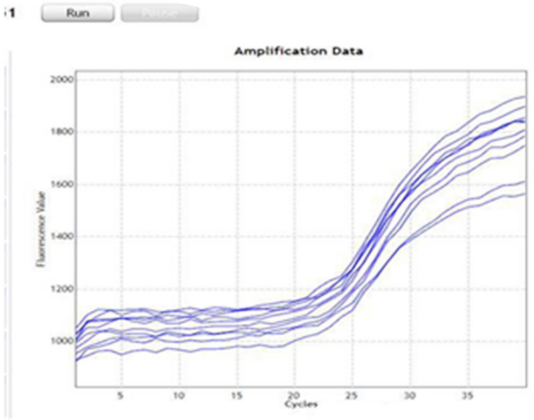


**Fig. 2.** Raw Data Plot of qPCR

**Table 3:** CT value and fold change of n65

| Well | CT Value (Mean) | Fold Change (Mean) |
|---|---|---|
| E1-E5 | 21.09 | 77.8 |
| E6-E10 | 20.47 | 77.7 |

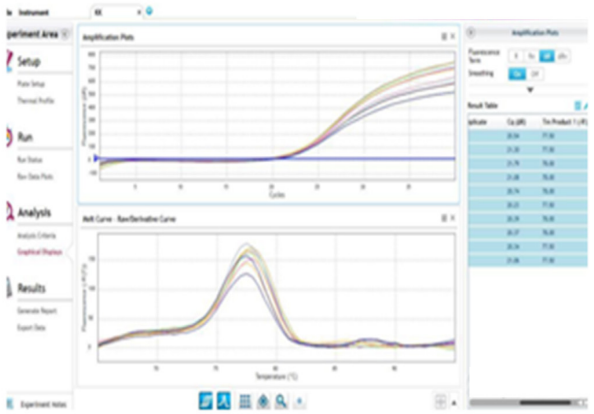

**Fig. 3.** Melting curve of qPCR

The CT Value and Fold Change of first five samples were 21.09 and 77.8. The CT Value and Fold Change of next five samples were 20.47 and 77.7

## CONCLUSION

The present study explored the extraction of DNA from two different cloth samples using phenol chloroform method. The results obtained through in study indicated that DNA can be successfully retrieved from both the cloths (cotton & Silk) during forensic investigations. Also, qPCR analysis has proved that the DNA extracted is functional and can be used for future forensics studies.

## REFERENCES

1. Anzai EK, Ozaki A, Nunes FD, Hirata M, Oliveira RN. Extração de DNA de saliva humanadepositadasobre a pele e suaaplicabilidadeaosprocessos de identificação individual. Odontol Soc. 2001;3(1/2):5-7.
2. Anzai EK, Hirata MH, Hirata RDC, Nunes FD, Melani RFA, Oliveira RN. DNA extraction from human saliva deposited on skin and its use in forensic identification procedures. Braz Oral Res. 2005;19(3):216- 22.
3. Wickenheiser RA (2002) Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. J Forensic Sci 47:442–450.
4. Goray M, van Oorschot RA (2015) The complexities of DNA transfer during a social setting. Leg Med (Tokyo) 17:82–91.
5. Phipps M, Petricevic S (2007) The tendency of individuals to transfer DNA to handled items. Forensic SciInt 168:162–168.
6. Van Oorschot RA, McArdle R, Goodwin WH, Ballantyne KN (2014) DNA transfer: The role of temperature and drying time. Leg Med (Tokyo) 16:161–163.
7. Kamphausen T, Fandel SB, Gutmann JS, Bajanowski T, Poetsch M (2015) Everything clean? Transfer of DNA traces between textiles in the washtub. Int J Legal Med 129:709–714.
8. van den Berge M, Ozcanhan G, Zijlstra S, Lindenbergh A, Sijen T (2016) Prevalence of human cell material: DAN and RNA profiling of public and private objects and after activity scenarios. Forensic SciInt Genet 21:81–89.
9. van Oorschot RA, Glavich G, Mitchell RJ (2014) Persistence of DNA deposited by the original user on objects after subsequent use by a second person. Forensic SciInt Genet 8:219–225.
10. Voskoboinik L, Amiel M, Reshef A, Gafny R, Barash M (2017) Laundry in a washing machine as a mediator of secondary and tertiary DNA transfer. Int J Legal Med. https://doi.org/10.1007/ s00414-017-1617-3.
11. Ensenberger, M. G., Hill, C. R., McLaren, R. S., Sprecher, C. J., &Storts, D. R. (2014). Developmental validation of the PowerPlex® 21 system. Forensic

Science International: Genetics, 9, 169–178. https://doi.org/10.1016/J.FSIGEN.2013.12.005.

12. Park, S. J., Kim, J. Y., Yang, Y. G., & Lee, S. H. (2008). Direct STR amplification from whole blood and blood- or saliva-spotted FTA without DNA purification. Journal of Forensic Sciences, 53(2), 335–341. https://doi.org/10.1111/j.1556-4029.2008.00666.x.
13. Waye, J. S., and Willard, H. F. (1986). Structure, organization, and sequence of alpha satellite DNA from human chromosome 17: evidence for evolution by unequal crossing-over and an ancestral pentamer repeat shared with the human X chromosome. Mol. Cell Biol. 6, 3156–3165.
14. Waye, J. S., Presley, L. A., Budowle, B., Shutler, G. G., and Fourney, R. M. (1989). A simple and sensitive method for quantifying human genomic DNA in forensic specimen extracts. BioTechniques 7, 852–855.
15. Walsh, P. S., Varlaro, J., and Reynolds, R. (1992). A rapid chemiluminescent method for quantitation of human DNA. Nucleic Acids Res. 20, 5061–5065.
16. Heid, C. A., Stevens, J., Livak, K. J., and Williams, P. M. (1996). Real time quantitative PCR. Genome Res. 6, 986–994
17. Holland, P. M., Abramson, R. D., Watson, R., and Gelfand, D. H. (1991). Detection of specific polymerase chain reaction product by utilizing the 5′–3′ exonuclease activity of Thermusaquaticus DNA polymerase. Proc. Natl. Acad. Sci. USA. 88, 7276–7280.
18. Livak, K. J., Flood, S. J., Marmaro, J., Giusti, W., and Deetz, K. (1995). Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. PCR Methods Appl. 4, 357–362.
19. Lyamichev, V., Brow, M. A., and Dahlberg, J.E. (1993). Structure-specific endonucleolytic cleavage of nucleic acids by eubacterial DNA polymerases. Science 260, 778–783.
20. J.A. Glasel, Validity of nucleic acid purities monitored by 260 nm/280 nm absorbanceratios, Biotechniques 18 (1995) 62–63.

*Original Article*

Journal of Forensic Chemistry and Toxicology
Volume 10 Number 1, January-June 2024
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.10124.4

# [1]H-NMR based Metabolic Fingerprinting in Forensic Investigations

**Pradeep Kumar[1], AK Jaiswal[2], Sujeet Kumar Mewar[3]**

**How to cite this article:**

Pradeep Kumar, AK Jaiswal, Sujeet Kumar Mewar. [1]H-NMR based Metabolic Fingerprinting in Forensic Investigations. J Forensic Chemistry Toxicol. 2024;10(1):27–33.

**Abstract**

Nuclear Magnetic Resonance (NMR) spectroscopy plays a crucial role in forensic sciences by providing detailed chemical information about substances present in biological samples such as blood. NMR spectroscopy is a versatile tool in forensic sciences, offering non-destructive and high-resolution analysis of biological samples like blood. Machine learning tools are increasingly employed in forensic investigations to enhance data analysis, pattern recognition, and decision-making processes. These tools aid in processing vast amounts of forensic data efficiently and uncovering hidden insights from complex datasets. Its ability to provide detailed chemical information aids in substance identification, quantitative analysis, and understanding of NMR data interpretation, making it invaluable in criminal investigations and forensic pathology.

**Keywords:** Nuclear Magnetic Resonance spectroscopy; machine learning tools; Blood sample; Metabolites, 1D CPMG; 2D TOCSY.

## INTRODUCTION

Forensic science is the application of several fields of science, such as biology, chemistry, physics, and medicine, to the domain of criminal investigation.[1] Through the forensic application and validation of scientific processes originally designed for diverse aims in a more confined framework, such as the legal medicine situation, it focuses mainly on providing evidence to be utilized in forensic science.[1]

**Author Affiliation:** [1]Post Doctoral, [2]Chemist, Department of Forensic Medicine and Toxicology, [3]Scientist Department of NMR, All India Institute of Medical Sciences, New Delhi 110029, India.

**Corresponding Author: Sujeet Kumar Mewar,** Scientist, Department of NMR, All India Institute of Medical Sciences, New Delhi 110029, India.

**E-mail:** sujeetmewar@gmail.com

**Received on:** 06.07.2024

**Accepted on:** 00.00.2024



Since the scientific instruments available had been insufficient, forensic science has addressed several challenges in recent decades that were unfulfilled in the past.[1] The development of gas chromatography, liquid chromatography, and mass spectrometry (MS or MS/MS) methods for the quantification of several drugs and their metabolites in human biological fluids.[2] Otheradvanced imaging tools like computed tomography with or without contrast medium and magnetic resonance imaging or MRI in the identification of the cause of death, have all contributed to the solution of challenges such as human individuality, and toxicological analysis on different biological fluids.[3]

The qualitative and quantitative study of low molecular weight compounds found in biological systems is known as metabolomics, and it offers insights into the metabolic changes brought on by external stimuli.[4] Metabolism enables one to get a global profile of the metabolome or execute a specific study, depending on the information needed. One significant benefit of metabolomics for

forensics is the ability to combine data from several bodily compartments and biological fluids.[5-9]

NMR spectroscopy exploits the magnetic properties of atomic nuclei, particularly those of hydrogen atoms in the context of blood analysis. When placed in a magnetic field and subjected to radiofrequency pulses, nuclei such as protons within blood molecules absorb and re-emit electromagnetic radiation at characteristic frequencies.[10-11] By detecting these signals, NMR spectroscopy can generate detailed spectra that reveal the chemical composition and molecular structure of the sample under investigation. The data obtained from NMR spectroscopy is quantitative, allowing for precise measurements of concentrations and providing insights into the interactions between different components within the blood sample.[12-13] NMR spectroscopy can identify and quantify various drugs and metabolites present in blood samples, even at trace levels. This capability is particularly critical in cases involving drug-related offenses or incidents where substance abuse is suspected. By analyzing metabolic profiles, NMR spectroscopy can provide information about an individual's physiological state, including factors such as nutrition, health status, and exposure to environmental toxins.[14] NMR can complement traditional DNA profiling techniques by providing additional information about the chemical environment of nucleic acids, which can aid in confirming the identity of individuals from blood samples. NMR spectroscopy enables the identification and quantification of toxic substances in blood, such as heavy metals or poisons, which can be crucial in cases involving poisoning or environmental exposure.[15-17]

**Advantages of NMR in forensic investigations include some important advantages Non-destructive Analysis using** NMR spectroscopy minimal sample preparation and not consuming the sample, allowing for subsequent analyses if necessary.[1-2] Thus, **high sensitivity and Specificity with** NMR can detect substances at very low concentrations, enhancing its utility in forensic toxicology and drug detection. Furthermore, **quantitative analysis** to quantify concentrations of substances in blood samples makes NMR spectroscopy highly reliable for forensic applications. **The versatility of** NMR spectroscopy applies to a wide range of forensic analyses beyond blood, including other bodily fluids and tissue samples.[3-4]

Many studies areattentiveto the pathophysiological effects of acute and long-term alcoholic toxicity on the metabolomes profiling of humans.[1,18] Furthermore, several studies have focused on using NMR-based metabolomics of biological fluids or tissues to identify putative PMI biomarkers and track changes in endogenous metabolites brought on by death.[19-22]

We mainly investigated the potential use of NMR as an analytical platform for forensic blood metabolomic, or metabolic fingerprinting in the present study. Additionally, 1D and 2D NMR investigations were used to characterize and identify the metabolites in blood samples. This analysis was performed extensively for forensic purposes, where $^{1}$H-NMR metabolic fingerprinting was presented as a new or alternative analytical approach.

### *Synopsis*

NMR spectroscopy plays a critical role in forensic sciences by providing precise chemical analysis of biological samples, aiding in drug identification, toxicological screening, and determining causes of death. NMR spectroscopy was employed to screen blood samples collected fromindividuals. The technique detected and identified various metabolites simultaneously, providing comprehensive information vitalrole in metabolic fingerprinting. The integration of machine learning tools enhances the capabilities of forensic investigations by enabling advanced data processing, pattern recognition, and decision support. The referenced machine learning applications underscore the combined potential of NMR spectroscopy and machine learning in modern forensic science practices.

## MATERIAL AND METHODS

### *Sample preparation*

Blood samples were collected from each subject in the morning pre-prandial after overnight fasting in a heparinized vacutainer on the day of endoscopy. Blood plasma was immediately separated by centrifugation at 5000rpm for 10 minutes at 4°C and was stored at -80°C until NMR-based fingerprinting analysis. For NMR spectroscopy, 200 µl of plasma was diluted with 400 µl of $D_2O$, containing 0.5 mM of TSP and 0.5 mM of sodium formate. Blood samplesone-dimensional (1D) 1H NMR spectra were obtained using a CPMG pulse sequence that pre-saturated the $H_2O$ peak. A spin-echo delay of 15 ms and a relaxation delay of 70 s was used for the experiment. A total of 64 scans were collected with 32K data points over a spectral width of 9124.1 Hz.A 70-second relaxation delay was used.

To facilitate chemical shift assignments, additional 2D NMR studies (2D TOCSY) were carried out. A typical parameter for the TOCSY experiments was acquired with a spectral width = 9124.1 Hz, data points = 2K in F2 dimension, 400 increments with 16 scans per increment, relaxation delay of 2.5 s, and mixing time of 80 ms.

### *NMR spectroscopy*

All the NMR spectra were acquired at 700 MHz spectrometers (Agilent Technologies) at the Department of NMR, AIIMS, New Delhi. 1D (presat followed by CPMG) and 2D (TOCSY) NMR spectra were acquired for the assignment of metabolites.

The peaks observed in the 1D spectrum were assigned with the help of 2D NMR experiments. A conventional method was utilized to acquire a one-dimensional spectrum. Some important metabolites were assigned using 1D and 2D NMR.

NMR data analysis using machine learning tool

Free induction decay (FID) signals were processed by applying an exponential function with a line-broadening factor of 0.3 Hz before Fourier transformation. Then, the 1D $^1$H-NMR spectra were manually phased and carefully corrected for baseline distortion. Chemical shift correction was referenced to the methyl group of TSP at 0.0 ppm. Each NMR spectrum was binned by 0.02 to 0.04 ppm and integrated using the Mestre Nova software (Version 9.0; Mestrelab Research S.L., La Coruña, Spain). Subsequently, the water resonance spectral area (δ5.5–4.5 ppm) was eliminated from the whole spectrum (δ10.0-0.5–0 ppm) to remove the skewed baseline caused by lacking water saturation.

The identification and characterization of blood metabolites and determined by comparing the intensity of metabolites isolated from peaks obtained by integration of the signal with that of formate.

The concentration of only those metabolites, that showed well-resolved peaks in the 1D spectrum of will be determined using the Chenomx software (Chenomx NMR Suite 7.1).

Statistical investigation was accomplished using SPSS (SPSS Inc., Chicago, IL, USA). The data were normalized and subjected to multivariate pattern recognition analysis using Metabo Analyst 6.0 software. Metabolites were considered significant at VIP >1.0 for further analysis of metabolomic data analysis. Partial least square discriminant analysis (PLS-DA) and orthogonal partial least square discriminant analysis (OPLS-DA) loading plots were carried out for significant metabolite identification. Metabolites corresponding to significant spectral features were identified and characterized using the chemical shift and splitting patterns of metabolites reported in the literature,[24-29] two-dimensional NMR spectra.

## RESULTS AND DISCUSSION

### *Spectral assignments and identification of metabolites of blood spectra*

The resonance assignment of various metabolites detected in the blood plasma was carried out using 2D $^1$H-NMR while the quantitative analysis of metabolites was carried out using peak integrals from the 1D $^1$H-NMR spectra. Representative 1D $^1$H-NMR spectrum of blood samples obtained from healthy individualsare shown in Fig. 1.

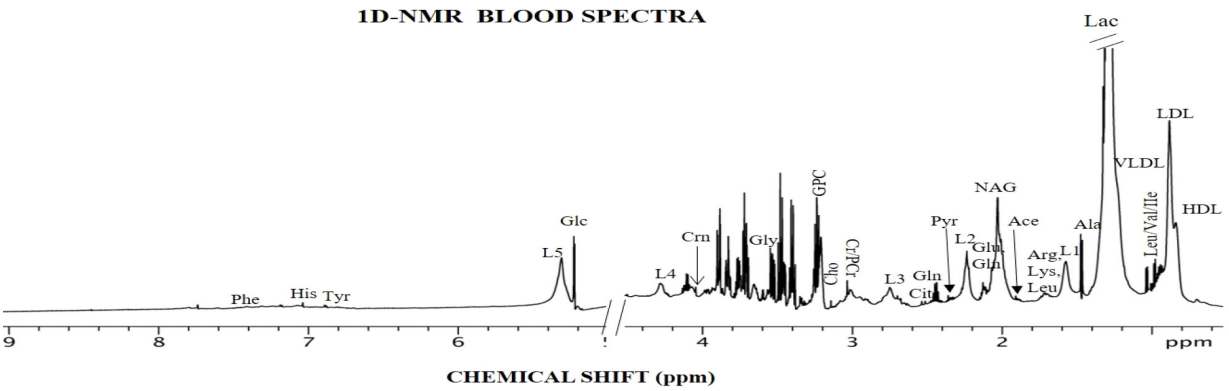


**Fig. 1:** Shows the representative of the one-dimensional (1D) Carr-Purcell-Meiboom Gill (CPMG) of $^1$H-NMR (700 MHz) spectra in D2O at 25 °C of the blood sample. HDL: High-density Lipoproteins; LDL: Low-density lipoproteins; VLDL: Very low-density lipoproteins; Leu: Leucine; IIe: Isoleucine Val: Valine; Lac: Lactate; OHB; hydroxybutyrate; Ala: Alanine; Ace: Acetate; NAG; N-acetyl glycoproteins; Glu: Glutamate; Gln: Glutamine; Pyr: Pyruvate; Cit: Citrate; Cr: Creatine; PCr: Phosphocreatine; Cho: Choline; GPC: Glycerophosphocholine; Glc: glucose; Gly: Glycine; Crn: Creatinine; Tyr: Tyrosine; His: Histidine; Phe; Phenylalanine.

The resonance assignments were made based on coupling connectivity observed in 2D spectra (TOCSY) and compared well with the chemical shift values of metabolites in blood samples. As an advantage of TOCSY, cross-peaks are formed between every component of a connected network. In all, metabolites could be unambiguously assigned in the blood plasma sampleusing 1D and 2D TOCSY spectra.

The 1D [1]H-NMR spectrum of blood plasma showed peaks due to metabolites such as amino acids, sugars phospholipids, organic acids, and other compounds.[23] Representative 2D TOCSY spectra obtained from blood used for metabolite assignments are shown in Fig. 2.

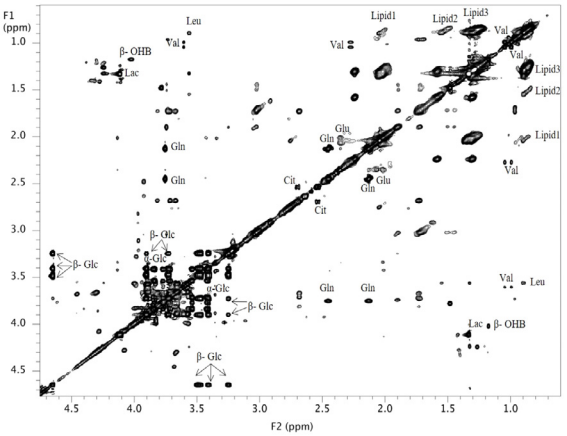


**Fig 2:** Shows representative region (0.7–4.6 ppm) of the two-dimensional total correlation (2D TOCSY) 1H NMR spectrum of the blood acquired at 700 MHz at 25 °C and assigned cross peak of various metabolites. VLDL: very low-density lipoproteins; Leu: Leucine; Ile: Isoleucine; Val: Valine; β-OHB: β-hydroxybutyrate; Lac: Lactate; Glu: Glutamate; Gln: Glutamine; Cit: Citrate; Glc: Glucose.

### *1D and 2D NMR spectra*

Representative 1D proton NMR spectra of blood samples obtained from a healthy individual are shown in Fig. 1. In all, metabolites were assigned unambiguously using 1D and 2D TOCSY spectra are shown in Fig. 1 and Fig. 2, respectively, and by comparing with the chemical shift values reported in the literature. The identification of metabolites that gave well-resolved signals in the 1D proton NMR spectrum was determined. In the 1D NMR spectrum, chemical shift and spin multiplicity are the most important parameters for resonance assignment. In 2D experiments, the resonances assignment is primarily dictated by the observation of cross peaks between coupled protons.[30-37] 2D TOCSY experiments provide a particularly powerful combination that may be sufficientto identify most metabolites in the blood. Various metabolites assigned and respective chemical shift positions of resonance arising from chemical entities, and blood are presented in Table 1.

Resonance peaks arising from ten amino acids could be identified unambiguously in the spectra ofthe blood sample. Between 0.90 and 1.00 ppm, the methyl protons of isoleucine (Ile), leucine (Leu), and valine (Val) were identified. The other protons of Ile, Leu, and Val were assigned using cross-peaks observed in TOCSY. Isoleucine$CH_3$ protons at 0.95 ppm showed coupling with its Gamma γ-$CH_2$ at 1.25 and 1.46 ppm, respectively. The methyl protons of alanine (Ala) at 1.48 ppm showed connectivity to its CH at 3.76 ppm. The 1D spectrum shows the resonances caused by the CH2 protons of glutamate (Glu) and glutamine (Gln) as a multiplet at 2.36 and 2.45 ppm, respectively. At 3.57 ppm, a glycine (Gly)-related singlet was detected. Furthermore, resonance was found in aromatic amino acids, such as tyrosine (Tyr) and phenylalanine (Phe). To identify Tyr's resonances, an unambiguous cross peak between H3, H5 (6.91 ppm) and H2, H6 (7.18 ppm) was identified. Similarly, H2, H6 resonances of Phe which were degenerate at 7.33 ppm showed coupling with its H3, H5 protons resonating at 7.43 ppm.

Resonances due to various organic acids such as lactate (Lac) and pyruvate (Pyr) were also observed. The doublet corresponding to methyl protons of Lac at 1.33 ppm showed connectivity with itsCH proton at 4.12 ppm in TOCSY. The $CH_3$ proton of Pyr was detected at 2.37 ppm. The AB pattern of citrate (Cit) was identified in the 1D spectrum by characteristics doublet of doublets cantered at 2.54 ppm and Formate (For) singlet at 8.46 ppm was observed.

The N $(CH_3)_3$ of choline (Cho) wasdetected at 3.21 ppm. The cross peaks due to glycerophosphocholine (GPC) were seen clearly in the TOCSY. The N $(CH_3)$ protons of GPC were observed at 3.23 ppm. A singlet peak at 1.91 ppm was assigned to the methyl protons of acetate (Ace). The signals due to hydroxybutyrate (βHOB) were observed at 1.20 ppm as a doublet. The connectivity between the $CH_3$ group at 1.20 ppm and CH resonance at 4.14 ppm of βHOB was observed.[30-37]

The H1′of-glucose(α-Glc) was assigned at 5.23 ppm which showed coupling to its H2′ proton at 3.53 ppm in TOCSY. Similarly, the H1′ of glucose (β-Glc) was observed as a doublet at 4.64 ppm. Resonances fromGlc were assigned following the connectivity pattern of H1′ (4.64 ppm) with H2′ (3.24 ppm), H4′ (3.41 ppm) and H5′ (3.47 ppm) protons. Other Metabolites Assignment Several resonances

**Table 1:** List of identified metabolites in the blood sample of healthy individuals using NMR spectroscopy and their chemical shift (ppm)position so fresonances1 H of metabolites. Code (identifiers) of the metabolites of data sets was searched indifferent metabolites Data bases (KEGGID and HMDB).

| Metabolites | Multiplicity (δ) ppm | KEGG ID | HMDB |
|---|---|---|---|
| Isoleucine (Ile) | 3.62 (α-CH), 1.97 (β-CH), 1.26 (γ CH2), 1.48 (γ′-CH2), 0.94 (δ-CH3) | C00407 | HMDB0000172 |
| Valine (Val) | 3.52 (α-CH), 2.21 (β CH),1.00 (γ-CH3), 1.04 (γ′-CH3) | C00183 | HMDB0000883 |
| Leucine (Leu) | 3.72 (α-CH), 1.72 (β-CH2), 1.69 (γ-CH2), 0.96 (δ-CH3) | C00123 | HMDB0000687 |
| Alanine (Ala) | 3.77 (α-CH), 1.48 (β-CH3) | C00041 | HMDB0000161 |
| Glutamate (Glu) | 3.65 (α-CH), 2.04 (β-CH2), 2.36 (γ-CH2) | C00025 | HMDB0000148 |
| Glutamine (Gln) | 3.65 (α-CH), 2.08 (β-CH2), 2.45 (γ-CH2) | C00064 | HMDB0000641 |
| Methanol (MeOH) | 3 | | |
| Glycine (Gly) | 3.56 (CH2) | C00037 | HMDB0000123 |
| Histidine (His) | 3.14 (β-CH2), 7.06 (H4), 7.83 (H2) | C00135 | HMDB0000177 |
| Tyrosine (Tyr) | 7.20 (H2, H6), 6.88 (H3, H5) | C00082 | HMDB0000158 |
| Phenylalanine (Phe) | 7.33 (H2, H6), 7.40 (H3, H5) | C00079 | HMDB0000159 |
| Choline (Cho) | 3.21 N(CH3)3, 3.52 (NCH2), 4.07 (CH2OH) | C00114 | HMDB0000097 |
| Glycerophosphocholine (GPC) | 3.23 N(CH3)3, 3.52 (NCH2 ) | C00670 | HMDB0000086 |
| Acetate (Ace) | 1.92 (CH3) | C00033 | HMDB0000042 |
| Acetoacetate (AcAc) | 2.23 (CH3) | C00164 | HMDB0000060 |
| Creatine (Cr) | 3.03 (NCH3), 3.93(CH2) | C00300 | HMDB0000064 |
| Phosphocreatine (PCr) | 3.04 (NCH3) | C02305 | HMDB0001511 |
| Creatinine (Crn) | 4.06 (CH2) | C00791 | HMDB0000562 |
| Lactate (Lac) | 4.12 (α-CH), 1.33 (β-CH3) | C00186 | HMDB0000190 |
| Pyruvate (Pyr) | 2.37 (CH3) | C00022 | HMDB0000243 |
| Glucose (Glc) | 5.23 (H1'), 4.64 (H1'), 3.53 (H2'), 3.72 (H3'), 3.42 (H4'), 3.24 (H2') | C00031 | HMDB0000122 |

such as methyl, methylene, allylic and olefinic protons from different lipids and triglycerides were also observed in the 1D CPMG spectra of lipids in blood plasma. The resonances from the terminal $CH_3$ protons of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) overlapped at 0.90 ppm. In TOCSY, a prominent cross peak was seen between the $CH_3$ and $CH_2$ protons of LDL and VLDL. The resonance corresponding to $CH_3$ of HDL was assigned at 0.84 ppm. Similarly, the resonances of Gly, Tyr, histidine (His), and Phe were assigned using TOCSY.

## CONCLUSION

In conclusion, NMR spectroscopy represents a powerful analytical technique that has revolutionized forensic investigations, particularly in the analysis of blood samples. Its ability to provide detailed chemical information with high sensitivity and specificity makes it invaluable in determining crucial aspects of criminal cases, ranging from drug detection to DNA analysis. As technology continues to advance, NMR spectroscopy is expected to play an increasingly prominent role in forensic science, contributing to enhanced accuracy and reliability in criminal investigations worldwide.

## ACKNOWLEDGMENT

Thanks, AIIMS, New Delhi for providing an NMR facility.

## REFERENCES


1. Locci E, Bazzano G,Chighine A, LoccoF, Ferraro E, DemontisR andd'Aloja, E2020Forensic NMR metabolomics: one more arrow in the quiver Metabolomics 16 11 118.
2. AmpanoziG,Halbheer D, Ebert L C, Thali M Jand HeldU2020 Postmortem imaging findings and cause of death determination compared with autopsy: a systematic review of diagnostic test accuracy and meta-analysis. International journal of legal medicine1341 321.
3. Garvin HM and Stock MK 2016 The Utility of Advanced Imaging in Forensic AnthropologyAcad Forensic Pathol. 201663499.
4. Liu R, Bao ZX, Zhao PJ and Li GH 2021Advances in the Study of Metabolomics and Metabolites in Some Species Interactions Molecules26113311.
5. SantosAD C, Dutra L M, MenezesLRA, Santos M F C and BarisonA2018 Forensic NMR spectroscopy: Just a beginning of a promising partnership TrAC Trends in Analytical Chemistry10731.
6. Groombridge C J1996 NMR spectroscopy in forensic science In Annual reports on NMR spectroscopy 32215297.
7. BeckonertO, Keun H C, Ebbels T M, Bundy J, Holmes E, Lindon J C and Nicholson J K2007 Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts Nature protocols 211 2692.
8. Bliziotis N G, Engelke UFH, Aspers RLEG, Engel J, Deinum J, Timmers HJLM, Wevers RA and Kluijtmans L A J 2020 A comparison of high-throughput plasma NMR protocols for comparative untargeted metabolomics. Metabolomics: Official journal of the Metabolomic Society 165 64.
9. Castillo-Peinado L S and Luque de Castro M D2016 Present and foreseeable future of metabolomics in forensic analysis Analytica chimica acta 925 1.
10. Dinis-Oliveira RJ 2019 Metabolism and metabolomics of opiates: A long way of forensic implications to unravel. Journal of forensic and legal medicine 61 128.
11. Emwas A H, Roy R, McKay R T, Tenori L, Saccenti E, Gowda G A N, Raftery D, Alahmari F, Jaremko L, Jaremko M and WishartDS2019 NMR Spectroscopy for Metabolomics Research Metabolites 97 123.
12. Procopio N and Bonicelli A 2024 From flesh to bones: Multi-omics approaches in forensic scienceProteomics 2413 e2200335.
13. Emwas A H, Roy R, McKay R T, Tenori L, Saccenti E, Gowda G A N, Raftery D, Alahmari F, Jaremko L, Jaremko M and Wishart D S 2019 NMR Spectroscopy for Metabolomics Research. Metabolites 97 123.
14. Lindon J C, Nicholson J K, Holmes E and Everett J R2000 Metabonomics: metabolic processes studied by NMR spectroscopy of biofluids. Concepts in Magnetic Resonance An Educational Journal125289.
15. HollywoodK, Brison D Rand Goodacre R2006 Metabolomics: current technologies and future trends Proteomics617 4716.
16. Wishart D S2007 Current progress in computational metabolomicsBriefings in bioinformatics 85 279.
17. WishartD S2008 Quantitative metabolomics using NMR. TrAC trends in analytical chemistry273 228.
18. IrwinC, van Reenen M, Mason S, Mienie L J, Wevers R A, Westerhuis J A and Reinecke C J2018 The [1]H-NMR-based metabolite profile of acute alcohol consumption: A metabolomics intervention study PloS one 135 e0196850.
19. Jawor P, Ząbek A, WojtowiczW, KrólD, StefaniakT andMłynarz P2019 Metabolomic studies as a tool for determining the post-mortem interval (PMI) in stillborn calvesBMC veterinary research151 189.
20. Mora-OrtizM, TrichardM, OregioniAand Claus SP2019 Thanatometabolomics: introducing NMR-based metabolomics to identify metabolic biomarkers of the time of death. Metabolomics: Official journal of the Metabolomic Society 153 37.
21. Hirakawa K, Koike K, Uekusa K, Nihira M, Yuta K and OhnoY 2009 Experimental estimation of postmortem interval using multivariate analysis of proton NMR metabolomic data. Legal medicine 1 11 S282.
22. Mazzatenta A, Pietrangelo T, Demontis R, and D'Ovidio C 2024 Volabolomic Fingerprinting for Post-Mortem Interval Estimation: A Novel Physiological Approach. Biomolecules 143 286.
23. PsychogiosN, Hau DD, Peng J, GuoA C, Mandal R, Bouatra S, Sinelnikov I, KrishnamurthyR,Eisner R, Gautam B, Young N, XiaJ, Knox C, Dong E, HuangP, Hollander Z, PedersenTL, Smith S R, BamforthF, GreinerR, … Wishart D S2011The human serum metabolome PloS one 62 e16957.
24. Lawton KA, Berger A, MitchellM, Milgram K E, Evans AM, Guo L, Hanson R W, Kalhan S C, Ryals J Aand MilburnMV2008 Analysis of the adult human plasma metabolomePharmacogenomics 94 383.
25. Lindon J C, Holmes E and Nicholson J K2007 Metabonomics in pharmaceutical R&D The FEBS journal2745 1140.
26. Zlatkis A, Bertsch W, Bafus D Aand Liebich H M1974 Analysis of trace volatile metabolites in serum and plasma. Journal of chromatography91 379.
27. Corsaro C, VasiS, Neri F, Mezzasalma A M, Neri Gand Fazio E2022 NMR in metabolomics: From conventional statistics to machine learning and neural network approaches Applied Sciences 126 2824.
28. Ebbels T M and Cavill R 2009 Bioinformatic methods in NMR-based metabolic profiling. Progress in nuclear magnetic resonance spectroscopy 554 361.

29. CaoJ, An G, Li J, Wang L, RenK, DuQ, Yun K., Wang Y and SunJ2023 Combined metabolomics and tandem machine-learning models for wound age estimation: a novel analytical strategy Forensic sciences research 81 50.

30. Kumar P, Kumar R, Kumar V, Kumaran SS, Sharma S, Thulkar S 2018 High-grade and low-grade prostate cancer discrimination via blood plasma NMR-based metabolomics. In: Proceedings of the Twenty-Seventh Annual Meeting of the International Society for Magnetic Resonance in Medicine (ISMRM) 2285.

31. Jagannathan NR, Kumar P, Kumar R, Kumar V, Kumaran SS, Thulkar S, 2018 Metabolic Profiling of Blood Plasma for Distinguishing Prostate Cancer in Patients with Positive Biopsy and Negative Biopsy using NMR Spectroscopy In Proceedings of the Twenty-Seventh Annual Meeting of the International Society for Magnetic Resonance in Medicine (ISMRM) 4007.

32. Jagannathan NR, Kumar P, Kumar R, Kumar V, Kumaran SS, Sharma S, 2018 Blood Plasma Metabolic Profiling Discriminates Prostate Cancer Patients with Metastases from those Without Metastases In Proceedings of the Twenty-Seventh Annual Meeting of the International Society for Magnetic Resonance in Medicine (ISMRM) 2277.

33. Kumar P, Kumar R, Sharma S, Thulkar S, Khan MA and Kumar V 2024 NMR-based metabolomics for early detection of prostate cancer biomarker/s Intl Soc Magn Reson Med (ISMRM) 3651.

34. Kumar P, Kumar R, Sharma S, Thulkar S, Khan MA and Kumar V 2023 Blood plasma metabolomics study of metastases prostate cancer patients reflects liver alterations involving carbon and nitrogen metabolism Intl Soc Magn Reson Med (ISMRM) 4264.

35. V Kumar, Kumar P, Kumar R, Gupta SD, Thulkar S, Jagannathan NR, and Jayasundar R 2022 Targeted Metabolomic Profiling of Blood Plasma in Aggressive Prostate Cancer Patients using NMR Spectroscopy In: Proceedings of the 31ST Annual Meeting of the International Society for Magnetic Resonance in Medicine (ISMRM) 0929.

36. Kumar P, Kumar R, Gupta SD, Thulkar S, Jagannathan NR, and Jayasundar R , and Kumar V 2020 Metabolic profiles of blood plasma in prostate cancer patients having different dietary patterns Proc 28TH Intl Soc Mag Reson Med 2960.

37. V Kumar, Kumar P, Kumar R, Gupta SD, Thulkar S, Jagannathan NR, and Jayasundar R 2020 Metabolic Differences in Blood Plasma of Type 2 Diabetic and Non-diabetic Prostate Cancer Patients using NMR Spectroscopy Proc Intl Soc Mag Reson Med 2953.

# Instructions to Authors

Submission to the journal must comply with the Guidelines for Authors.
Non-compliant submission will be returned to the author for correction.

To access the online submission system and for the most up-to-date version of the Guide for Authors please visit:

http://www.rfppl.co.in

Technical problems or general questions on publishing with **JFCT** are supported by Red Flower Publication Pvt. Ltd.'s Author Support team (http://rfppl.co.in/article_submission_system.php?mid=5#)

Alternatively, please contact the Journal's Editorial Office for further assistance.

**Editorial Manager**
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi - 110 091(India)
Mobile: 9821671871, Phone: 91-11-79695648
E-mail: info@rfppl.co.in

*Original Article*

Journal of Forensic Chemistry and Toxicology
Volume 10 Number 1, January-June 2024
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.10124.5

# The Estimation of Genome Potential for STR Analysis in bloodstain collected at different temperatures

**Samruddhi Payas[1], Anita Yadav[2], Tanushree Dheer[3], Rajesh Kumar[4]**

**How to cite this article:**

Samruddhi Payas, Anita Yadav Tanushree Dheer *et al*. The Estimation of Genome Potential for STR Analysis in bloodstain collected at different temperatures. J Forensic Chemistry Toxicol. 2024;10(1):35–38.

**Abstract**

DNA analysis is used in forensic investigations to identify individuals and solve crimes. Important pieces of evidence are frequently found at crime scenes, including bloodstains. However, both the quantity and the quality of the extracted DNA can vary depending on the conditions in which bloodstains are exposed before analysis, such as different temperatures.

This study, to evaluate the genome potential for performing short tandem repeat (STR) analysis on bloodstains collected from three different temperature settings: $-20^0$, $4^0$, and room temperature. The study will evaluate how temperature affects DNA extraction and typing. Previous studies have demonstrated that burns and high heat can cause DNA to degrade and lower DNA quantities in bloodstains. Additionally, research has shown that even after being heated to specific temperatures, cleaned bloodstains can still produce sufficient DNA for analysis. This research will help to clarify the challenges and limitations involved in conducting STR analysis on bloodstains exposed to a range of temperatures by examining the effects of various temperatures on DNA quantity and quality in bloodstains. The results will help investigators in the forensic field increase DNA recovery techniques and raise the accuracy of DNA profiling in criminal investigations.

**Keywords:** Short Tandem Repeat (STR analysis on bloodstain); Temperatures; DNA extraction; Criminal investigations.

## INTRODUCTION

Forensic DNA analysis is crucial in criminal investigations, with Short Tandem Repeat (STR) analysis being a widely used method. However, temperature changes can affect the quality and quantity of DNA extracted from blood stains. This thesis aims to investigate the impact of temperature variations on STR analysis in blood stains and develop reliable estimation models. The research will involve collecting samples under controlled temperatures, aiming to improve the accuracy and reliability of forensic DNA analysis in criminal investigations.[1,2] Blood's history spans millions of years, with scientific understanding evolving from ancient beliefs to modern discoveries. Microscopy in the 17th century revolutionized blood circulation, while Karl Landsteiner's 1901 discovery revolutionized transfusion treatment. Hematology advanced in the late 19th and early 20th centuries, with specialized magazines like "Blood" promoting interdisciplinary collaboration. Blood has significantly impacted medical practices,

**Author Affiliation:** [1]M.Sc Student, [2]Associate Professor, [3]Assistant Professor, Department of Forensic Medicine and Toxicology, Sanjeev Agarwal Global Educational University, Bhopal 462022, Madhya Pradesh, [4]Associate Professor, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, Deoghar 814152, Jharkhand, India.

**Corresponding Author: Rajesh Kumar,** Associate Professor, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, Deoghar 814152, Jharkhand, India.

**E-mail:** sujeetmewar@gmail.com

**Received on:** 06.07.2024 **Accepted on:** 27.06.2024

particularly in transfusion medicine.[3,4,5] Blood is a crucial biological evidence found at crime scenes, serving various functions such as transporting vital materials, removing waste, protecting the body from pathogens, regulating body temperature, transporting hormones, and preventing excessive bleeding. It is composed of cells, enzymes, proteins, water, and inorganic substances. Blood stains provide information on the circumstances surrounding violent crimes, driving criminal investigations and supporting the conclusion of criminal cases. Blood detection in forensic science can help determine the source of blood, such as foetal blood, pregnancy and abortion blood, menstrual blood, and arterial blood. Bloodstain analysis from clothing is a crucial technique in forensic science for extracting valuable information from bloodstains in criminal investigations.[6,7,8] It helps in reconstructing details, identifying patterns, and developing conclusions by examining the size, shape, distribution, and characteristics of bloodstains.[9,10,11] Proper documentation, careful collection, and storage of garments are essential for maintaining the integrity of evidence. Ageing of blood can be determined by colour changes over time. Bloodstain analysis provides valuable data for criminal investigations and judicial actions. Factors affecting genomic potential for STR analysis include DNA degradation, detection rate, and quantitative PCR assays. Experimental data and analysis are required to determine the impact of temperature on DNA degradation and the success rate of STR analysis.[12-15]

## METHODOLOGY

### *Material Required*

***Chemicals:*** 1x TAE buffer, Sol. B (pH 8.0), 20% SDS, Proteinase K, Phenol-chloroform-Isoamyl alcohol mixture (25:24:1), 5M Sodium Acetate (pH 5.2), Chilled Isopropanol (IPA), 70% ethanol, Nuclease free water, Primers, dNTPs + Buffer etc.

***Instruments:*** Microcentrifuge, Autoclave, Refrigerator**,** PCR Machine, RT-PCR Machine, Pipette/Tips, Eppendorf tubes 2ml, Scissors, Forceps etc.

***Sample Collection:*** Blood collected from a single person after taking consent, a total of 30 samples of bloodstains were spotted in a cotton cloth/fabric. Out of which 10 blood stains were kept at Room temperature (RT), 10 at $-20^0$, and 10 at $4^0$. The samples were incubated at respective temperatures for 11 days before processingfurther.

***Sample Pretreatment:*** The sample was collected on 25th April 2023 and kept each 10 samples at three temperatures Room Temperature (RT), $-20^0$, $4^0$ for 10 days. From 11th day DNA extraction from each sample was done having an interval of one day between three samples.

### *Procedure*

#### *DNA extraction from blood stains*

The study involved cutting a stain-free cloth and adding 1.5ml of Solu. B, 50% SDS, and Proteinase K. The mixture was incubated overnight and centrifuged at 10,000 RPM for 15 minutes. The supernatant was transferred to a fresh tube, mixed with Sodium Acetate and PCI, and incubated overnight. The pellet was washed with 70% ethanol, air-dried, and dissolved in nuclease-free water. The dissolved DNA was visualized under UV light using agarose gel electrophoresis.

#### *Agarose Gel Electrophoresis*

Agarose gel was created by diluting 10X TAE buffer to 1X and mixing 300 mg of agarose with 30 ml of 1X TAE buffer. The flask was heated, and the agarose was dissolved, while boiling was avoided. A gel-casting tray was sealed with biohazard tape. To the cooled agarose solution, 0.5 µl of ethidium bromide was added. The agarose was poured to a depth of 1 cm and allowed to solidify. A 500 µl DNA sample, 2.5 µl loading dye, and a marker were added to the gel. The gel was placed in a tank filled with TAE buffer. The electrophoresis was run at 80V for an hour until the front dye reached the gel's bottom. The electrodes were checked for bubbling, and the gel was photographed under UV transillumination.

#### *DNA Quantification*

The quantification was done using the Qubit 3.0 fluorometer kit and its instructions. The assay tubes for the standards and one assay tube for each user sample were set up. The working solution was prepared by diluting the Qubit reagent 1:200 in Qubit buffer. The assay tube and standard tubes were prepared according to the table given below.

**Table 1:** Assay tube and standard tubes protocol

| Components | Standard Assay Tubes (µl) | Sample assay tubes (µl) |
|---|---|---|
| Working solution (from step 2) | 190 | 199 |
| Standard (from kit) | 10 | - |

*Table Cont...*

| | | |
|---|---|---|
| Vol. of sample | - | 1 |
| Total volume in each tube | 200 | 200 |

The tubes were vortexed for 2-3 seconds and incubated for 2 minutes at room temperature (RT). The tubes were inserted into the Qubit Fluorometer, and readings were taken. The readings were in ng/µl.

### *q-PCR*

***Procedure:***Reaction mixture is prepared by adding all the components in one tube except sample and dividing the content equally in all wells and then adding sample in last. Reaction mixture was prepared for the total volume of 10µl.

**Table 2:** Table Reaction mixture for q-PCR

| S/No. | Content | Volume (µl) |
|---|---|---|
| 1 | 2X qPCR Mix | 5 |
| 2 | Fwd. Primer NEAT 1 | 0.5 |
| 3 | Rev Primer NEAT 1 | 0.5 |
| 4 | RNAse free water | 3.8 |
| 5 | Sample (DNA) | 0.2 |

***Running of q-PCR:*** The 2-step q-PCR involved a hot start at 950°C for 5 minutes, followed by denaturation, primer annealing, and extension at 600°C for 20 seconds. The amplification cycle was 45 cycles, culminating in melting.

**Table 3:** Steps in q-PCR

| Sr/No. | Content | Temp (°C) | Duration (sec) |
|---|---|---|---|
| 1 | Pre-denaturation | 95 | 3 min |
| 2 | Denaturation | 95 | 10 |
| 3 | Annealing &Extension | 60 | 20 |
| 4 | Melting | ------ | ------ |

**STR Analysis:** STR analysis compares allele repeats at specific DNA loci in samples, used in forensic analysis, human sample authentication, and cell line authentication. It detects and measures short tandem repeat sequences, 2-7 base pairs long.[16]

### *Procedure*

***DNA Extraction:*** The DNA is extracted from the biological sample of interest using established protocols.

***Primer Design:***Primers target specific STR loci for amplification, allowing repeat sequence amplification.

***Polymerase Chain Reaction (PCR):*** PCR amplifies targeted STR loci using primers, generating multiple copies of the STR region through denaturation, annealing, and extension cycles.

***Fragment Separation:*** PCR products are separated using electrophoresis or

capillary electrophoresis to determine STR repeat lengths.

***Data Analysis:*** Using software or genetic analysers, separated fragments are analysed to identify STR locus repeat units and establish links.

STR analysis is utilized in forensic science for human identification and cell line authentication, enabling discrimination and accuracy in unrelated individuals.

## RESULTS AND DISCUSSION

### *Gel-Electrophoresis Results RT-PCR Results*

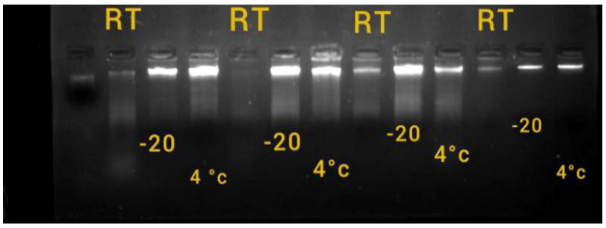


**Fig. 1:** 1-12 Gel-Electrophoresis samples visible as bands in results

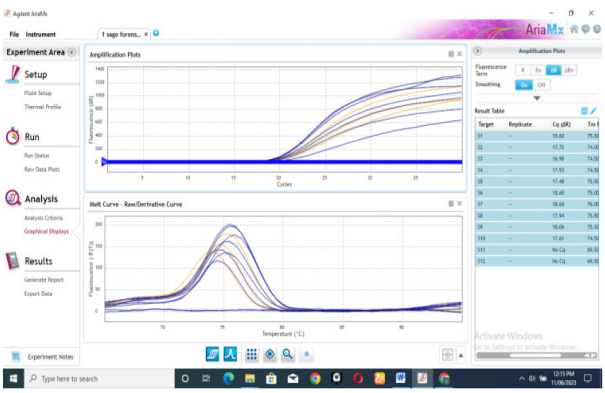


**Fig. 2:** Melt curve (Fold change) of NEAT 1 gene expression

***Note:*** *CT values range from 17.49 to 19.32, indicating NEAT1 gene expression in all samples, with lower values indicating higher abundance.*

In the given dataset, the CT values for the NEAT1 gene range from **17.49 to 19.32.** Sample B3 (S3) has the lowest CT value of **17.49**, suggesting the highest expression level of NEAT1 among the tested

samples. Sample B1 (S1) and B10 (S10) have the highest CT values of **19.04** and **19.32**, respectively, indicating relatively lower expression levels of NEAT1.

**Table 4:** Statistical Summary of qPCR Data.

| Parameters | CT values | Fold change values |
|---|---|---|
| Mode | 18.21 | 74.5 |
| Median | 1.245 | 75.25 |
| Mean | 18.25 | 74.85 |
| Range | 17.49 – 19.32 | 69.5 – 76 |

***STR Analysis Results***

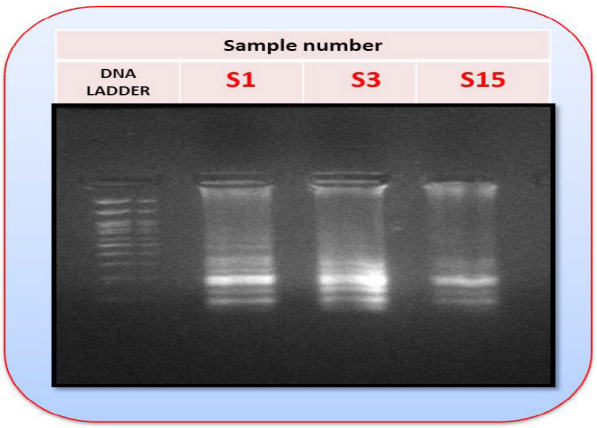


**Fig. 3:** Amplification of human genome STR region from blood-stained cloth samples using molecular marker-based primer set yields easily distinguishable bands, providing baseline data for forensic purposes

## CONCLUSION

The study investigates the application of Short Tandem Repeats (STRs) for temperature-dependent blood stain analysis. For locating genetic variations and mutations in Mendelian illnesses, researchers found STR analysis to be reliable. The effect of temperature on the degradation of DNA and detectable loci was also investigated. The results of these investigations have implications for forensic research, improving methods for diagnosis and treatment, and benefiting society.

## REFERENCES

1. Bukyya JL, Tejasvi MLA, Avinash A, P CH, Talwade P, Afroz MM, Pokala A, Neela PK, Shyamilee TK, Srisha V. DNA Profiling in Forensic Science: A Review. GlobMed Genet. 2021May31;8(4): 135143. doi:10.1055/s00411728689. PMID: 34877570; PMCID: PMC8635824.
2. Blood at 70: its roots in the history of haematology and its birth.
3. The History of Blood | The New Yorker.
4. https://www.visiblebody.com/learn/circulatory/circulatory-functions-of-the-blood
5. https://open.oregonstate.education/aandp/chapter/18-1-functions-of-blood/
6. Composition of blood:- Indian J Anaesth. 2014 SepOct;58(5): 529537.Overview of blood components and their preparation - PMC.
7. Khan Academy. Components of blood (article) | Khan Academy.
8. Components of the Blood - Plasma and Serum.
9. Field, F., & Field, F. (2022). Examination of Blood in Forensics - Forensic 'blog. Forensic 'Blog.
10. https://doi.org/10.1016/j.forsciint.2022.111411
11. DNA Profiling in Forensic Science: A Review - PMC (nih.gov)
12. https://www.nist.gov/system/files/documents/2019/06/07/bpa_bibliography_by_topic_2019.pdf [date: -17-06-23 time: -11:49 pm]
13. De Castro TC. Forensic Textile Science || Forensic Interpretation of Bloodstains on Fabrics. 2017;127–67.
14. Singh P. Age Estimation of a Dried Bloodstain Using Different Techniques - A Review Article. Academic Journal of Forensic Sciences. 2018;1(1):23–7.
15. Kitayama T, Fujii K, Nakahara H, Mizuno N, Kasai K, Yonezawa N, *et al*. Estimation of the detection rate in STR analysis by determining the DNA degradation ratio using quantitative PCR. Leg Med (Tokyo) [Internet]. 2013;15(1):1–6. A.
16. Products on Blood Dna Retrieval From Cloth. The Egyptian Journal of Forensic Sciences and Applied Toxicology [Internet]. 2018;18(2):53–66.
17. A. Barbaro *et al.* / International Congress Series 1261 (2004) 562–5640).
18. Simbolo M, Gottardi M, Corbo V, Fassan M, Mafficini A, Malpeli G, *et al*. DNA qualification workflow for next generation sequencing of histopathological samples. PLoS One [Internet]. 2013;8(6): e62692.
19. M. Simbolo *et al.*, 2013, B.G. Wang *et al.*, 2003, E. Sunami AT *et al.*, 2009
20. Simbolo *et al.*, 2013.

*Review Article*

Journal of Forensic Chemistry and Toxicology
Volume 10 Number 1, January-June 2024
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.10124.6

# Flavored Hookah abuse: An Alarming Toxicity of Concern among Youths

**Neha Jain**

**How to cite this article:**

Neha Jain. Flavored Hookah abuse: An Alarming Toxicity of Concern among Youths. J Forensic Chemistry Toxicol. 2024;10(1):41–44.

**Abstract**

The hookah use among present day generation creates an alarming need considering its negative health effects. Hookah tobacco (also known as water pipe tobacco, maassel, shisha, narghile, or argileh) is a type of combustible tobacco that is smoked with a hookah (waterpipe). It is popular among the youths as these hookah smokers smoke at hookah lounges as well as in private homes, bars, cafes and restaurants. In all the flavored products of tobacco, nicotine is the most potent and one of the addictive drug present including hookah tobacco. The concentration show a variation although the average nicotine content ranges between 1.8–6.3%of 3.4 mg/g tobacco depends upon the brand. In this article efforts have been made to highlight the toxic effects of Flavored Hookah abuse among youths.

**Keywords:** Flavored Hookah; Toxicity; Youths; Tobacco; Alarming.

## INTRODUCTION

The problem of smoking tobacco in form of cigarettes, hookahs is the major concern among youths in relation to its adverse effects on health. It is well known fact that tobacco being one of the most widely abused substancein the world because of presence of a highly addictive major alkaloid stimulant nicotine in it. This nicotine abuse leads to the problem of adverse health effects including cancer, pulmonary and cardiovascular diseases.[1]

**Author Affiliation:** Former Senior Scientific Officer, Sherlock Institute of Forensic Science, New Delhi 110009, India.

**Corresponding Author: Neha Jain**, Former Senior Scientific Officer, Sherlock Institute of Forensic Science, New Delhi 110009, India.

**E-mail:** neha.jain258992@gmail.com

**Received on:** 23.03.2024

**Accepted on:** 28.06.2024



The hookah use among present day generation creates an alarming need considering its negative health effects.Hookah smokers smoke at hookah lounges as well as in private homes. Hookah tobacco (also known as water pipe tobacco, maassel, shisha, narghile, or argileh) is a type of combustible tobacco that is smoked with a hookah (waterpipe). Water pipe tobacco smoking obsession has again spread worldwide. Especially, the younger generation is besotted with mu'assel (shisha) and avidly use these for smoking.[3,8]

Flavors profiling in flavored hookah tobacco is an issue of increasing scrutiny for the health sector owing to its adverse effects on humans, especially being heated to produce smoke. Tobacco smoke comprises a large number of chemicals and brand-specific flavors included hundreds of volatiles complex mixture attributed towards its toxicity and carcinogenic properties. These additives added in commercial brands of mu'assel

(shisha) imparts diverse taste with amusing aroma.[4]

Hookah (water pipe) smoking is the inhaling of hookah tobacco smoke that has been generated by heating hookah tobacco with burning charcoal and passed through a partially filled water jar. The mechanism of hookah tobacco smoking is unique. First, the tobacco is heated indirectly with charcoal, then the smoke passes through a water bowl and finally is inhaled by the smoker through a rubber hose fitted with a mouthpiece. Hookah tobacco products come in different flavors, such as apple, mint, cherry, chocolate, coconut, licorice, cappuccino, and watermelon, paan, raas, strawberry, orange, paan masala, etc.[1,9]


**Fig. 1:** Hookah (water pipe) Smoking Apparatus.

***Source:*** (Middha and Negi Egyptian Journal of Forensic Sciences (2019) 9:39 https://doi.org/10.1186/s41935-019-0146-2)

### *Toxic Effects on Human Health*

Generally it is thought that smoking non – tobacco hookah is safe and does not causes toxicity but it is reported by researchers and scientists during their experimental studies conducted that even non-tobacco hookahs released smoke in the environment same like that of tobacco products.[4,6]

In all the flavored products of tobacco, nicotine is the most potent and one of the addictive drug present including hookah tobacco. The concentration show a variation although the average nicotine content ranges between 1.8–6.3% of 3.4 mg/g tobacco depends upon the brand.

In contrast to the misleading false ingredient statement '0.05% nicotine' that is portrayed on most hookah tobacco packages. The hookah smokers smoke 10–20 g hookah tobacco head per one hookah smoking session which leads to an average amount from 36 to 126 mg.[1,2]

The major chemicals attributed to toxicity are fragranced ones, synthetically derived from petroleum the smoking of them leads to major health hazards. The common signs of toxicity among youths involves cancer, birth defects, Central Nervous System (CNS) disorders and allergic reactions. Some of these chemicals are addictive in nature for ex-menthol which increases the nicotine receptor density.[5]

In fact, research shows that waterpipe smokers may absorb even more of the harmful chemicals found in cigarette smoke because smoking sessions are typically longer. A typical one-hour hookah session involves inhaling 100–200 times the volume of smoke from a single cigarette.[3] Hookah tobacco smoking has been associated with increased risk for lung and oral cancers, coronary heart disease, and pulmonary disease, cough, respiratory problems, reduced lung function and decreased fertility. The heating of charcoal not only create smoke but it also leads to production of highly toxic gases like carbon monoxide, certain metals and other carcinogenic chemicals which can increase the risk of cancers and heart problems.[2,6]

The burning of these flavored items not only produces smoke but also releases a small amount

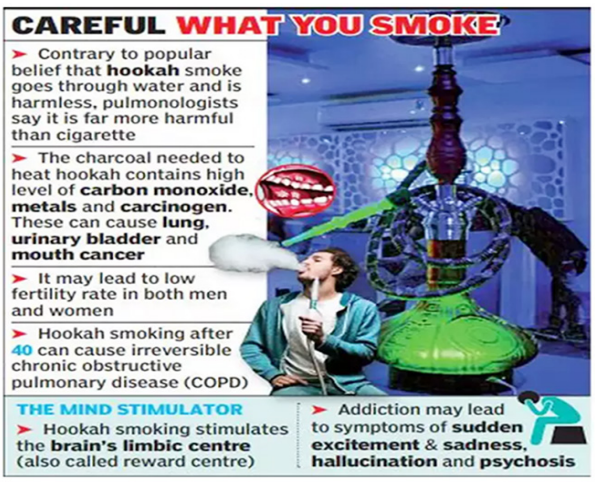


**Fig. 2:** Harmful Effects of Smoking Hookah

***Source:*** (Doctors caution youngsters on peril of Smoking Hookah – By RiyanRamanathan V) (http://timesofindia.indiatimes.com/articleshow/95900704.cms?utm_source=contentofinterest&utm_medium=text&utm_campaign=cppst)

of secondary smoke consisting of very fine particles spreading in air and pose serious toxicity to persons present in vicinity.

These Hookah pipes may leads to clotting in the pulmonary and coronary arteries and thereby obstruct the blood supply which results in Chronic Obstruction Pulmonary Disorder (COPD). The same pipe being used multiple times by multiple people may also increase the chances of oral herpes and other infectious diseases.[9]

### *Continuous Abuse leading Problems among Youths*

The continuous and prolonged use of present day well known non-tobacco hookahs or flavored hookahs are prevalent among youths and adults.As per the data presented by National Adult Tobacco Survey (NATS) in year 2013–2014, 20.2% of the youth population between the age group of 18–24 years used hookah 'every day', 'some days', or 'rarely'.[8]

The abuse of these items increased manifolds in the younger generation as per the report published on "Smoking and Tobacco Use" by Centre for Disease Control and Prevention, United States as the studies showing a vast increase between 22% to 40% from the past-year. There occurs a great upsurge in the abuse among youths aged from 19-30 years. The studies are not limited to this as the surveys conducted reveals a significant rise in the hookah abuse i.e. nearly 1 in every 13 (7.8%) high school students in the United States had used a hookah to smoke.[7]

The young adults who are not currently enrolled in college may also show similar behaviour and risk perceptions as do college students who are abusing it. Hence, young population are at the apex of abusing these addictive substances due to a large number of factors including motivational, socialization and many more which may pose an alarming threat to their life.

## CONCLUSION

Researchers and scientist according to a report published on internet by Express News Service on 12th February, 2021 states that present day widely available substances in the form of herbal cigarettes, bidis, a blend of certain herbs rolled in tendu leaves etc. marketed with the tagline of 'safety" and considered as a healthier alternative to traditional cigarettes are unhealthier and poses serious risk to the user.[5]

Usually the packets of all the varieties of these flavored items of hookah does not contain any detail regarding the chemical constituents and other multiple flavoring additives.[4]

The chemical constituents of these products is not been completely known to the users because the manufactures remain silent about mentioning the chemical ingredients of the flavoring agents. The short falls in the measures owing to technical procedures for identification these additives are masking the data showing the presence of these carcinogenic flavored additives in such toxic herbal compounds and hookahs and thereby increase the hazard potential for human health. The need of the hour is to unequivocally establish a technique for chemical profiling of the flavoring additives using state of art.[4]

Henceforth, directives must be taken by various state and central bodies and conventions must be made, policies must be framed to regulate tobacco products production in form of these ***Flavoured Hookahs*** and their abuse especially among youths to protect public health because ***"Health is Wealth"***

## REFERENCES


1. Farag, M.A., Elmassry, M.M. & El-Ahmady, S.H. The characterization of flavored hookahs aroma profile and in response to heating as analyzed via headspace solid-phase microextraction (SPME) and chemometrics. Sci Rep 8, 17028 (2018). https://doi.org/10.1038/s41598-018-35368-6.
2. Kassemet al, Levels of Urine Cotinine from Hookah Smoking and Exposure to Hookah Tobacco Secondhand Smoke in Hookah Lounges and Homes. Int J High Risk Behav Addict. 2018 Mar;7(1):e67601. doi: 10.5812/ijhrba.67601. Epub 2018 Feb 19. PMID: 29805963; PMCID: PMC5967845.
3. https://www.fda.gov/tobacco-products/products-ingredients-components/hookah-tobacco-shisha-or-waterpipe-tobacco
4. Middha Deepak and ArchnaNegi, Forensic chemical profiling of flavouring additives in seized mu'assel (shisha) by gas chromatography-mass spectrometry (GC-MS),Egyptian Journal of Forensic Sciences (2019) 9:39. https://doi.org/10.1186/s41935-019-0146-2
5. https://indianexpress.com/article/cities/chandigarh/herbal-flavoured-hookah-cigarettes-sold-as-healthcare-products-not-safe-says-study-by-oral-health-sciences-centre-pgimer-7184989/
6. https://recipes.timesofindia.com/articles/health/8-facts-about-hookah-that-will-shock-you/photostory/64477316.cms?picid=64477330

7. https://www.cdc.gov/tobacco/data_statistics/fact_sheets/tobacco_industry/hookahs/index.htm
8. Nicksic NE, Ly C, Loukas A, Perry CL. Hookah Use and Perceptions among Young Adult Hookah Users. J Addict BehavTherRehabil. 2018 Jun;7(2):10.4172/2324-9005.1000178. doi: 10.4172/2324-9005.1000178. Epub 2018 Mar 12. PMID: 31692999; PMCID: PMC6831090.
9. http://timesofindia.indiatimes.com/articleshow/95900704.cms?utm_source=contentofinterest&utm_medium=text&utm_campaign=cppst

*Case Report*

Journal of Forensic Chemistry and Toxicology
Volume 10 Number 1, January-June 2024
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.10124.7

# Natural Death Turned Unnatural

**Kamalkant Sahu[1], Dheeraj Abhaykumar Binodkumar[2]**

**How to cite this article:**
Kamalkant Sahu, Dheeraj Abhaykumar Binodkumar. Natural Death Turned Unnatural. J Forensic Chemistry Toxicol. 2024;10(1):47–50.

**Abstract**

Acute airway obstruction or choking is reportedly commoner in children than adults. Most commonly aspirated material among the children is either vegetable matter or inorganic objects such as a toy.Foreign body aspiration (FBA)amounts to the fourth leading cause of death occurring at either home or community places, in the United States of America.The death rate in cases of FBA in adults is seven times higher among people of age >65 years than in children, though the prevalence of it is less. Cardiovascular diseases (CVD) including ischaemic heart disease and cerebrovascular such as stroke account for 17.7 million deaths. Such a case where the diagnosis of cardiovascular disease was much eminent, turned out to be of choking on autopsy is discussed in this case report.

**Keywords:** FBA; Choking; Elderly; CVD.

## INTRODUCTION

Out of the exhaustive list of emergency health conditions, acute airway obstruction is a condition which is first handled by the general public at the time of occurrence and later by the health care providers. Literature suggests that the outcome of such a condition can be positively increased by proper public education.[1] The aetiology behind this condition is varying according to the age group and so are the methods to handle it. Strategies followed by the other developed countries can be of use in India, in spite of the lesser available data on the condition.

Acute airway obstruction or choking is reportedly commoner in children than adults. Most commonly aspirated material among the children is either vegetable matter or inorganic objects such as a toy. Foreign body asphyxia (FBA) is a well-known aspect to the physician.

FBA is the fourth leading cause of death at home or community places in the United States of America. As compared to children FBA in adults occur due to aspiration of food particles, medicine tablets, loose dental implants or other inorganic materials.[2] The death rate in cases of FBA in adults is seven times higher among people of age >65 years than in children, though the prevalence of it is less.[3]

60% of all deaths occur due to non-communicable diseases such as cardiovascular disease (CVD),

**Author Affiliation:** [1]Senior Resident, Department of Forensic Medicine & Toxicology, All India Institute of Medical Sciences Raipur, Chattisgarh 492099, [2]Assistant Professor, Department of Forensic Medicine & Toxicology, All India Institute of Medical Sciences, Deoghar, 814152 Jharkhand, India.

**Corresponding Author: Dheeraj Abhaykumar Binodkumar**, Assistant Professor, Department of Forensic Medicine & Toxicology, All India Institute of Medical Sciences, Deoghar, 814152 Jharkhand, India.

**E-mail:** kamalkant2302@gmail.com

**Received on:** 23.03.2024

**Accepted on:** 28.06.2024

various cancers, chronic respiratory illnesses, and diabetes.Cardiovascular diseases including ischaemic heart disease and cerebrovascular such as stroke account for 17.7 million deaths.[4]

## CASE PRESENTATION

A 60-year male was brought to the casualty of a tertiary care hospital, by his son in an unconscious state, for the last 30 minutes (Image 1). As per the history provided by the relatives, he was a known case of hypertension for the last 15 years, diabetic for the last 10 years and was on regular prescribed medication. All of a sudden he collapsed complaining of chest pain at night time. During the period of resuscitation, he was tested for Troponin-T, which turned out to be non-significant. Also, there were no obvious changes on the electrocardiogram and normal blood sugar level. Resuscitation attempts were made but were futile. He was pronounced dead after the examination by the on-duty doctor. Since the cause of death could not be established, the body was subjected to a post-mortem examination.

On post-mortem examination, the heart weighed 378 grams. On dissection of the heart, 50% narrowing of the lumen of the left anterior descending artery was noted. The rest of the coronaries, valves and walls were unremarkable [Image 2]. The lungs were then examined, the right lung weighed 487 grams and the left weighed 468 grams. On dissection of the trachea, greyish-coloured soft gelatinous material was noted obstructing both primary bronchi, with hyperemia [Image 3]. On further dissection of the lungs, the greyish-coloured material was found till the terminal bronchus [Image 4].

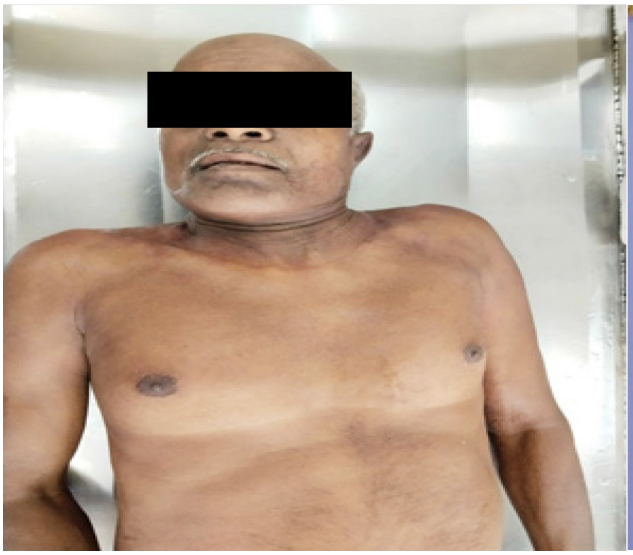

**Image 1:** Elderly Patient

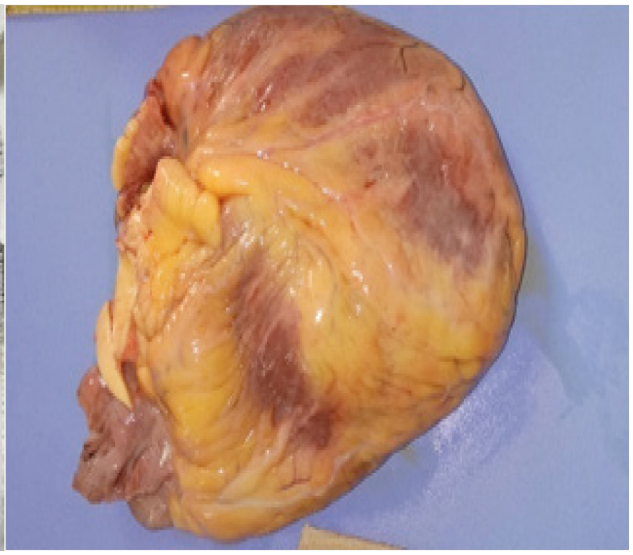

**Image 2:** Heart

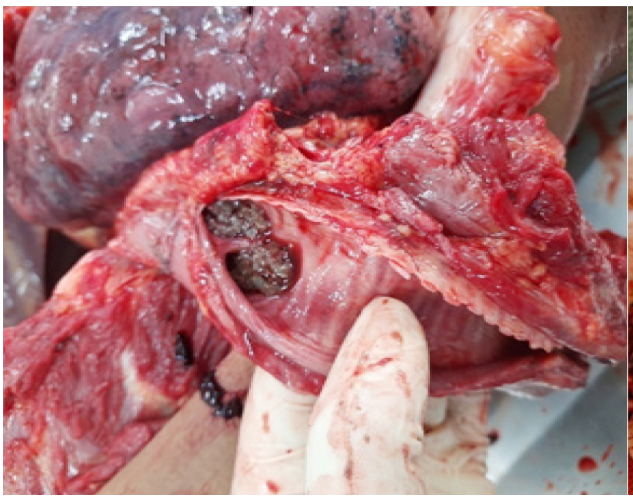

**Image 3:** Choking material in Trachea

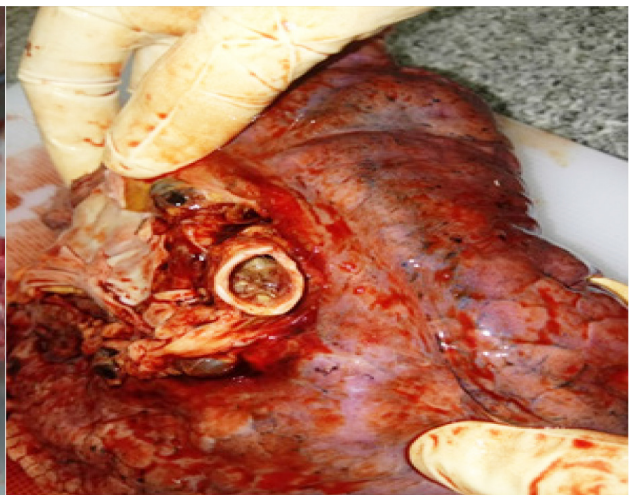

**Image 4:** Choking material inside Lung

## DISCUSSION

In 1963, Haugen was the first to coin the term "Café Coronary".[5] FBA at the time of eating presents with severe dyspnoea. Hence, even after this long duration passed after the coining of the term, people still do not suspect choking and wrongly label them as coronary artery disease, when the patient is of old age.Home is the most common place where FBA occurs other than the old age homes in cases of elderly patients.[6] This may due to the non- affordability of trained personnel at home to take care of elderly people in India.

Berzlanovich AM *et al.* found in their study that among the cases brought to the emergency during their research study period, observers were present near the patient of FBA in 63% of cases. Moreover, only in 8% of cases, the fatal emergency was correctly identified. Most commonly misdiagnosis related to symptoms of FBA are epileptic seizures, and cardiac arrest in adult patients.[7] The special aspect of FBA in the elderly is still at large not investigated in elderly patients in the current medical textbooks.

Elderly individuals take a long time in processing the food in their mouth before the small pieces can be swallowed.[8,9] Hence, life-threatening conditions can occur if the feeding rate is increased leading to the overloading of deglutition. Thus, such fatal events may be preventable, subject to knowledge of the nature and frequency of FBA and its causes. The data to which can be collected from the medical health care centres and providers maintaining records of all such sudden and unexpected deaths.[10]

Various neurological conditions such as Parkinsonism, Alzheimer's disease, or haemorrhagic stroke leading to difficult mastication and incoordination can lead to increased incidences of FBA. Also, the usage of sedative drugs in certain conditions, with anti-dopaminergic or anticholinergic activity has been directly linked to an increased incidence of FBA.[11,12]

Heimlich manoeuvre is life-saving in cases of FBA where the obstruction is at the level of the glottis and supra-glottic region. [13,14] Simple insertion of fingers or specially designed curved plastic forceps insertion into the mouth and hypopharynx can also be used to remove the obstruction.[15] The range of possible outcomes from the tracheobronchial foreign body includes self-resolution, pneumonia, lung abscess, asphyxia and death.

India is the fifth contributor to the world's death. Among the causes, cardiovascular disease (CVD) is the most common. Moreover, among Indians CVD occurs a decade earlier compared to the western population. The conventional risk factors fail to explain the increased risk of Indians towards cardiovascular diseases.[16] 28% of deaths and 14% of total disability-adjusted life years in 2016 were due to CVD as compared to 15% and 6.9% respectively in 1990.[17]

## CONCLUSION

Most incidences of FBA occurring in presence of the public is preventable. Proper training of the general public in applying the Heimlichmanoeuvre, can prevent the fatal outcome of choking. Thus, it is suggested for all first-handphysicians be well-oriented and trained to differentiate between choking and CVD. Both being fatal to the individual warrants urgent management.

## REFERENCES


1. Sadan N, Raz A, Wolach B. Impact of community educational programmes on foreign body aspiration in Israel. Eur J Pediatr. 1995;154(10):859-62.
2. Baharloo F, Veyckemans F, Francis C, Biettlot MP, and Rodenstein DO, "Tracheobronchial foreign bodies: presentation and management in children and adults," Chest, vol. 115, no. 5, pp. 1357–62, 1999.
3. Kramarow E, Warner M, Chen L-H. Food-related choking deaths among the elderly. Injury Prevention. 2014;20(3):200-03.
4. WHO. Global Health Estimates 2015: Deaths by Cause, Age, Sex, by Country and by Region, 2000e2015. Geneva: World Health Organization; 2016.
5. Haugen RK. The cafe coronary—sudden deaths in restaurants. JAMA 1963;186:142–3.
6. Dolkas L, Stanley C, Smith AM, Vilke GM. Deaths associated with choking in San Diego county. J Forensic Sci. 2007 Jan;52(1):176-9.
7. Ahmed SM, Garg R, Divatia JV, *et al.* Compression-only life support (COLS) for cardiopulmonary resuscitation by layperson outside the hospital. Indian Journal of Anaesthesia. 2017;61(11):867.
8. Hu T, Huang L, Cartwrigth WS. Evaluation of the costs of caring for the senile demented elderly: a pilot study. Gerontologist 1986;26:158–62.
9. Fioritti A, Giaccotto L, Melega V. Choking incidents among psychiatric patients: retrospective analysis of thirty-one cases from the west Bologna psychiatric wards. Can J Psychiatry 1997;42:515–20.
10. Berzlanovich AM, Dorner BF, Waldhoer T, Fasching P, Keil W. Foreign Body Asphyxia A Preventable

Cause of Death in the Elderly. Am J Prev Med 2005;28(1):65–9.

11. Pontoppidan H, Beecher HK. Progressive loss of protective reflexes in the airway with the advance of age. JAMA 1960;174:77–81.
12. Kendall KA, Leonard RJ. Bolus transit and airway protection coordination in older dysphagic patients. Laryngoscope 2001;111:2017–21.
13. Feinberg MJ, Ekberg O. Deglutination after near-fatal choking episode: radiologic evaluation. Radiology 1990;176:637–40.
14. Hsieh HH, Bhatia SC, Andersen JM, Cheng SC. Psychotropic medication and nonfatal cafe coronary. J ClinPsychopharmacol 1986;6:101–2.
15. Eller WC, Haugen RK. Food asphyxiation—restaurant rescue. New Engl J Med 1973;289:81–2.
16. Prabhakaran D, Jeemon P, Roy A. Cardiovascular diseases in India. Circulation. 2016;133:1605-20.
17. Ruhil R. India has reached on the descending limb of tobacco epidemic. Indian J Community Med. 2018;43:153-6.

*Case Report*

Journal of Forensic Chemistry and Toxicology
Volume 10 Number 1, January-June 2024
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.10124.8

# Don't Cry Over Spilled Milk: A Case Report on Intoxicating Mystery for Forensic Chemistry

**Vivekanshu Verma**

**How to cite this article:**

Vivekanshu Verma. Don't Cry Over Spilled Milk: A Case Report on Intoxicating Mystery for Forensic Chemistry. J Forensic Chemistry Toxicol. 2024;10(1):51–54.

**Abstract**

Under the Bharatiya Nyaya Sanhita 2023, Section 274, adulterating milk with hazardous chemicals including hydrogen peroxide is a punishable crime. This caustic chemical poses significant health risks, particularly to children, causing corrosive harm to their developing bodies. The law explicitly targets those who endanger public health by adulterating food or drink intended for sale, imposing strict penalties to deter such malicious practices. Ensuring the safety and integrity of consumables, especially milk, is crucial for protecting innocent children and maintaining public trust.

**Keywords:** Corrosive; Caustic; Adulterated Milk; Crime; Hydrogen peroxide.

## INTRODUCTION

Milk adulteration has long been a concern in India, impacting public health and undermining trust in essential food supplies. One alarming practice is the deliberate adulteration of milk with hydrogen peroxide, a caustic chemical. In some countries hydrogen peroxide is added to milk as an adulterant for shelf-life extension. However, in most countries the concentration of hydrogen peroxide in milk is limited or it is even prohibited to add this substance to the product.[1] Under the Bharatiya Nyaya Sanhita (Indian Penal Code) 2023, Section 274,[2] addresses this issue explicitly, criminalizing the adulteration of food or drink intended for sale. This legislation underscores the severe repercussions of such actions, particularly due to the corrosive harm caused to innocent children.

Hydrogen peroxide, a powerful oxidizing agent, is sometimes added to milk to extend its shelf life by preventing bacterial growth. However, this practice is not only unethical but also highly dangerous. Hydrogen peroxide can cause significant health issues when ingested, including irritation and damage to the gastrointestinal tract, vomiting, and abdominal pain. In children, whose bodies are still developing, the impact is even more severe, posing risks of long-term health complications or even life-threatening conditions.

The adverse effects of hydrogen peroxide in milk are particularly pronounced in children, who are more vulnerable to the toxic effects of chemicals. The ingestion of adulterated milk can lead to immediate

**Author Affiliation:** Associate Consultant & Fellow, Department of Emergency & Trauma Care, Medanta - The Medicity, Gurugram 122001, Haryana, India.

**Corresponding Author: Vivekanshu Verma**, Associate Consultant & Fellow, Department of Emergency & Trauma Care, Medanta - The Medicity, Gurugram 122001, Haryana, India.

**E-mail:** vivekanshu@yahoo.co.in

**Received on:** 19.06.2024

**Accepted on:** 18.07.2024

symptoms such as burns in the mouth and throat, difficulty swallowing, and severe digestive issues. Long-term exposure can impair organ function and stunt growth, underscoring the critical need for robust legal protections.

By criminalizing the adulteration of milk with hydrogen peroxide, Section 274 of the Bharatiya Nyaya Sanhita 2023 plays a pivotal role in ensuring the safety and well-being of the population, particularly children. It sends a strong message to milk producers and distributors about the serious consequences of compromising food safety. This legislative measure is part of a broader effort to enhance food safety standards in India, promoting public health and reinforcing consumer confidence.

## CASE REPORT

In the midnight, a 4-year-old child was brought by his parents with alleged history of hazardous chemical poisoning, in acute distress and crying state, with profuse transparent secretions from oral cavity, compromising his airway. On arrival, he was immediately received in resuscitation bay in our emergency department in Medanta the Medicity, sector 38, Gurugram. As per Pediatric resuscitation guidelines, Airway was secured, and breathing supported by mechanical ventilator. On local examination, the mucosa in oral cavity was reddened, sluffed and fragile, with whitish deposits adherent to the airway. ABG reported severe metabolic acidosis with raised lactates. For suspected foul play, medicolegal report prepared, and the concerned police authority informed for further medicolegal investigation of the case. A detailed history was noted, in which the parents of child stated that they are owner of a milk dairy commercially, working from home, and there they have kept a hazardous chemical: concentrated Hydrogen peroxide ($H_2O_2$) in liquid jar, which is accidently ingested by their child, while playing with it. When enquired further for why $H_2O_2$ is kept in their home, the patient's father explained that they used $H_2O_2$ as an adulterant to increase the quantity of buffalo's milk, for commercial gains. The patient was shifted to the Intensive Care Unit and underwent Upper Gastrointestinal Endoscopy, which reported corrosive caustic lesions in the stomach and food pipe. The patient started on antibiotics, proton pump inhibitors with intravenous fluids and kept Nil per orally. In the meanwhile, we requested the patient's father to bring the chemical jar of concentrated Hydrogen peroxide, which was consumed by the patient, so that the jar was sent to chemical analysis for identifying the hazardous chemical, in the biochemistry laboratory at Medanta Hospital, where the senior Biochemist reported that it contains concentrated Hydrogen peroxide. Later the alleged chemical Jar and blood samples were sent to the Forensic Science Lab and medicolegal investigation. After intensive therapy and closed monitoring of vital parameters, the patient recovered and was discharged in stable condition.

## CASE DISCUSSION

Worldwide, 80% of caustic ingestions occur in young children.[3] Adulterated milk in circulation and consumption poses a bigger threat to regulators, consumers, and the milk industry equally in the implementation of food safety standards. Adulteration in most food products is a rising challenge and a matter of concern in front of the authorities of countries, especially the developing ones.[4] Adulteration of Buffalo milk, white in colour with similar looking but hazardous caustic chemical: Hydrogen peroxide, which turns white in contact with urea and water. Although it looksalike the white milk, and to make it taste sweet, an artificial sweetener is added. Hydrogen peroxide is an oxidizing agent, but it is very unstable and readily breaks down to oxygen and water. Generation of oxygen gas in closed body cavities can potentially cause mechanical distension that results in gastric or intestinal perforation, as well as venous or arterial gas embolization.

### *$H_2O_2$ is acidic, not an alkali*

Hydrogen peroxide typically has a pH between 3 and 6, influenced by its concentration and production method. In aqueous solution, $H_2O_2$ releases two hydrogen ions, whereas water releases one. Because $H_2O_2$ protonates more extensively than water, it has a higher acid dissociation constant (Ka), making it a stronger Brønsted-Lowry acid compared to water. Acids cause coagulation necrosis, forming an eschar that limits further damage, primarily affecting the stomach more than the esophagus. In contrast, alkalis cause rapid liquefaction necrosis without forming an eschar, allowing damage to persist until the alkali is neutralized or diluted, and they typically affect the esophagus more than the stomach.[5]

Hydrogen peroxide is commonly used in dental products like mouth rinses and tooth whiteners, as well as in skin disinfectants, hair treatments, and earwax removers. It also has numerous industrial

applications. In veterinary medicine, it is used to induce vomiting. Hydrogen peroxide for household use is available in 3–5% solutions and causes only mild throat and gastric irritation with ingestion of less than 1oz.[6] However, gas embolization has occurred with low concentrations used in surgical irrigations.[7] Hair-bleaching solutions may contain hydrogen peroxide concentrations above 10%, which can be corrosive. Most reported fatalities involve ingesting undiluted 35% hydrogen peroxide, sold as "hyperoxygen therapy" in health food stores or labeled "food grade" in industrial settings.

Consult an expert pediatric gastroenterologist for a potential Upper Gastrointestinal endoscopy after children ingesting corrosive agents like concentrated hydrogen peroxide or potassium permanganate. Most ingestions are benign, causing only mild, self-limited irritation. However, for gas emboli resulting from concentrated peroxide ingestion, consider hyperbaric oxygen treatment.

Concentrated hydrogen peroxide solutions (20-30%) are strong irritants to the skin and mucous membranes, causing significant irritation upon contact. Even at lower concentrations, such as 6%, it acts as a weak irritant, releasing 20 vol% oxygen on contact with skin or mucous membranes. When used in colonic lavage, hydrogen peroxide can cause severe complications like gas embolism and intestinal gangrene, even at concentrations as low as 0.75%.

For treating hydrogen peroxide ingestion, it is crucial to immediately provide water to dilute the substance. Using a gastric tube is recommended to prevent increased internal pressure and mitigate potential damage. Given the risks associated with concentrated hydrogen peroxide, particularly for medical or cosmetic uses, handling should always be cautious, and appropriate protective measures should be taken. Additionally, seeking prompt medical attention is vital to manage any adverse effects effectively, especially in cases of high-concentration exposure or internal use.

### *Legal aspects of Adulteration of edible natural product with hazardous chemical*

As per BNS (Bharatiya Nyaya Sanhita) 2023, **Section 274:** Whoever adulterates any article of food or drink, so as to make such article noxious as food or drink, intending to sell such article as food or drink, or knowing it to be likely that the same will be sold as food or drink, shall be punished with imprisonment of either description for a term which may extend to six months, or with fine which may extend to five thousand rupees, or with both.[8] During the recent legal update by the Parliament, the cost of penalty is increased from one thousand,[9] to five thousand rupees, although it's still peanuts for anyone adulterating milk, who are earning in lakhs every month,[10] by illegal act, harming the others.

Section 274 of the Bharatiya Nyaya Sanhita 2023 is a critical piece of legislation designed to protect consumers from such hazardous practices. The section stipulates strict penalties for anyone found guilty of adulterating food or drink with harmful substances. By explicitly mentioning "adulteration of food or drink intended for sale," the law aims to safeguard public health and ensure that consumables meet safety standards. This provision is especially pertinent in the context of milk, a staple in the diet of children across India.

The inclusion of hydrogen peroxide in milk constitutes a clear violation of Section 274. The law recognizes the act of adding harmful substances to food as a deliberate and malicious attempt to deceive consumers and endanger their health. The perpetrators of such acts are subject to stringent legal consequences, reflecting the gravity of their offenses. This legal framework serves as a deterrent, aiming to curb the prevalence of food adulteration by imposing severe punishments on offenders.

### *Toxic Detective's analysis of intoxicating idiom: Do not cry over spilled milk!*

The phrase 'Don't Cry over Spilt Milk' means there's no use in worrying over past events which cannot be changed, to feel sorry or sad about something that has already happened.[11] Toxic Homonyms are similar sounding words with different meanings, causing impact in thoughtful investigation by a toxic detective. The title of the riddle is very titillating, for the youth, as it indicates revenge by injuring an eye for an eye. But as preachers like Mahatma Gandhiji said, injuring an eye for an eye, in revenge and the whole world will go blind. So, forgiveness is a bigger way of forgetting the harm suffered than taking petty revenge. But nature has its own way to handle the balance of right and wrongs done by greedy people, who adulterate the food supplied for commercial purposes, and make easy money, after diluting its contents, not only reducing the nutritional value, but increasing its harming capacity. As mostly cheap adulterants are synthetic chemicals, which are hazardous for ingestion. Don't Cry Over Spilled Milk: A Case Report on Intoxicating Mystery for Forensic Chemistry says it all about its adulteration, thus creating toxic emergencies, from time to time, resulting in untimely deaths of innocent population.

**Table 1.** Toxic Riddle in Rhymes

| **Toxic Proverb in Rhymes: Tit for Tat.**[12] |
|---|
| Do U know an intoxicating Case Scenario to Chat! |
| A strategy for iterated Prisoner's Dilemma in splat. |
| Toxic Riddle in Rhymes based on homonyms Scat. |
| Where there's Equivalent Retaliation, by Tit for Tat! |
| When Milk is Adulterated After Milking Teat to Get? |
| Extra amount of white coloured content alike that. |
| Which kills its consumer whether it's a Cat or Rat. |
| As it acts as an oxidizing agent when it's diluted at! |
| 1-2% of its strength derived from industrial combat, |
| By nature's revenge, Adulterator's child drank that, |
| Brought to Emergency, in Comatose State, full flat, |
| Thus, Milkman who misused, the harmful HAZMAT! |
| To dilute, pure milk, and increase its quantity STAT. |
| Didn't thought that it is dangerously toxic to hepat, |
| But when his own Child admitted in ICU, where at, |
| & he realized criminal act harming human habitat, |
| Child suffered corrosive ulcers needing hemostat, |
| Underwent urgent laparotomy to seal leaked spat, |
| Lets' Decode this toxic Riddle & win a cocked Hat. |
| Where chemical is adulterated in milk to look fat |

## CONCLUSION

In conclusion, the adulteration of milk with hydrogen peroxide is a grievous offense under the Bharatiya Nyaya Sanhita 2023, Section 274. This practice not only deceives consumers but poses significant health risks, especially to children. The law's stringent penalties for such actions are essential in safeguarding public health and maintaining the integrity of the food supply. Ensuring the safety of milk and other consumables is a critical responsibility, and the legal framework provided by Section 274 is a vital tool in achieving this goal.

***Conflict of Interest: Nil***

## REFERENCES


1. Ivanova AS, Merkuleva AD, Andreev SV, Sakharov KA. Method for determination of hydrogen peroxide in adulterated milk using high performance liquid chromatography. Food Chem. 2019 Jun 15;283:431-436. doi: 10.1016/j.foodchem.2019.01.051. Epub 2019 Jan 17. PMID: 30722894.
2. BNS (Bharatiya Nyaya Sanhita) 2023, Section 274-Adulteration of food or drink intended for sale.
3. Gerald F. O'Malley. Caustic Ingestion-Injuries; Poisoning-MSD Manual Professional Edition (msdmanuals.com)Last accessed on 18th June 2024. Link:https://www.msdmanuals.com/en-in/professional/injuries-poisoning/poisoning/caustic-ingestion
4. Yadav AK., *et al*. Key Milk Adulterants in India and their Detection Techniques: a Review.Food Analytical Methods. Springer 2022. Link https://doi.org/10.1007/s12161-022-02427-8
5. Henry MC, *et al*. Hydrogen peroxide 3% exposures. J Toxicol Clin Toxicol1996;34:323
6. Gerald F. O'Malley. Caustic Ingestion-Injuries; Poisoning - MSD Manual Professional Edition (msdmanuals.com) Last accessed on 18th June 2024. Link: https://www.msdmanuals.com/en-in/professional/injuries-poisoning/poisoning/caustic-ingestion
7. Mullins ME, Beltran JT. Acute cerebral gas embolism from hydrogen peroxide ingestion successfully treated with hyperbaric oxygen. J Toxicol Clin Toxicol1998;36:253
8. BNS(Bharatiya Nyaya Sanhita) 2023, Section 274-Adulteration of food or drink intended for sale.
9. IPC (Indian Penal Code) 1860, Section 272-Adulteration of food or drink intended for sale. Read more at: https://devgan.in/ipc/section/272/
10. Tomar, Shruti. Hindustan Times. 2 brothers turned millionaires in 7 yrs, sold synthetic milk in Delhi, UP | Latest News India-Hindustan TimesLast accessed on 18th June 2024. https://www.hindustantimes.com/india-news/how-synthetic-milk-powered-the-meteoric-rise-of-chambal-traders/story-baBu9ldQfrz2H6SFCHyzCK.html
11. Cry Over Spilled Milk - Cambridge English Dictionary. Last accessed on 18th June 2024. Link: https://dictionary.cambridge.org/dictionary/english/cry-over-spilled-milk.
12. Poisoned Tit for Tat: Toxic Homonyms eBook : Verma, Vivekanshu: Amazon.in: Kindle Store.5 April 2024, Kindle Book Publications (KDP) Link to the Riddle Book: https://www.amazon.in/dp/B0D128NLZW

# Instructions to Authors

Submission to the journal must comply with the Guidelines for Authors.
Non-compliant submission will be returned to the author for correction.

To access the online submission system and for the most up-to-date version of the Guide for Authors please visit:

http://www.rfppl.co.in

Technical problems or general questions on publishing with **JFCT** are supported by Red Flower Publication Pvt. Ltd.'s Author Support team (http://rfppl.co.in/article_submission_system.php?mid=5#)

Alternatively, please contact the Journal's Editorial Office for further assistance.

**Editorial Manager**
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi - 110 091(India)
Mobile: 9821671871, Phone: 91-11-79695648
E-mail: info@rfppl.co.in

# Guidelines for Authors

Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by international committee of medical Journal Editors

**Types of Manuscripts and Limits**

Original articles: Up to 3000 words excluding references and abstract and up to 10 references.

Review articles: Up to 2500 words excluding references and abstract and up to 10 references.

Case reports: Up to 1000 words excluding references and abstract and up to 10 references.

**Online Submission of the Manuscripts**

Articles can also be submitted online from http://rfppl.co.in/customer_index.php.

I) First Page File: Prepare the title page, covering letter, acknowledgement, etc. using a word processor program. All information which can reveal your identity should be here. use text/rtf/doc/PDF files. Do not zip the files.

2) Article file: The main text of the article, beginning from Abstract till References (including tables) should be in this file. Do not include any information (such as acknowledgement, your name in page headers, etc.) in this file. Use text/rtf/doc/PDF files. Do not zip the files. Limit the file size to 400 Kb. Do not incorporate images in the file. If file size is large, graphs can be submitted as images separately without incorporating them in the article file to reduce the size of the file.

3) Images: Submit good quality color images. Each image should be less than 100 Kb in size. Size of the image can be reduced by decreasing the actual height and width of the images (keep up to 400 pixels or 3 inches). All image formats (jpeg, tiff, gif, bmp, png, eps etc.) are acceptable; jpeg is most suitable.

Legends: Legends for the fig.s/images should be included at the end of the article file.

If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks from submission. Hard copies of the images (3 sets), for articles submitted online, should be sent to the journal office at the time of submission of a revised manuscript. Editorial office: Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091, India, Phone: 91-11-22754205, 45796900, 22756995. E-mail: author@rfppl.co.in. Submission page: http://rfppl.co.in/article_submission_system.php?mid=5.

**Preparation of the Manuscript**

The text of observational and experimental articles should be divided into sections with the headings: Introduction, Methods, Results, Discussion, References, Tables, fig.s, fig. legends, and Acknowledgment. Do not make subheadings in these sections.

**Title Page**

The title page should carry

1) Type of manuscript (e.g. Original article, Review article, Case Report)

2) The title of the article, should be concise and informative;

3) Running title or short title not more than 50 characters;

4) The name by which each contributor is known (Last name, First name and initials of middle name), with his or her highest academic degree(s) and institutional affiliation;

5) The name of the department(s) and institution(s) to which the work should be attributed;

6) The name, address, phone numbers, facsimile numbers and e-mail address of the contributor responsible for correspondence about the manuscript; should be mentoined.

7) The total number of pages, total number of photographs and word counts separately for abstract and for the text (excluding the references and abstract);

8) Source(s) of support in the form of grants, equipment, drugs, or all of these;

9) Acknowledgement, if any; and

l0) If the manuscript was presented as part at a meeting, the organization, place, and exact date on which it was read.

**Abstract Page**

The second page should carry the full title of the manuscript and an abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Materials, Statistical analysis used, Results and Conclusions. Below the abstract should provide 3 to 10 keywords.

**Introduction**

State the background of the study and purpose of the study and summarize the rationale for the study or observation.

**Methods**

The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http:// www. consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma. net/e/policy/17-c_e.html).

**Results**

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

**Discussion**

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

**References**

List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_ requirements.html) for more examples.

**Standard journal article**

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

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

**Article in supplement or special issue**

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

**Corporate (collective) author**

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

**Unpublished article**

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

**Personal author(s)**

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

**Chapter in book**

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

**No author given**

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

**Reference from electronic media**

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

More information about other reference types is available at www.nlm.nih.gov/bsd/uniform_requirements.html, but observes some minor deviations (no full stop after journal title, no issue or date after volume, etc).

**Tables**

Tables should be self-explanatory and should not duplicate textual material.

Tables with more than 10 columns and 25 rows are not acceptable.

Table numbers should be in Arabic numerals, consecutively in the order of their first citation in the text and supply a brief title for each.

Explain in footnotes all non-standard abbreviations that are used in each table.

For footnotes use the following symbols, in this sequence: *, ¶, †, ‡‡,

**Illustrations (fig.s)**

Graphics files are welcome if supplied as Tiff, EPS, or PowerPoint files of minimum 1200x1600 pixel size. The minimum line weight for line art is 0.5 point for optimal printing.

When possible, please place symbol legends below the fig. instead of to the side.

Original color fig.s can be printed in color at the editor's and publisher's discretion provided the author agrees to pay.

Type or print out legends (maximum 40 words, excluding the credit line) for illustrations using double spacing, with Arabic numerals corresponding to the illustrations.

**Sending a revised manuscript**

While submitting a revised manuscript, contributors are requested to include, along with single copy of the final revised manuscript, a photocopy of the revised manuscript with the changes underlined in red and copy of the comments with the point to point clarification to each comment. The manuscript number should be written on each of these documents. If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks of submission. Hard copies of images should be sent to the office of the journal. There is no need to send printed manuscript for articles submitted online.

**Reprints**

Journal provides no free printed reprints, however a author copy is sent to the main author and additional copies are available on payment (ask to the journal office).

**Copyrights**

The whole of the literary matter in the journal is copyright and cannot be reproduced without the written permission.

**Declaration**

A declaration should be submitted stating that the manuscript represents valid work and that neither this manuscript nor one with substantially similar content under the present authorship has been published or is being considered for publication elsewhere and the authorship of this article will not be contested by any one whose name (s) is/are not listed here, and that the order of authorship as placed in the manuscript is final and accepted by the co-authors. Declarations should be signed by all the authors in the order in which they are mentioned in the original manuscript. Matters appearing in the Journal are covered by copyright but no objection will be made to their reproduction provided permission is obtained from the Editor prior to publication and due acknowledgment of the source is made.

**Approval of Ethics Committee**

We need the Ethics committee approval letter from an Institutional ethical committee (IEC) or an institutional review board (IRB) to publish your Research article or author should submit a statement that the study does not require ethics approval along with evidence. The evidence could either be consent from patients is available and there are no ethics issues in the paper or a letter from an IRB stating that the study in question does not require ethics approval.

**Abbreviations**

Standard abbreviations should be used and be spelt out when first used in the text. Abbreviations should not be used in the title or abstract.

**Checklist**

- Manuscript Title
- Covering letter: Signed by all contributors
- Previous publication/ presentations mentioned, Source of funding mentioned
- Conflicts of interest disclosed

**Authors**

- Middle name initials provided.
- Author for correspondence, with e-mail address provided.
- Number of contributors restricted as per the instructions.
- Identity not revealed in paper except title page (e.g.name of the institute in Methods, citing previous study as 'our study')

**Presentation and Format**

- Double spacing
- Margins 2.5 cm from all four sides
- Title page contains all the desired information. Running title provided (not more than 50 characters)
- Abstract page contains the full title of the manuscript
- Abstract provided: Structured abstract provided for an original article.
- Key words provided (three or more)
- Introduction of 75-100 words
- Headings in title case (not ALL CAPITALS).
  References cited in square brackets
- References according to the journal's instructions

**Language and grammar**

- Uniformly American English
- Abbreviations spelt out in full for the first time. Numerals from 1 to l0 spelt out
- Numerals at the beginning of the sentence spelt out

**Tables and fig.s**

- No repetition of data in tables and graphs and in text.
- Actual numbers from which graphs drawn, provided.
- fig.s necessary and of good quality (color)
- Table and fig. numbers in Arabic letters (not Roman).
- Labels pasted on back of the photographs (no names written)
- fig. legends provided (not more than 40 words)
- Patients' privacy maintained, (if not permission taken)
- Credit note for borrowed fig.s/tables provided
- Manuscript provided on a CDROM (with double spacing)

**Submitting the Manuscript**

- Is the journal editor's contact information current?
- Is the cover letter included with the manuscript? Does the letter:

1. Include the author's postal address, e-mail address, telephone number, and fax number for future correspondence?
2. State that the manuscript is original, not previously published, and not under concurrent consideration elsewhere?
3. Inform the journal editor of the existence of any similar published manuscripts written by the author?
4. Mention any supplemental material you are submitting for the online version of your article. Contributors' Form (to be modified as applicable and one signed copy attached with the manuscript)