Original Article

DNA Extraction from Postmortem Blood: A Pilot Study for Advancing Molecular Diagnostics in Forensic Medicine Casework

Sadhu Rama Mohana Rao[1], Sravani Yandava[2], T. Mohit Kumar Moses[3], Kattamreddy Ananth Rupesh[4], K. Satyasree[5], K. Mamatha[6], Anuradha Argi[7]

[1]Associate Professor, [2,3,4]Assistant Professor of Forensic Medicine and Toxicology, Andhra Medical College, Visakhapatnam, India, [5]Professor of Pathology, Andhra Medical College, Visakhapatnam, India, [6]Professor of Forensic Medicine and Toxicology, Andhra Medical College, Visakhapatnam, India, [7]Scientist C, Multi-Disciplinary Research Unit, Andhra Medical College, Visakhapatnam, India.

How to cite this article: Sadhu Rama Mohana Rao, Sravani Yandava, T. Mohit Kumar Moses et. al. DNA Extraction from Postmortem Blood: A Pilot Study for Advancing Molecular Diagnostics in Forensic Medicine Casework. Indian Journal of Forensic Medicine and Toxicology/Volume 18 No. 1, January-March 2024.

Abstract

Background: DNA's role in forensic practice is widely acknowledged for its unparalleled accuracy in identification. While developed countries have established molecular autopsy programs as early as two decades ago, India is yet to initiate such a program. The isolation of DNA serves as the crucial first step in the molecular autopsy protocol. The postmortem blood sample is one of the good sources for DNA extraction which wasn't considered with rigor by the scientific community so far.

Aims and Objectives: The aim of this study was to investigate the specific time period within which DNA can be effectively extracted from postmortem blood samples. The objective was to identify if there are patterns in the quality and purity of the extracted DNA based on the postmortem interval. Additionally, the study aimed to investigate if the cause of death influenced DNA extractability.

Observation and Results: DNA can be extracted from postmortem blood within a timeframe of up to 72 hours after death, given that the deceased body was preserved in cold storage within 12 hours after death. Both the salting out method and the phenol chloroform method yielded bands of comparable quality, with the phenol chloroform method showing a slightly higher DNA yield. The average absorbance ratio was 1.4 for the salting out method and 1.6 for the phenol chloroform method, as determined using a Nanodrop.

Conclusion: This study concluded that DNA extraction from postmortem blood samples is feasible within 72 hours after death. The integrity of the DNA remained intact during this time, but the quality and purity gradually decreased as the postmortem interval increased. The cause of death did not significantly affect DNA extractability.

Keywords: DNA, Molecular Autopsy, Postmortem Blood, Sudden Death, Genomics.

Introduction

The role of DNA in the field of forensics is indispensable and it has attained the status of being referred to as the 'crystal ball of forensic science'. Traditionally, DNA data has been extensively used for identification in forensic pathology practice as

Corresponding Author: Kattamreddy Ananth Rupesh, Assistant Professor of Forensic Medicine and Toxicology, Andhra Medical College, Visakhapatnam, India.

E-mail: ananth.kattam@gmail.com

Mobile: +91 82977 16897

Submission Date: Jul 18, 2023 **Revision Date:** Jul 27, 2023 **Publication date:** Jan 19, 2024

well. However, with ever-increasing published data in molecular forensic medicine, postmortem DNA extraction will attain the status of a routine investigation like the vitreous humor chemistry, at least in all cases of sudden deaths in young individuals.

In Indian settings, the use of blood as a specimen for postmortem DNA caseworkis less due to the challenges associated with maintaining a cold chain and ensuring sample quality. As the extraction of DNA is usually carried out by forensic science laboratories, forensic pathologists regularly preserve the femur, sternum, or other bones as they withstand decomposition well and can be employed for the extraction of DNA successfully. The role of using blood for DNA extraction in Indian autopsy practice is often neglected and warrants exploration owing to its highly cost-effective nature.

The objective of this study is to investigate the timeframe within which DNA can be successfully extracted from postmortem blood samples for subsequent analysis. The study aimed to determine if there is a discernible pattern in the quality and purity of extracted DNA based on the time elapsed since death. Additionally, an attempt was made to identify any variations in DNA extractability based on the cause of death.

Materials and Methods

The present study was conducted at the Department of Forensic Medicine, Andhra Medical College, utilizing a convenient sample of 32 autopsy cases with known time since death. The study period spanned from May to July 2023. DNA isolation was performed at the Multi-Disciplinary Research Unit (MDRU) using two manual techniques: the salting out method and the phenol-chloroform method. Equipment employed in the study included a table top centrifuge, cooling centrifuge, electrophoresis unit, and Nanodrop. The consumables used were 5ml, 10 ml disposable syringes, EDTA vacutainers, 1.5 ml tubes, and pipette tips. Postmortem blood was collected directly from the heart of the deceased during the dissection and preserved at 4 degrees Celsius in EDTA vacutainers until they are transported to the laboratory. The process of extraction was carried out within a week after the collection of the specimen. The time required for each extraction method ranged from 1 to 1.30 hours. Ethical considerations were addressed through the inclusion of consent from the next of kin, ensuring adherence to ethical guidelines.

Results

The age and sex data were not collected to maintain utmost privacy in view of the fact that DNA is sensitive data, and they don't have any relevance to our study. The profile of the cause of death of cases in our study amongst the 32 cases is depicted in Figure 1.

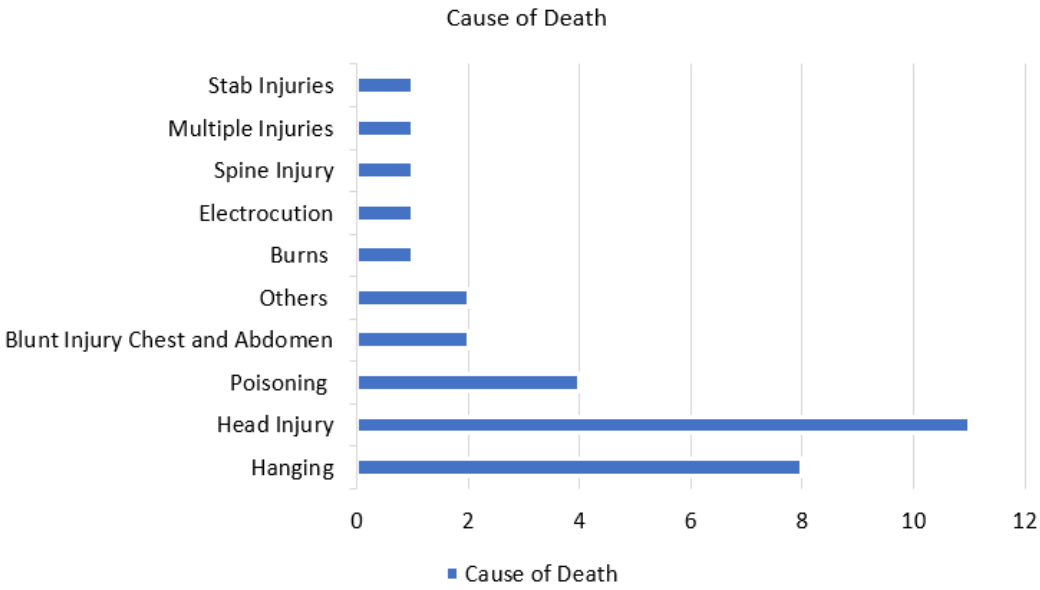

Figure 1: Medicolegal Profile of Cases in our Study

The two cases in the others section include an anaphylactic death due to a drug and crush injury of the pelvis.

In all cases, the time elapsed between the occurrence of death and the deposition of the deceased body in the freezer box was less than 12 hours. The timeframe varied, with some bodies being deposited as early as 1 hour after death, while others were placed in the freezer box within a maximum of 12 hours after the time of death.

DNA isolation was carried out using the salting out method and the phenol chloroform method to maintain cost efficiency. Throughout the process, challenges arose as most samples emitted unpleasant odors and exhibited clot formation, distinguishing them from the typical samples handled in the laboratory in clinical casework from live individuals.

The isolated DNA underwent assessment for both quality and quantity. The quality evaluation involved 0.8% Agarose gel electrophoresis to determine the intensity of DNA bands. Nucleic acid concentration, indicating DNA purity, was estimated using Nanodrop by calculating the absorbance ratio at 260 nm and 280 nm. A ratio of 1.8 is commonly accepted as indicative of "pure" DNA. Both the salting out method and the phenol chloroform method yielded bands of comparable quality. The phenol chloroform method showed a slightly higher DNA yield compared to the salting out method. The average absorbance ratio for the salting out method was 1.4, whereas, for the phenol chloroform method, it was 1.6.

The data pertaining to the relationship between time since death and extractability of DNA from postmortem blood using the salting out method and phenol-chloroformiso amyl alcohol method is tabulated in Table 1.

We encountered difficulties in obtaining blood samples without clots in cases where the time elapsed since death exceeded 72 hours, despite some of them being preserved in cold storage shortly after death. As a result, our attempts to extract DNA from these samples were unsuccessful. The quality evaluation of DNA using 0.8% agarose gel electrophoresis is shown in Figure 2.

Table 1: DNA Extractability (Different Methods) From Postmortem Blood and Time Since Death.

S.No.	Time Since Death Range	Number of Cases	Quality of DNA(Nanodrop) The ratio of absorbance at 260 nm and 280 nm	
			Salting out method	Phenol chloroform isoamyl alcohol method
1	0-12 hours	5	1.5 -1.6	1.6-1.7
2	12-24 hours	14	1.4 -1.5	1.6-1.7
3	24-48 hours	7	1.4-1.5	1.5-1.6
4	48-72 hours	6	1.3-1.4	1.5-1.6

The average values of the purity of the DNA and the corresponding quantity of the DNA are mentioned in Table 2.

Table 2: Average Purity and Quantity of DNA Extracted Using Different Methods

Extraction Method	Nanodrop (Absorbance at 260nm/280nm)	Quantity of DNA in ng/µl
Salting out method	1.4	104.276
Phenol chloroform isoamyl alcohol method	1.7	105.235

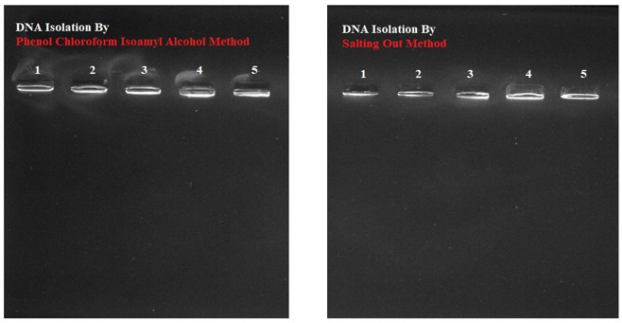

Figure 2: Quality Evaluation of DNA using 0.8% Agarose Gel Electrophoresis

Discussion

DNA extraction from nucleated cells can be carried out by various methods. The salting out method and phenol chloroform methods are time-tested and very economical in a low-cost setting wet lab. The commercial kit-based extraction is common in most laboratories these days.[1,2]

Molecular autopsy has become an integral part of modern forensic pathology, expanding the applications of DNA beyond its traditional role in identification. Over the past two decades, several developed countries have embraced the use of molecular autopsy programs, specifically through the development of genetic test panels aimed at investigating cases of sudden unexplained deaths in young individuals, believed to be of cardiac origin. In light of these developments, it is becoming essential to extract DNA from autopsy cases for ancillary investigations other than just for DNA profiling used in the identification business.[3] It is also worth a mention that researchers established a link between DNA degradation and Post Mortem Interval (PMI). The extracted DNA can be utilized for the purpose of studying the same in forensic casework.[4] The extracted DNA has to be stored at -20 degrees Celsius for further investigations.

Blood is widely regarded as an optimal source for DNA extraction, particularly when compared to challenging samples such as bone. In terms of ease of extraction, blood on FTA paper is highly recommended, while blood on gauze in a dried format also serves as a viable alternative. These blood-based samples offer the advantage of being relatively straightforward to collect and extract, making them cost-effective options in forensic investigations. However, to ensure the preservation of high-quality DNA for forensic casework, it is essential to establish the precise duration during which DNA can be reliably extracted from postmortem blood. This knowledge is crucial for developing robust protocols and maximizing the utility of blood samples in forensic applications.

The costs involved in the process of extracting the DNA and setting up a Molecular Forensic Medicine Unit (MFMU) in Forensic Medicine departments for that purpose are not as exorbitant as supposed by several people. The first step in molecular autopsy protocol is to extract high-quality and pure DNA which can be done by the forensic pathologist and his team with the bare minimum resources as demonstrated by us. Once the DNA is extracted, it can be subjected to a number of investigations like gene expression/polymorphism studies/mutation analysis using advanced genetic sequencing methods by collaborating with the molecular laboratory chain of ICMR.

This importance of genetic testing cannot be undermined in postmortem investigations, particularly in cases where there is uncertainty regarding the cause of death. Genetic testing can serve various purposes in postmortem work, including toxicogenomics studies to identify genetic factors associated with drug toxicity. Additionally, it plays a crucial role in investigating sudden cardiac death attributed to cardiomyopathies and other indications requiring molecular autopsy.[5,6]

In the context of India, the next critical step is to compile a comprehensive list of genes that should be included in molecular testing, considering the incidence and prevalence of arrhythmogenic cardiac diseases and other cardiomyopathies prevalent within the country. This preparatory phase is essential before launching a nationwide molecular autopsy program. It is of utmost importance to ensure that genetic testing is not conducted in isolation, but rather is accompanied by screening and counseling services for the next of kin.

The DNA isolation from samples collected within 72 hours of the time since death proved to

be effective. Beyond this timeframe, factors such as clotting of blood and contamination make the extraction of DNA more challenging. Furthermore, the DNA obtained through manual methods may not meet the quality requirements for Next Generation Sequencing work. It is important to emphasize that this study serves as a preliminary demonstration of the feasibility of isolating DNA from deceased individuals using minimal tools and resources.

We did not find any similar studies for comparison. There was no relation between the cause of death and the extractability of DNA from postmortem blood. Further studies are needed on higher samples to study this aspect of DNA extraction. Moreover, the preservation of the corpse in cold storage within 12 hours of death is one of the factors responsible for the successful extraction of DNA up to 72 hours after death in our study.

In a similar study, the integrity and extraction yield of DNA were higher in samples collected by emergency staff immediately after failed resuscitation whereas the DNA stability in autopsy specimens was highly variable and had unpredictable quality.[7]In contrast, our study established that postmortem blood can be a good source for DNA extraction up to 72 hours after death.

In another study, researchers reported that It was not possible to collect blood from 38 % of the autopsy cases (Post Mortem Interval ranging from 1 to 14 days) due to severe coagulation and hemolysis, whereas muscle tissue was available for all cases.[8]The consistency of DNA yields from blood samples varied due to sample inhomogeneity, although blood clots were found to be rich in DNA.[9]

The ability to extract DNA from postmortem blood samples is influenced by various factors that affect the decomposition process. It is important to consider these factors when applying the findings of our study in practical settings. Additionally, further studies with larger sample sizes should be conducted to explore seasonal variations and the impact of temperatures below 4 degrees Celsius on preserving corpses, with the aim of extending the timeframe for successful DNA extraction.

Conclusion

In conclusion, this study demonstrated that DNA can be successfully extracted from postmortem blood samples within a timeframe of up to 72 hours after death. The integrity of the DNA remained preserved during this period, although a gradual decline in the quality and purity of the extracted DNA was observed with increasing time since death. No significant variations in DNA extractability were found based on the cause of death. The departments of Forensic Medicine and Toxicology should consider the establishment of Molecular Forensic Medicine Units (MFMU) for advancing the cause of molecular autopsy in our routine casework in the interest of justice.

Ethical considerations: Addressed by the authors.

Conflicts of interest: None to declare

Financial Support: Nil

Acknowledgments: We sincerely thank the Department of Forensic Medicine and Multi-Disciplinary Research Unit (MDRU) faculty and staff for their constant support in materializing this project.

References

1. Gautam A. Phenol-Chloroform DNA Isolation Method. DNA and RNA Isolation Techniques for Non-Experts, Cham: Springer International Publishing; 2022, p. 33–9.
2. Miller SA, Dykes DD, Polesky HF. A simple salting-out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215–1215. https://doi.org/10.1093/nar/16.3.1215.
3. Martínez-Barrios E, Grassi S, Brión M, Toro R, Cesar S, Cruzalegui J, et al. Molecular autopsy: Twenty years of post-mortem diagnosis in sudden cardiac death. Front Med (Lausanne) 2023;10. https://doi.org/10.3389/fmed.2023.1118585.
4. Tozzo P, Scrivano S, Sanavio M, Caenazzo L. The Role of DNA Degradation in the Estimation of Post-Mortem Interval: A Systematic Review of

the Current Literature. International Journal of Molecular Sciences 2020;21:3540. https://doi.org/10.3390/ijms21103540.

5. Torkamani A, Muse ED, Spencer EG, Rueda M, Wagner GN, Lucas JR, et al. Molecular autopsy for sudden unexpected death. JAMA 2016;316:1492. https://doi.org/10.1001/jama.2016.11445.
6. Cunningham KS. The promise of molecular autopsy in forensic pathology practice. Acad Forensic Pathol 2017;7:551–66. https://doi.org/10.23907/2017.047.
7. Stanasiuk C, Milting H, Homm S, Persson J, Holtz L, Wittmer A, et al. Blood taken immediately after fatal resuscitation attempts yields higher-quality DNA for genetic studies as compared to autopsy samples. Int J Legal Med [Internet]. 2023; Available from: http://dx.doi.org/10.1007/s00414-023-02966-7
8. Hansen J, Lesnikova I, Funder AMD, Banner J. DNA and RNA analysis of blood and muscle from bodies with variable postmortem intervals. Forensic Sci Med Pathol [Internet]. 2014;10(3):322–8. Available from: http://dx.doi.org/10.1007/s12024-014-9567-2
9. Bär W, Kratzer A, Mächler M, Schmid W. Postmortem stability of DNA. Forensic Sci Int [Internet]. 1988;39(1):59–70. Available from: http://dx.doi.org/10.1016/0379-0738(88)90118-1.