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Suitability of post mortem cardiac blood for forensic genetic testing

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Abstract: The study shows the expediency of post mortem (PM) cardiac blood samples in forensic DNA profiling. Sufficient quantities of DNA were extracted from the PM cardiac blood samples taken from the dead bodies at various time intervals. PM blood samples were easy to obtain and with lesser incubation periods, were analyzed in a short span of time. Samples were taken from the accidental cases and burn cases, which included electric and thermal burns. Total of 35 cases were studied which included accidental cases at various time intervals and burn cases, in all the cases the samples showed positive amplification, which further lead to the successful genotyping. Complete and balanced DNA profiles were generated with the commercially available kits, AmpFlSTR Identifiler Plus, PowerPlex 16HS, PowerPlex Y 23 and Investigator Argus X-12 multiplex kit, which are being commonly used in forensic DNA typing.

Key Words: Forensic, STR, Post mortem Blood, Dead body DNA profiling, dead body identification.

INTRODUCTION

Identification of dead body is a prime concern for medico-legal purposes. After establishing the crime, the dead body has to be identified. Various techniques have been tried, tested and employed for the purpose of identification. Almost after three decades of its inception, at present DNA fingerprinting is the most trusted and established technology for the purpose of identification. Advent of polymerase chain reaction (PCR) technique has turned out to be a revolution in the process of identification. It can amplify the forensic samples in traces and render them identifiability. Successful attempts have been made to generate DNA profile from the biological samples like nail [1-2], tissue [3], teeth [4-6] and bones [4, 7-8]. Saliva and blood samples are the most common samples that are used for the data basing and reference purposes because of their ease of collection. Reports are there for successful PCR based identification of burnt dead bodies and even of the charred bodies [9-10]. Teeth and bone fragments taken from the burnt dead body for the purpose of identification have given positive results [11]. It has also been reported that DNA extraction and subsequent STR typing from the ashes of a cremated person is possible in some cases, though not reliable [12].

The post mortem (PM) samples have reportedly been analyzed to find out the cause of sudden unexplained deaths and for the diagnosis of hereditary heart disease [13-15]. PCR based PM analysis of virus and bacterial pathogens in human tissue and body fluids have also been reported to reveal infectious causes of death [16]. For genetic analysis of PM samples, teeth and bones can be a good source of DNA from months and years old bodies, albeit they are difficult to process and are time consuming too [17]. Bone if not turned in to ashes, can be a good source of DNA and teeth are reported to generate DNA profiles even after being kept at very high temperature [18]. As it is said that Justice delayed is justice denied, keeping this in mind, Swiftness is need of the hour in forensic DNA testing laboratories. As the blood sample is comparatively easier to process and doesn't require long incubation/lysis periods, the present study has been dedicated towards using the PM blood samples from the heart for human identification (HID) purpose. PM blood samples for toxicological analysis are usually collected from

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Table 1. Group wise distribution of samples used in the analysis

Groups	Type of case	Postmortem Interval	Number of samples
Group 1	Accident	Up to 24 Hours	10
Group 2	Accident	24-48 hours	10
Group 3	Accident	48-90 hours	05
Group 4	Burn cases include thermal (up to 90%) burns and electric burns as well	Up to 24 hours	10
	Total number of samples		**35**

the femoral vein [19]. Peripheral blood has been shown to be more reliable for toxicological analysis than the conventional heart blood [20]. It is reported that blood collection from the heart should be avoided for toxicological analysis, as substances in the stomach and intestine can diffuse to the organs in the thorax, causing false raise in the blood level after death [20-21]. But no such issues have been reported for DNA profiling from PM cardiac blood. The study shows the suitability of PM blood from heart for DNA extraction, quantification, amplification and genotyping at various time intervals. The study is approved by the ethical committee of the PGIMER, Chandigarh, India vide letter number: INT/IEC/2015/611, dated: 13-10-2015.

MATERIALS AND METHODS

Sampling

At medico legal post mortem, after obtaining the written informed consent from the legal heir of the deceased, 2 mL blood was collected by the medical practitioner from the right ventricle in an EDTA vial. The blood samples were stored at 4°C till analysis. Samples were analyzed within 24 hours of collection.

Samples were taken only from the patients who succumbed to the injuries during treatment in PGIMER, Chandigarh, India. Dead bodies were stored at 4°C in the mortuary of the institute. The difference between the time of death and keeping the dead body in the mortuary at 4°C was different every case, as the time taken to follow the institutional protocol to send the body to the mortuary varied in every case, though the time difference was not too large.

A total of 35 samples were collected from dead bodies with post mortem interval (PMI) ranging from 15 hours to 90 hours. The samples were divided into 4 groups according to their PMI (Table 1). The PMI is calculated as time interval between the death and autopsy.

DNA extraction and quantitation

The DNA was extracted using silica column based, QIAamp Blood mini kit (Qaigen) as per manufacturer's recommended protocol and was stored at -20°C till further processing. The quality of the extracted DNA was checked by Agarose gel electrophoresis and was further confirmed and quantified by Applied Biosystems / Thermo 7500 Real Time PCR by using Quantifiler Human DNA Quantification Kit (Applied Biosystems /Thermo), following the manufacturer's recommended protocol to check the quantity of amplifiable DNA. Each sample was processed in triplicates.

Amplification

The extracted DNA samples were amplified with four different types of multiplex systems that are available commercially. First multiplex system used was AmpFlSTR Identifiler plus kit (ABI/LT/Thermo), for autosomal STRs which simultaneously amplifies 15 autosomal STR loci (D3S1358, TH01, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, CSF1PO, vWA, D8S1179, TPOX, FGA, D2S1338 and D19S433) and amelogenin marker. Second multiplex system used was Investigator Argus 12 X STR kit (Qaigen) which amplifies 12 X STR loci (DXS10103, DXS8378, DXS7132, DXS10134, DXS10074, DXS10101, DXS10135, DXS7423, DXS10146, DXS10079, HPRTB and DXS10148) and amelogenin marker. The extracted DNA samples were also amplified for 23 Y STRs (DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385a/b, DYS456 and Y-GATA-H4) by using the third multiplex system i.e. PowerPlex Y 23 STR kit (Promega). Fourth multiplex system was again for autosomal STRs, PowerPlex 16HS kit (Promega), with a set of different markers (D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, CSF1PO, D16S539, D7S820, D13S317, D5S818, Penta D, Penta E and amelogenin marker). All the amplifications were done as per the respective manufacturer's recommended protocol except half reaction volume was used. A total 8 μL of final reaction mix was used for PCR with 0.5 ng of DNA template.

All the amplifications were performed on ABI thermal cycler HID Veriti 96-Well (Applied Biosystems / Thermo). These kits are being routinely used in forensic DNA analysis. The amplified products were checked on 3% Agarose gel for multiplex amplification (Fig. 2) and further a few samples were run on genetic analyzer for genotyping.

Genotyping

In all the cases, 0.3 μL of the PCR product was separated on an Applied Biosystems 3500/3100 Genetic Analyzer (Applied Biosystems /Thermo), using standard parameters and with their respective size standards/allelic ladder with POP-4 polymer using internal size standards supplied with respective multiplex kits. All steps were performed in concordance with the laboratory's internal

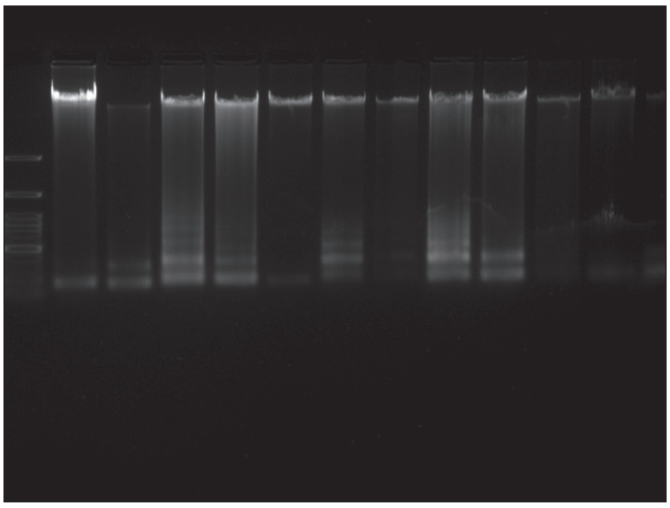

Figure 1. Quality assessment of samples by Gel electrophoresis.

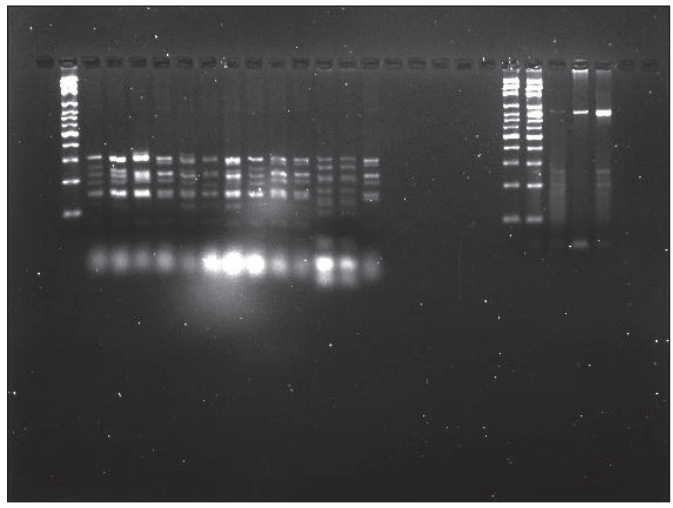

Figure 2. Gel image of amplified product showing multiple bands and thus confirming the multiplex amplification of autosomal and X-STR loci.

Table 2. Observed DNA yield from different samples and their amplification suitability with the commercially available multiplex systems for forensic purpose

Group	Sample	DNA Quanity (ng/μL) Replicate I	II	III	Mean	SD	Total loci in the various multiplex kits/Amplified loci IDPlus	X12 Kit	PPY23	PP16 HS
							16/16	13/13	23/23	16/16
	S1	157.6	156	157	156.8	0.79	16/16	13/13	23/23	16/16
	S2	121.9	120.3	121.4	121.1	0.81	16/16	13/13	23/23	16/16
	S3	147.8	145.7	146.9	146.8	1.05	16/16	13/13	23/23	16/16
	S4	42.8	43.5	42.5	42.9	0.51	16/16	13/13	23/23	16/16
Group 1	S5	132.5	132.3	132.6	132.4	0.16	16/16	13/13	23/23	16/16
	S6	64.52	64.2	64.5	64.4	0.18	16/16	13/13	23/23	16/16
	S7	35.07	35.5	35.6	35.3	0.28	16/16	13/13	23/23	16/16
	S8	30.07	30.6	30.8	30.49	0.38	16/16	13/13	23/23	16/16
	S9	82.87	81.8	82.5	82.3	0.54	16/16	13/13	23/23	16/16
	S10	74.7	74.5	75.5	74.9	0.53	16/16	13/13	23/23	16/16
	S11	101.2	101.5	100.5	101	0.51	16/16	13/13	23/23	16/16
	S12	346.6	345.5	346.3	346.1	0.57	16/16	13/13	23/23	16/16
	S13	167.8	167.5	166.3	167.2	0.79	16/16	13/13	23/23	16/16
	S14	63.42	62.43	61.5	62.45	0.96	16/16	13/13	23/23	16/16
Group 2	S15	78.32	77.6	76.4	77.44	0.97	16/16	13/13	23/23	16/16
	S16	82.4	81.4	82.8	82.2	0.72	16/16	13/13	23/23	16/16
	S17	166.4	165.3	166.8	166.1	0.78	16/16	13/13	23/23	16/16
	S18	171.9	171.3	170.9	171.3	0.5	16/16	13/13	23/23	16/16
	S19	56.04	55.9	56.3	56.08	0.2	16/16	13/13	23/23	16/16
	S20	78.5	77.9	78.5	78.3	0.35	16/16	13/13	23/23	16/16
	S21	104.4	103.8	104.6	104.2	0.42	16/16	13/13	23/23	16/16
	S22	131.4	131.8	130.8	131.3	0.5	16/16	13/13	23/23	16/16
Group 3	S23	39.69	39.9	38.7	39.4	0.64	16/16	13/13	23/23	16/16
	S24	35.7	35.9	34.6	35.4	0.7	16/16	13/13	23/23	16/16
	S25	40.8	40	39.2	40	0.8	16/16	13/13	23/23	16/16
	S26	15.4	15.6	14.9	15.3	0.36	16/16	13/13	23/23	16/16
	S27	27.7	26.7	27.5	27.3	0.53	16/16	13/13	23/23	16/16
Group 4	S28	30.6	30.5	31.2	30.7	0.38	16/16	13/13	23/23	16/16
	S29	78.5	77.9	78.5	78.3	0.35	16/16	13/13	23/23	16/16
	S30	35.3	34.4	33.6	34.4	0.85	16/16	13/13	23/23	16/16
	S31	42.4	41.8	41.3	41.8	0.39	16/16	13/13	23/23	16/16
	S32	35.4	33.4	35.7	34.8	0.88	16/16	13/13	23/23	16/16
	S33	53.5	53.4	52.2	53.0	0.51	16/16	13/13	23/23	16/16
	S34	44.5	42.4	42.5	43.1	0.83	16/16	13/13	23/23	16/16
	S35	31.4	33.4	32.6	32.4	0.71	16/16	13/13	23/23	16/16

IDPlus-AmplFLSTR Identifiler Plus kit (Applied Biosystems /Thermo), X12- Investigator X 12 STR kit (Qiagen), PPY23-PowerPlex Y23 Kit (Promega), PP16HS- PowerPlex 16HS Kit (Promega),

Table 3. Average DNA yield observed in different groups

Group	PMI	Average DNA yield (ng/μL)	Range (ng/μL) Inter sample variation	Amplification and genotyping
Group 1	Up to 24 hours	88.739	30.49 to 156.8	10/10 samples
Group 2	24-48 hours	130.81	56.08 to 346.1	10/10 samples
Group 3	48-90 hours	126.2	34.5 to 131.3	5/5 samples
Group 4	Up to 24 hours	37.2	15.3 to 78.3	10/10 samples

control standards and kit controls. According to the DNA recommendations [22], alleles were designated on the basis of number of allele repeats with the help of allelic ladder provided by the manufacturers. Peak detection threshold was set to 50 RFUs for allele designation.

RESULTS

All the samples from various groups yielded good quality DNA as checked by Agarose gel electrophoresis (Fig. 1). Concentrations of extracted DNA obtained from various groups as quantified by Real-time PCR are presented in Table 2. A minimum 15.3 to maximum 346.1 ng/μL DNA was observed in all the 35 samples processed (Table 2). In group 1,2,3 to 4 the observed DNA yield ranged between 30.4 to 156.8, 56.0 to 346.1, 35.4 to 131.3, 15.3 to 48.3 ng/μL respectively (Table 3). The larger variation in the DNA yield is the result of varying temperatures that every dead body has been exposed to before reaching the mortuary after death but despite of this factor, every sample from all the groups yielded sufficient DNA to carry out further analyses required for Human Identification (HID), as the minimum requirement of DNA for genotyping for the commonly used genotyping multiplex systems is less than 1 ng. We suggest that QIAamp Blood mini kit (Qaigen) is good option as semi automated mode of DNA isolation of forensic samples and this can be a good alternative for the labs which either follow the standard phenol chloroform procedure or do not have the automated DNA extraction facility to fasten the process of isolation. Amplification of DNA with all the multiplex systems was confirmed on 3% Agarose gel. Results obtained are presented in Figure 2. DNA from all the samples showed multiplex amplification thus confirming that the quality and quantity of the DNA obtained from all the samples were good enough to generate complete and balanced DNA profiles.

DISCUSSIONS

All the commercially available multiplex kits for autosomal, Y and X STR analysis of Forensic DNA are very sensitive and require very little amount of DNA (from 250 to 500 pg/ μL) to generate complete DNA profiles. Jain *et al.* [23] recently reported to have generated full and balanced Y STR DNA profile by using Powerplex Y 23 with even 16 pg DNA. Though various attempts have been made even for direct amplification of samples using multiplex kits to reduce the processing time [24-26], but either specific kits are needed or the samples are to be pretreated before the analysis. Also blood has already been a sample of choice for many Indian DNA testing laboratories as the yield of DNA from blood is quite high with less processing time. So for the identification of dead bodies if the PM blood from the heart is available, it can be used for genetic testing. Even in the cases of thermal and electric burns the cardiac blood gives sufficient DNA to create full DNA profiles which further can be used to establish the identity of the individual. We further recommend that these PM blood samples can be processed by automated system available for forensic DNA analysis to fasten the process and also to process multiple samples at the same time to avoid chances of contamination by manual multiple handing of the sample.

CONCLUSION

The obtained results suggest that PM blood from the heart is a good alternative for DNA analysis required for HID purpose. The method is less time consuming, as the whole process of extraction takes just 3-4 hours and is efficient enough to generate full profiles from the post mortem blood samples till 90 hours in case of road accidents and up to 24 hours from burnt bodies. However the larger PMI windows are yet to be explored. This study concludes that blood samples from dead bodies can be sent to Forensic laboratories rather than bone or teeth samples which take 2-3 days of pre-processing and results in the pendency of cases.

Conflict of interest. The authors declare that there is no conflict of interest.

Acknowledgement. All three authors share the same credentials in committing this scientific work.

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Quality control

Passed proficiency testing of the GITAD, Spain http://gitad.ugr.es/principal.htm), quality control exercise of the YHRD, Germany (http://www.yhrd.org) and US Y-STR Database (http://www.usystrdatabase.org).

References

1. Preuner S, Danzer M, Pröll J, Pötschger U, Lawitschka A, Gabriel C, Lion T. High-quality DNA from fingernails for genetic analysis. J Mol Diagn. 2014;16(4):459-466.
2. Yoshida-Yamamoto S, Nishimura S, Okuno T, Rakuman M, Takii Y. Efficient DNA extraction from nail clippings using the protease solution from Cucumis melo. Mol Biotechnol. 2010;46(1):41–48.
3. Romero R, Juston A, Ballantyne J, Henry B. The Applicability of Formalin-Fixed and Formalin Fixed Paraffin Embedded Tissues in Forensic DNA Analysis. J Forensic Sci.1997;42(4):708-714.
4. Rohland N, Hofreiter M. Ancient DNA extraction from bones and teeth. Nat Protoc. 2007;2(7):1756–1762.
5. Tran-Hung L, Tran-Thi N, Aboudharam G, Raoult D, Drancourt M. A new method to extract dental pulp DNA: Application to universal detection of bacteria. PLoS One. 2007;2(10):e1062.
6. Higgins D, Austin JJ. Teeth as a source of DNA for forensic identification of human remains: A Review. Sci Justice. 2013;53(4):433–441.
7. Jakubowska J, Maciejewska A, Pawłowski R. Comparison of three methods of DNA extraction from human bones with different degrees of degradation. Int J Legal Med. 2012;126(1):173–178.
8. Pressure Biosciences Inc. DNA Extraction from Bone Fragments without Pulverization. Appl Note Forensics. :1–2. Retrieved from: http://www.pressurebiosciences.com/downloads/app_notes/AN-10003.v1.pdf
9. Sajantila A, Ström M, Budowle B, Karhunen PJ, Peltonen L. The polymerase chain reaction and post-mortem forensic identity testing: Application of amplified D1S80 and HLA-DQα loci to the identification of fire victims. Forensic Sci Int. 1991;51(1):23–34.
10. Soares-Vieira JA, Billerbeck AE, Iwamura ES, Cardoso L, Romero Muñoz D. Post-mortem forensic identity testing: application of PCR to the identification of fire victim. Sao Paulo Med J. 2000;118(3):75–77.
11. Schwark T, Heinrich A, Preuße-Prange A, Von Wurmb-Schwark N. Reliable genetic identification of burnt human remains. Forensic Sci Int Genet. 2011;5(5):393–399.
12. Von Wurmb-Schwark N, Simeoni E, Ringleb A, Oehmichen M. Genetic investigation of modern burned corpses. Int Congr Ser. 2004;1261:50–52.
13. Basso C, Carturan E, Pilichou K, Rizzo S, Corrado D, Thiene G. Sudden cardiac death with normal heart: molecular autopsy. Cardiovasc Pathol. 2010;19(6):321–325.
14. DiPaolo M, Luchini D, Bloise R, Priori SG. Postmortem molecular analysis in victims of sudden unexplained death. Am J Forensic Med Pathol. 2004;25(2):182–184.
15. Madea B, Saukko P, Oliva A, Musshoff F. Molecular pathology in forensic medicine--Introduction. Forensic Sci Int. 2010;203(1-3):3-14.
16. Gaaloul I, Riabi S, Harrath R, Evans M, Salem NH, Mlayeh S, Huber S, Aouni M. Sudden unexpected death related to Enterovirus myocarditis: histopathology, immunohistochemistry and molecular pathology diagnosis at post-mortem. BMC Infect Dis. 2012;12(1):212.
17. Kaneko Y, Ohira H, Tsuda Y, Yamada Y. Comparison of hard tissues that are useful for DNA analysis in forensic autopsy. Leg Med.2015;17(6):547–552.
18. Devaraju R, Gantala R, Ambati M, Vemula A, Kubbi J, Gotoor S. DNA detection in tooth exposed to different temperatures: An *in vitro* study. J Indian Acad Oral Med Radiol. 2014;26(4):393-397.
19. Özgür Can İ, Özkara E, Salaçin S, Gümüştekin M. Importance of sampling sites for postmortem evaluation of ethyl alcohol. J Forensic Res. 2012;3(7):1-3. http://dx.doi.org/10.4172/2157-7145.1000156
20. Sylvester PA, Wong NA, Warren BF, Ranson DL. Unacceptably high site variability in postmortem blood alcohol analysis. J Clin Pathol. 1998;51(3):250-252.
21. Kugelberg FC, Jones AW. Interpreting results of ethanol analysis in postmortem specimens: a review of the literature. Forensic Sci Int. 2007;165(1):10-29.
22. Mayr WR. DNA Recommendations- 1994 Report concerning further recommendations of the DNA Commission of the ISFH regarding PCR-based polymorphisms in STR (short tandem repeat) systems.Vox Sang. 1995;69(1):70-71.
23. Jain T, Shrivastva P, Bansal DD, Dash HR, Trivedi VB. PowerPlex Y23 System: A Fast, Sensitive and Reliable Y-STR Multiplex System for Forensic and Population Genetic Purpose. J Mol Biomark Diagn. 2016;7(3):1-7. http://dx.doi.org/10.4172/2157-7145.1000156
24. Hall DE, Roy R. An evaluation of direct PCR amplification. Croatian Med J. 2014;55(6):655–661.
25. Wieczorek D, Krenke BE. Direct Amplification from Buccal and Blood Samples Preserved on Cards Using the PowerPlex 16HS system. Promega Corp. 2009;1-6.
26. Shrivastava P, Jain T, Trivedi VB. DNA fingerprinting: A substantial and imperative aid to forensic investigation. Euro J Forensic Sci. 2016 3: 3.