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Case Study

Establishment of paternity from scalp muscles of burnt unidentified dead body – a case study

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Abstract

Burning cases include homicides, forest fires, vehicles, mass disasters etc. Visual identification of victim's in such types of cases is difficult. DNA profiling can play important role in the identification of victims. After post-mortem, charred bones are usually received in the forensic laboratories for establishment of identity and paternity/maternity. However, success rates of DNA profiling from these bones are very less. Moreover, contamination of the bones is also a problem. Alternately, burnt scalp muscles can be a good source of DNA for establishment of identity, paternity/maternity of unidenified dead bodies. In this study, DNA profiles were generated from scalp muscles of a burnt deceased person and paternity was established by comparing the DNA profile from blood sample of putative son. DNA from burnt scalp muscles of the deceased person and blood sample of putative son was isolated using magnetic bead based method with Qiagen EZ1 Advanced XL BioRobot. The DNA was subjected to Multiplex PCR amplification using PowerPlex®21 kit (Promega Corporation, U.S.A.). Capillary electrophoresis was done with 3130 Genetic Analyzer (Applied Biosystems, U.S.A.) and data were analyzed using GeneMapper® ID Software Version 3.2. The autosomal DNA analysis confirmed the deceased person was the biological father of putative son.

Keywords: DNA, maternity, paternity, scalp muscles.

Introduction

There are a number of cases where charred bones are received in forensic laboratories[1,2]. Such types of cases include homicides[3], mass disasters[4,5], burning after vehicle accident[6], forest fires[7], plane accidents[8], terrorist attacks[9], wars[10], armed conflicts[11], etc. After fire, the skin and muscles of victim are lost so abruptly that visual identification is impossible[12,13]. DNA profiling can play an important role in the identification of such victims. Usually in burning cases, doctors after post-mortem send charred bones for DNA profiling. But bones reduce weight due to combustion of organic materials and evaporation of water[14,15]. Shrinkage and deformation of bones also occur during burning of body. Since organic matrix disappears after burning of bone, which results in loss of DNA.

Hence, DNA profiling from charred bones is less successful. The scalp muscles can be an alternative to such types of cases. The muscles found in the head, face and neck region of the human body are called as scalp. These muscles remain distributed as a dense layer beneath the skin. These muscles also contain abundant cells and are a good source of DNA profiling. DNA profiles generated from these burnt scalp muscles can be used for identification of victims and to solve paternity/ maternity disputes.

Case history: In this case, the house of the victim (deceased person) caught fire late in the night. The body of deceased was completely burnt and it was not possible to identify the person. The relatives claimed that the deceased person was their family member. The body was sent to post-mortem and concerned medical officer sent scalp muscles of deceased person to the DNA Division, State Forensic Science Laboratory, Junga, Himachal Pradesh to generate DNA profile. In order to establish paternity, the blood sample of the putative son was also received in forensic laboratory. The DNA profiling of both the samples was done. The clean DNA profiles were obtained from scalp muscles of the deceased person and blood sample of putative son. By comparing their DNA profiles, the paternity was established.

Materials and methods

Materials: Scalp muscles of the burnt deceased person and blood sample of putative son were labeled as A and B, respectively. EZ1 DNA Investigator Kit was purchased from QIAGEN India Pvt. Ltd. - New Delhi, India.

Methods: The DNA isolation from scalp muscles of the deceased person and blood sample of the putative son was done by magnetic bead based method with slight modifications[16].

In brief, scalp muscles were cut into pieces with sterilized blades and put into a microvial (1.5ml). In another microvial, blood sample of putative son (50µl) was added. To both tubes, buffer G2 (350µl) and proteinase (15µl) K were mixed and tubes were lysed in a NB 20 water bath (Nuve, Ankara, Turkey) at 56°C for 48h. After lysis, lysate was poured into two separate sample tubes for DNA extraction. Elution tubes, tip holders containing filter-tips and reagent cartridges were inserted in EZ1® Advanced XL BioRobot (QIAGEN, Hilden, Germany) as per manual. The "Large-Volume Protocol" was used for DNA isolation. The isolated DNA was stored at -20°C in a refrigerator (Celfrost, India) for further use.

PCR amplification: The amplification of DNA was done as per protocol given in PowerPlex® 21 System kit[17]. In brief, master mix (5µl) and primer mix (5µl) was added in two separate PCR tubes. The contents were mixed thoroughly and DNA (15µl) from scalp muscles and blood sample (15µl) was added. The contents were mixed and spun in SPINWIN microcentrifuge (Tarsons, India). The amplification was done with GeneAmp® PCR System 9700 thermo cycler (Applied Biosystems, U.S.A.). 2800 M DNA was used as positive control as per kit manual, whereas, nuclease free water was used as negative control. The following protocol was set for PCR amplification: 96°C for 1 minute, 94°C for 10 seconds, 59°C for 1 minute, 72°C for 30 seconds for 30 cycles, then 60°C for 10 minutes and 4°C soak. The amplified products were quantified using agarose gel electrophoresis (2%) at 200V (Bio-Rad, Hercules, California, United States).

After amplification, appropriate dilutions were made with Hi-Di™ Formamide (Thermo Fisher Scientific, Waltham, Massachusetts, United States) for capillary electrophoresis.

Capillary electrophoresis: Capillary electrophoresis of PCR products were done with ABI 3130 Genetic Analyzer (Applied Biosystems, U.S.A.) using POP-4 at 15 ampere current and genotyping was carried out using GeneMapper® ID Software Version 3.2.

Results and discussion

The genotypes of the scalp muscles of deceased person (A) and blood sample of putative son (B) are given in Table-1. As shown in the table, Amelogenin marker of sample A and B depicted "XY" alleles, which confirmed that both were males. Complete and clean DNA profiles were obtained from both samples, which showed amplification at 21 loci. The electropherograms of sample A and sample B are given in fig. 1 and Figure-2, respectively. One of the two alleles found in the genotype of deceased person showed match with one of two alleles in the genotype of putative son, which are highlighted in bold and underline. This data confirmed that deceased person (A) was the biological father of putative son (B). The positive control showed alleles as given in the kit manual, whereas no amplification was observed in negative control. There are no reports available in literature to compare data obtained from this study.

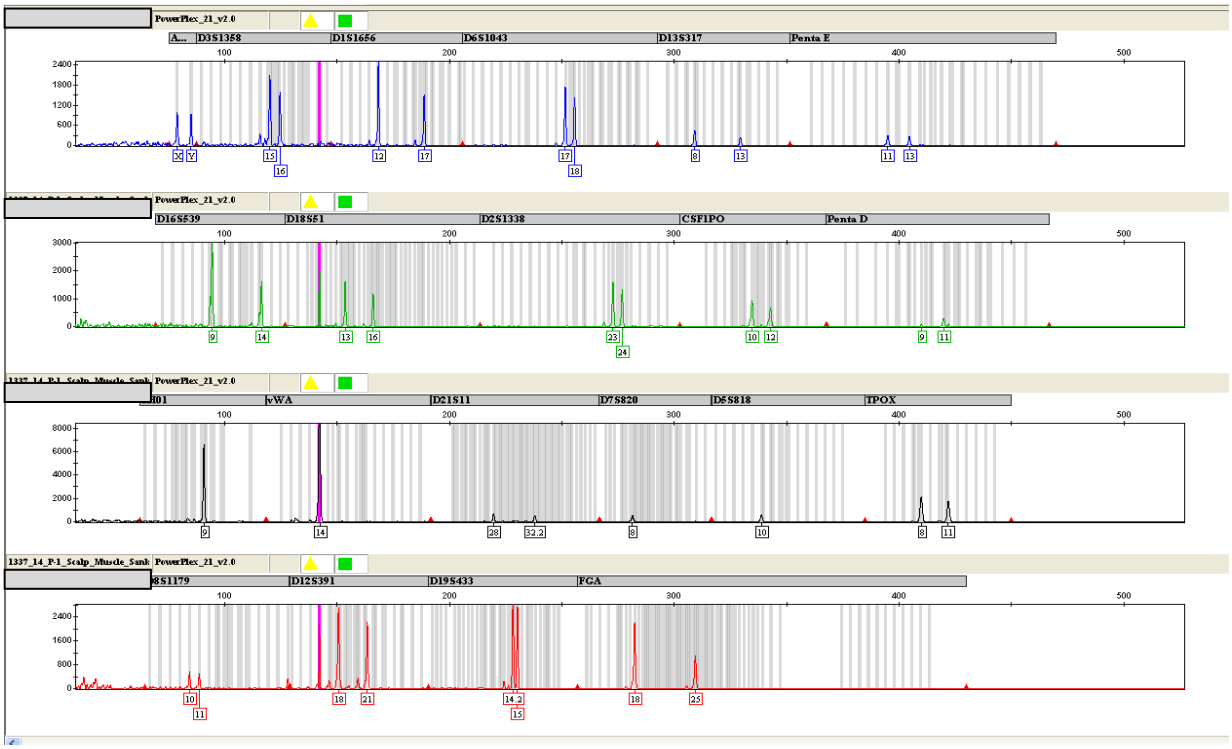

Figure-1: Electropherogram of DNA isolated from scalp muscles of deceased person (A).

Table-1: The genotypes of scalp muscles of deceased person (A) and blood sample of putative son (B).

Genetic markers	Positive control		Negative control	Scalp muscles of deceased person (A)		Blood sample of putative son (B)	
	Allele 1	Allele 2	Alleles	Allele 1	Allele 2	Allele 1	Allele 2
Amelogenin	X	Y	-	X	Y	X	Y
D3S1358	17	18	-	15	16	16	17
D1S1656	12	13	-	12	17	12	17
D6S1043	12	20	-	17	18	13	18
D13S317	9	11	-	8	13	8	11
Penta E	7	14	-	11	13	11	12
D16S539	9	13	-	9	14	10	14
D18S51	16	18	-	13	16	12	13
D2S1338	22	25	-	23	24	19	23
CSF1PO	12	12	-	10	12	10	11
Penta D	12	13	-	9	11	9	11
TH01	6	9.3	-	9	9	9	9
vWA	16	19	-	14	14	14	19
D21S11	29	31.2	-	28	32.2	29	32.2
D7S820	8	11	-	8	8	8	12
D5S818	12	12	-	10	10	9	10
TPOX	11	11	-	8	11	9	11
D8S1179	14	15	-	10	11	11	15
D12S391	18	23	-	18	21	18	22
D19S433	13	14	-	14.2	15	15	15
FGA	20	23	-	18	25	22	25

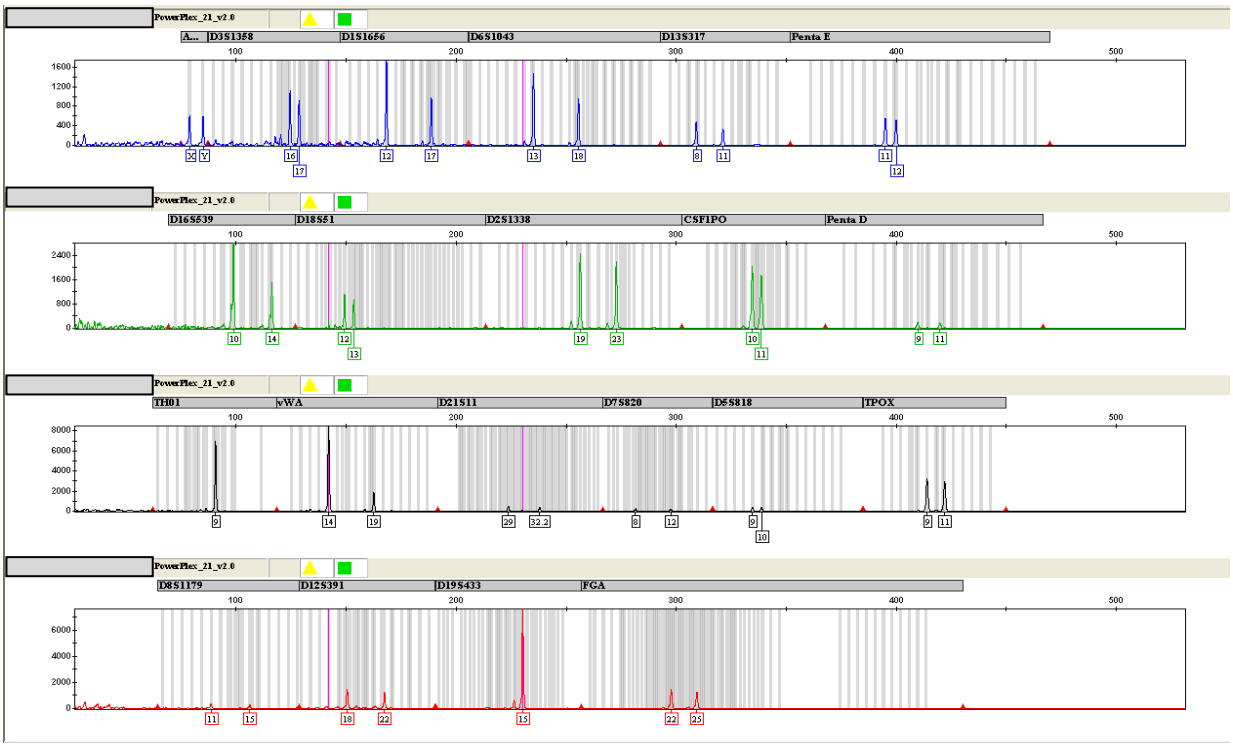

Figure-2: Electropherogram of DNA isolated from blood sample of putative son (B).

Discussion: To get DNA profile from burnt bones is a challenging task, as bones become brittle and friable[18]. However, scalp muscle can be alternative to charred bones. The results of the present study established paternity from burnt scalp muscles of deceased person and putative son. Hence, scalp muscles are a very good source of DNA profiling in case of burnt dead bodies. Scalp muscles of burnt dead bodies should be preferred by medical practitioners for DNA profiling in forensic laboratories. The DNA profiles generated from scalp muscles of dead bodies can be helpful in the generation of DNA databases.

Conclusion

Scalp muscles are an important exhibits for DNA profiling from burnt dead bodies. The scalp muscles contain abundant cells from where DNA can be extracted and profiles can be generated. Usually, charred bones are received in the forensic laboratories from burnt cases. These bones contain very less quantity of DNA and are also prone to contamination. Hence, medical practitioners should try to send scalp muscles in addition to other exhibits to forensic laboratories depending on burning condition of the dead body.

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