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Establishment of paternity from humerus bone of an unidentified skeleton

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Abstract

DNA typing from decomposed human body remains a challenge to forensic DNA scientists. Degraded human body parts are received in forensic laboratories from mass disasters, bombings, terrorist attacks, volcanoes, etc. Identification of dead bodies from these cases are important from the social and legal perspective. In this study, identity and paternity were established from the humerus bone of unidentified skeleton. The DNA from humerus bone was isolated using organic method. The blood sample of putative son of the deceased was also received on cotton gauge and DNA was isolated using Qiagen EZ1® Advanced XL BioRobot. The isolated DNA was subjected to Multiplex PCR amplification with PowerPlex®21 kit (Promega Corporation, U.S.A.). Capillary electrophoresis of amplified products was done with 3130 Genetic Analyzer (Applied Biosystems, U.S.A.) and data were analyzed using GeneMapper® ID Software Version 3.2. By comparing DNA profiles of the humerus bone of unidentified skeleton and blood on cotton gauge of putative son helped in the establishment of paternity. Hence, humerus bones are a good exhibit for identification from decomposed human body.

Keywords: Blood, Bone, DNA, Humerus, Paternity.

Introduction

DNA profiling from degraded human remains is still a challenge to forensic DNA scientists in the purification and interpretation of profiles[1]. The decomposed samples are received in forensic laboratories from disasters such as earthquakes[2], tsunami[3], homicides[4], mass disasters[5,6], terrorist attacks[7], plane crashes[8], wildfire[9], etc. The identification of human remains from such types of disasters is an important from legal and social perspective. Visual identification of victims by clothings, personal belongings and eyewitness reports are usually unreliable. Such type of identifications needs support from ante-mortem as well as post-mortem examination data. Hence, identification of humans from decomposing fragments are still challenging to forensic geneticists, pathologists and forensic odontologists[6,10]. The International Commission on Missing Persons (ICMP) at Netherlands is an international organization which addresses the issues of missing persons in forensic science[11]. DNA typing can play an important role in the identification of such types of persons from mass disaster such as 9/11 World Trade Center Attack at U.S.A. in 2001. From DNA typing, it is possible to identify degraded human remained samples. DNA profiles can be generated from many types of samples such as blood, hair, nails, tissue, bone etc., which largely depends on forensic scenarios[6]. However, bones are one of the common exhibit received in forensic laboratories for DNA profiling from disasters. However, there are certain limitations as extracted DNA is low in quantity and degradation decrease the chance of clean DNA profile. In addition to this, old and poor conditions of bones also pose problems in processing. Bone tissue is composed of proteins (collagen and osteocalcin) and minerals. The majority of mineral portion consists of hydroxyapatite such as calcium hydroxide, calcium fluoride, calcium phosphate, calcium carbonate and citrate[12]. Among different human bones such as femur, humerus, tibia, radius, ulna etc., humerus bone is preferred by the forensic scientists for DNA profiling. Humerus bone is the longest and thickest of the upper extremity in body, which forms the skeleton of the arm and help in connecting the shoulder and elbow joints to each other. Also, this bone is very important for forensic and anthropological studies[13,14]. DNA profiles generated from humerus bone can be helpful in the establishment of identity, maternity and paternity.

In the present study, paternity was established from the humerus bone of unidentified skeleton as a routine case work. According to investigating officers, one person in plain area of Himachal Pradesh went missing. His relative suspected that he committed suicide in the river. Later a skeleton was found in the river. After post-mortem exmaination, medical officer sent humerus bone of unidentified skeleton for DNA profiling to DNA Division, State Forensic Science Laboratory, Junga, Himachal Pradesh (Figure-1).

In order to establish paternity, the blood sample of the putative son on cotton gauze was also received in the laboratory. Clean DNA profiles were obtained from the humerus bone of unidentified skeleton and blood sample on cotton gauze from putative son. Paternity was established by comparing their DNA profiles.

Figure-1: Humerus bone from unidentified skeleton.

Materials and methods

Materials: Humerus bone from unidentified skeleton and blood sample of putative son on cotton gauze were labeled as A and B, respectively. EZ1 DNA Investigator Kit was purchased from QIAGEN India Pvt. Ltd. - New Delhi, India.

Methods: DNA from humerus bone was isolated by the organic method with slight modifications[15,16], whereas, DNA from blood sample on cotton gauze of putative son was isolated by magnetic bead based method using Qiagen EZ1 Advanced XL BioRobot[17]. Following is the summary of these methods:

Organic method: A piece of humerus bone was cleaned properly from outer side with sterilized blade. The bone was cut into pieces with hammer and put in absolute alcohol for ovenight. The alcohol was drained out and bone was dried at room temperature for 3-4 days. The bone was ground into powder using tissue lyser (Qiagen, Hilden, Germany). The powder was taken into a falcon tube (15ml) and approx. 2ml of DNA extraction buffer, 2ml of EDTA (0.5M) and 50µl of proteinase was added. The falcon tube was vortexed and incubated for 72h at 56°C in a NB 20 water bath (Nuve, Ankara, Turkey). After incubation, the lysate was taken into microvial (1.5ml) and 500µl of phenol:chloroform:isoamyl alcohol (25:24:1) was added into the vial, vortexed for few seconds and spun in a rotospin for 10 minutes (Tarsons, India). The microvial was centrifuged at 12000rpm for 10 minutes in a 5430 R refrigerated centrifuge (Eppendorf, Hamburg, Germany). The aqueous layer was removed carefully with a micropipette and added in another microvial. To this microvial, 500µl of phenol:chloroform:isoamyl alcohol (25:24:1) was added and previous step was repeated. To the aqueous layer, 500 µl of sodium acetate (2M) and chilled absolute alcohol (500µl) was added. The vial was kept at -20°C for overnight precipitation in a refrigerator (Celfrost, India). Then, microvial was centrifuged at 14000rpm for 15 minutes a 5430 R refrigerated centrifuge (Eppendorf, Hamburg, Germany). The supernatant was discarded carefully and 70% alcohol (500µl) was added. The microvial was centrifuged at 10000 rpm for 5 minutes and supernatant was discarded. This step was repeated once and microvial was dried in a digital dry bath (Labnet, New Jersey, United States) at 56°C for 30 minutes. To the vial, 20µl of TE buffer was added and dried in a dry bath at 56°C for 10 minutes. The DNA was stored at -20°C in refrigerator (Celfrost, India) for further use.

Magnetic bead method: Blood sample of putative son of the deceased on cotton gauze was cut into pieces with sterilized blades and put into a microvial (1.5ml). To the tube, buffer G2 (350µl) and proteinase (15µl) K were added and lysed in a NB 20 water bath (Nuve, Ankara, Turkey) at 56°C for 48h. After lysis, lysate was poured into a sample tube for DNA extraction. Elution tube, tip holder containing filter-tip and reagent cartridge 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[18]. In brief, master mix (5µl) and primer mix (5µl) was added in two separate PCR tubes. The contents were mixed thoroughly and DNA (15 µl) from humerus bone and blood sample of putative son was added. The contents were mixed and spun in SPINWIN microcentrifuge (Tarsons, India). The amplification was done with GeneAmp®PCR System 9700 thermal cycler (Applied Biosystems, U.S.A.). 2800 M DNA was used as positive control as per kit manual, whereas nuclease free water as a 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 32 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 humerus bone of unidentified skeleton (A) and blood sample of putative son on cotton gauze (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. Clean DNA profiles were obtained from both samples showing amplification at 21 loci. The electropherograms of sample A and sample B are given in Figure-2 and Figure-3, respectively. The genotype obtained from the humerus bone of unidentified skeleton showed match with one of the two alleles in the genotype obtained from blood sample of the putative son on cotton gauze which followed the law of Mendelian inheritance. This data confirmed that unidentified person was the biological father of putative son. The positive control showed alleles as given in the kit manual, whereas no amplification was observed in negative control. There are less reports on DNA profiling from the humerus bone. However, some groups have done genetic analysis of degraded bone samples. Siriboonpiputtana et al.[1] isolated DNA from different bone samples. They also isolated DNA from right and left humerus bones and obtained complete DNA profiles. These results were similar to our results. Hence it can be concluded that humerus bone from the skeleton are a good source of DNA profiling.

Table-1: The genotypes of humerus bone of unidentified skeleton and blood sample of putative son on cotton gauze.

Genetic markers	Positive control (2800 M DNA)		Negative control	Humerus bone of unidentified skeleton (A)		Blood sample of putative son on cotton gauze (B)	
	Allele 1	Allele 2	Alleles	Allele 1	Allele 2	Allele 1	Allele 2
Amelogenin	X	Y	-	X	Y	X	Y
D3S1358	17	18	-	17	18	15	17
D1S1656	12	13	-	15	16.3	11	16.3
D6S1043	12	20	-	11	13	11	13
D13S317	9	11	-	12	12	11	12
Penta E	7	14	-	5	11	11	19
D16S539	9	13	-	9	13	9	11
D18S51	16	18	-	19	19	17	19
D2S1338	22	25	-	18	23	18	19
CSF1PO	12	12	-	10	13	13	13
Penta D	12	13	-	9	10	9	10
TH01	6	9.3	-	8	9	6	8
vWA	16	19	-	17	19	18	19
D21S11	29	31.2	-	27	29	28	29
D7S820	8	11	-	8	13	8	11
D5S818	12	12	-	11	12	11	12
TPOX	11	11	-	11	11	11	11
D8S1179	14	15	-	12	14	12	16
D12S391	18	23	-	17	18	17	18
D19S433	13	14	-	13.2	15.2	14.2	15.2
FGA	20	23	-	23	24	22	24

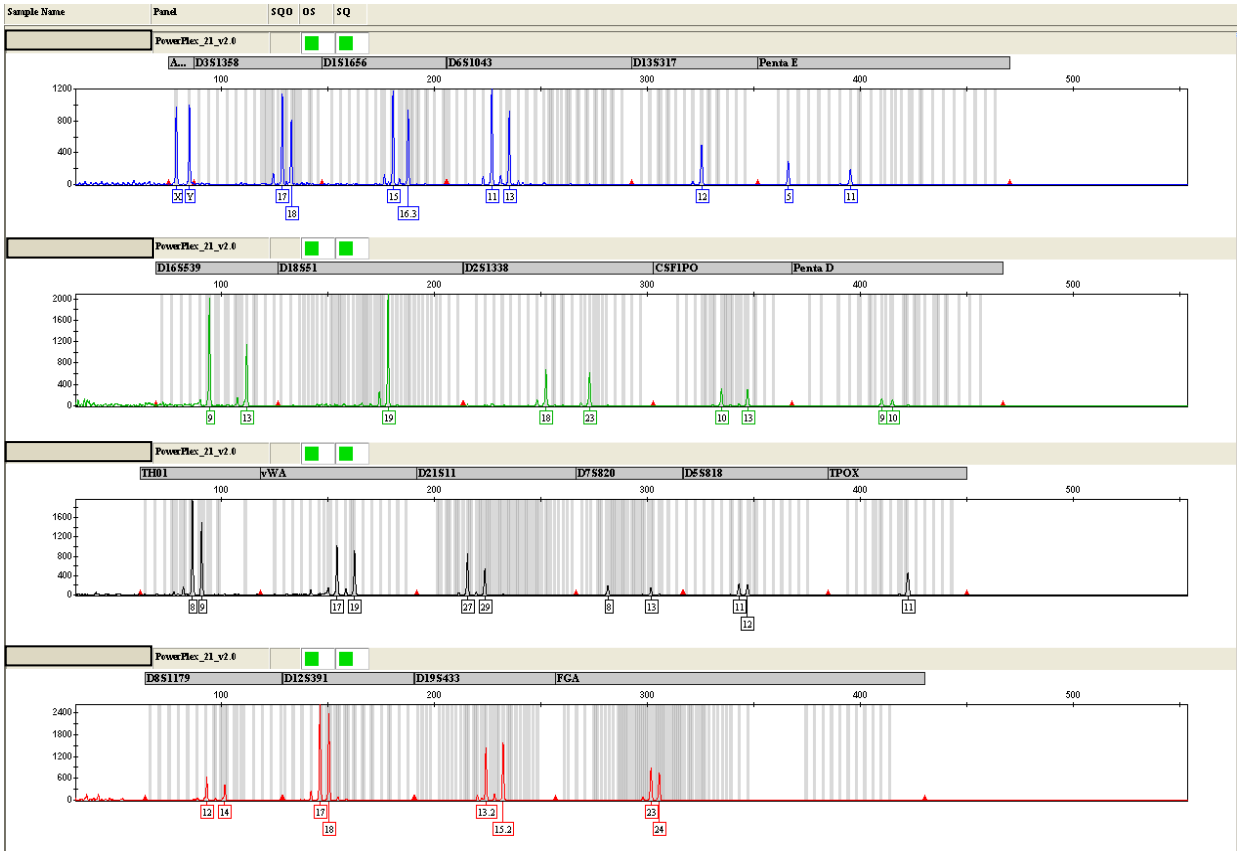

Figure-2: Electropherogram of DNA isolated from humerus bone of unidentified skeleton (A).

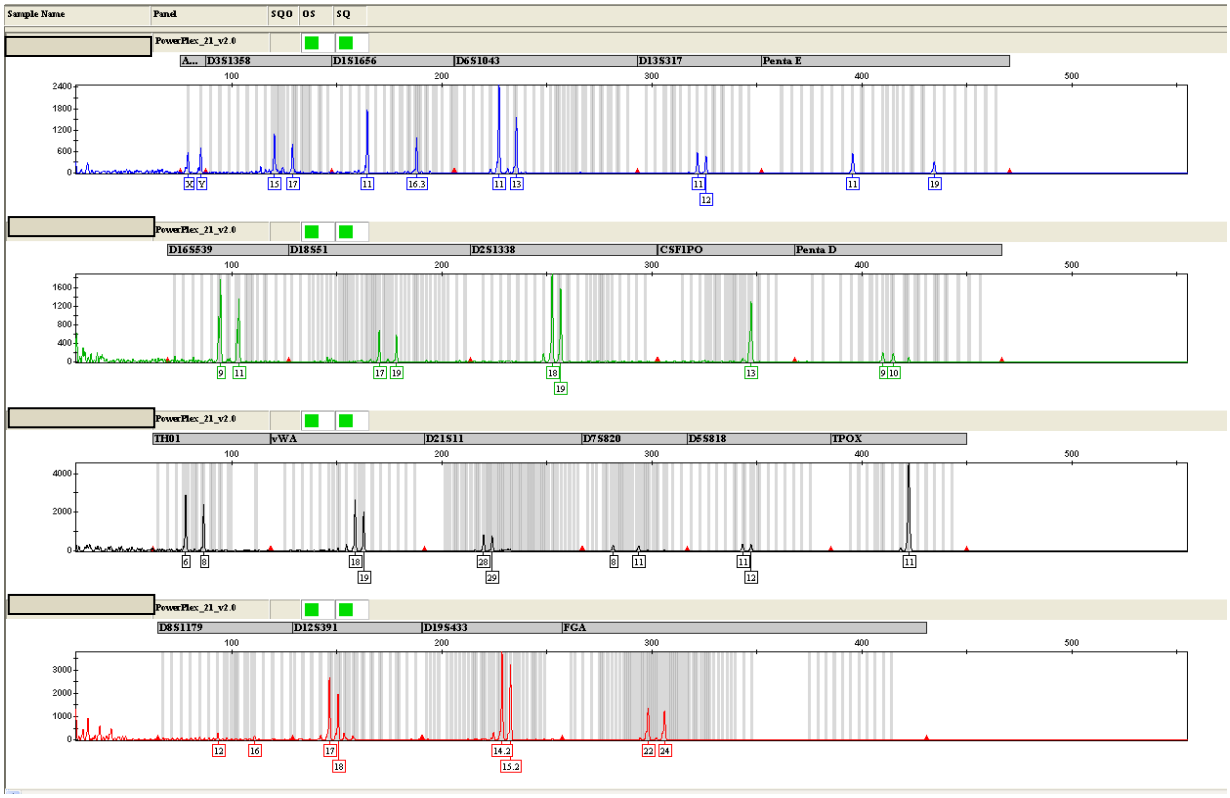

Figure-3: Electropherogram of DNA isolated from blood sample of putative son on cotton gauze (B).

Conclusion

Humerus bones of the decomposed human bodies are a good source for DNA profiling. It has also been reported that preservation of DNA in humerus bone is better as compared to flat and spongy bones, such as skull, vertebrae etc. Humerus bones are also easy to process for DNA extraction. Hence, medical officers should prefer humerus bone to forensic laboratories for identification of unidentified dead bodies. The DNA profiles obtained from these bones can be helpful in establishing paternity and maternity. Also, DNA profiles obtained from humerus bones can be helpful in making DNA databases.

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