Research Journal of Forensic Sciences ISSN 2321–1792
Vol. **9(1),** 7-10, January **(2021)** *Res. J. Forensic Sci.*

Case Study

Establishment of maternity from sternum bone of an unidentified dead body – A case study

Naresh Kumar[1*] and Arun Sharma[2]
[1]DNA Division, Regional Forensic Science Laboratory, Central Range, Mandi – 175002, Himachal Pradesh, India
[2]Directorate of Forensics Services, Junga, Shimla – 171218, Himachal Pradesh, India
nareshkumarbiotech85@gmail.com

Available online at: www.isca.in, www.isca.me
Received 13th September 2020, revised 8th November 2020, accepted 17th December 2020

Abstract

Sternum bone is one of the most common exhibits received in forensic laboratories from unidentified dead bodies. These bones are received for identification of dead bodies or establishment of maternity and paternity. Sternum bones are a good source for DNA profiling as they contain abundant cells. The costal cartilage tissue attached with sternum bone act as a source of clean DNA profile. In the present study, maternity was established from sternum bone of an unidentified dead body. DNA from sternum bone was isolated using magnetic bead based method with Qiagen EZ1 Advanced XL BioRobot, whereas DNA from FTA card of putative son was purified using FTA purification buffer reagent. The isolated and purified DNA was subjected to Multiplex PCR amplification using PowerPlex® 21 System kit (Promega Corporation, Wisconsin, United States). Capillary electrophoresis of amplified products was done with 3130 Genetic Analyzer (Applied Biosystems, U.S.A.). The data were analyzed using GeneMapper® ID Software Version 3.2. The autosomal short tandem repeats (STR) DNA analysis confirmed the maternity of unidentified dead bodies. Hence, human sternum bones are a good source of DNA for establishment of identity, maternity and paternity.

Keywords: DNA, FTA, Maternity, Paternity, Sternum bones.

Introduction

One of the most challenging tasks in forensic science is the identification of unidentified dead bodies. There are numbers of cases such as homicides[1], mass disasters[2,3], traffic accidents[4], wildfire[5], plane accidents[6], terrorist attacks[7], wars[8] in which parts of unidentified dead bodies are received in forensic laboratories for identification purposes. Sometimes, it becomes very difficult to identify the body by visual perspective[9]. However, identification of these dead bodies is important from legal perspective. DNA profiling can play a crucial role in this aspect and help poor family to get compensation from government authorities. There are several exhibits received in forensic laboratories from unidentified dead bodies such as teeth[10], femur bone[11], humerus bone[11], sternum bone[12], clavicle bone[13], etc. However, sternum bone is one of the common exhibit. The sternum is a T-shaped bone present in the anterior thoracic part of the human body[14]. It mainly consists of manubrium, body and xiphoid process[15]. The sternum bone is a good source for DNA profiling as they contain a large number of cells. The costal cartilage part of sternum bone, where ribs attach to bone, yields a clean DNA profile.

In the present study, maternity was established from sternum bone of an unidentified dead body. According to investigating officers, the unidentified dead body of a lady was found on the side of a channel in a decomposed condition. The body was also eaten by animals and it was difficult to identify the body. However, his husband claimed that she was his wife. Medical officers sent sternum bone of unidentified dead body and blood sample of the putative son on FTA card as routine laboratory work in the DNA Division, State Forensic Science Laboratory, Junga, Shimla, Himachal Pradesh, India. Maternity was established by comparing DNA profiles of sternum bone of the dead body and putative son.

Materials and methods

Materials: The sternum bone of unidentified dead body and blood sample of putative son was labelled as X, Y, respectively. EZ1 DNA Investigator Kit was purchased from QIAGEN Hilden, Germany. The PowerPlex® 21 System kit was procured from Promega Corporation, Wisconsin, United States.

Methods: The DNA isolation from sternum bone was done by magnetic bead based method with slight modifications[16]. In brief, costal cartilage tissue attached with sternum bone was cut into pieces with sterilized blades. The pieces were added in a micro vial (1.5ml) and washed once with autoclaved distilled water by centrifuging in a 5430R refrigerated centrifuge (Eppendorf, Hamburg, Germany) at 10000 rpm for 5 minutes. The supernantant was discarded. In the pellet, buffer G2 (350µl) and proteinase (25µl) K was added. The bone pieces were lysed at 56°C in an NB 20 water bath (Nuve, Ankara, Turkey) for 48

hours. The lysate was poured into a sample tube (2ml). 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 without adding MTL buffer because of sufficient lysate. The isolated DNA was stored at -20°C in a refrigerator (Celfrost, India) for further use.

The DNA from blood sample on FTA card of the putative son was purified as per method with slight modifications[17]. FTA card bearing blood sample was punched with the help of Harris 1.2mm micro punch. The punches were added in a micro vial and novel FTA purification reagent (150µl) and proteinase K (15µl) was added. The punches were incubated at 56°C in an NB 20 water bath (Nuve, Ankara, Turkey) for two hours, then washed with autoclaved water and dried in a digital dry bath (Labnet International, U.S.A.). The punches were stored at -20°C in a refrigerator (Celfrost, India) for further use.

PCR amplification: The amplification of DNA was done using 25µl reaction volume as per protocol given in PowerPlex® 21 System (Promega Corporation, U.S.A.)[18]. In brief, master mix (5µl) and primer mix (5µl) was added in two PCR tubes followed by the addition of isolated DNA (15µl) from sternum bone (X) and one punch of the FTA card from putative son (Y). To complete the reaction volume with FTA card, 15 µl of nuclease free water was also added. The contents were mixed thoroughly and spun in SPINWIN microcentrifuge (Tarsons, India). The PCR tubes were put into GeneAmp®PCR System 9700 thermocycler (Applied Biosystems, U.S.A.). 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%). The appropriate dilutions were made for further capillary electrophoresis. 2800 M DNA in the kit was used as positive control, whereas nuclease free water was used as negative control to check the quality management.

Capillary electrophoresis: Capillary electrophoresis of amplified products were done with ABI 3130 Genetic Analyzer (Applied Biosystems, U.S.A.) and genotyping was carried out using GeneMapper® ID Software Version 3.2.

Results and discussion

The Punnett square table of analyzed samples using PowerPlex®21 kit is given in Table-1. As shown in the table, Amelogenin marker of X (unidentified dead body) depicted "XX" allele, which confirmed that the person was female. A clean DNA profile showing amplification at all the 21 loci was obtained from this exhibit. Amelogenin marker of Y sample showed "XY" allele, which confirmed male individual. From this exhibit, also a clean DNA profile was obtained with amplification at all the 21 loci. One of the two maternal alleles obtained from the genotype of sample X (unidentified dead body) showed match with one of the two alleles obtained from the genotype of Y (blood on FTA card of putative son). The result followed the law of Mendelian Inheritance. It suggested that X (unidentified dead body) was the biological mother of Y (blood on FTA card, putative son). Hence, maternity was established. Positive control (2800M) showed alleles as per the kit manual, whereas no amplification was observed in negative control. There is very less literature available for the identification of dead bodies from sternum bones by DNA profiling. Recently, De Donno et al.[19] identified a saponified dead body, which was recovered from the sea, without arms and legs. They isolated DNA from sternum bone by NucleoSpin® DNA Trace Kit (Macherey Nagel™) protocol and developed DNA profile of an individual. They compared the DNA profile obtained from the sternum bone of unidentified dead body with the DNA profile of the putative son. The DNA profiles did not match; hence the possibility of kinship was excluded. Hence, it can be concluded that sternum bones are also effective in the generation of DNA profiles from decomposed dead bodies.

Table-1: Punnett square table of the analyzed samples.

Genetic markers	Positive control (2800 M)		Negative control	X (Unidentified dead body)		Y (blood on FTA card, putative son)	
	Allele 1	Allele 2	Alleles	Allele 1	Allele 2	Allele 1	Allele 2
Amelogenin	X	Y	-	X	X	X	Y
D3S1358	17	18	-	16	18	16	18
D1S1656	12	13	-	15	16	15	15
D6S1043	12	20	-	12	18	13	18
D13S317	9	11	-	9	10	8	9
Penta E	7	14	-	15	15	15	17
D16S539	9	13	-	9	10	9	11

D18S51	16	18	-	13	17	13	13
D2S1338	22	25	-	18	21	21	24
CSF1PO	12	12	-	10	11	10	11
Penta D	12	13	-	10	11	11	11
TH01	6	9.3	-	6	7	6	9.3
vWA	16	19	-	15	17	15	17
D21S11	29	31.2	-	30	32.2	32.2	32.2
D7S820	8	11	-	10	11	8	10
D5S818	12	12	-	10	11	10	12
TPOX	11	11	-	8	8	8	12
D8S1179	14	15	-	13	14	14	15
D12S391	18	23	-	19	19	19	21
D19S433	13	14	-	13	15	13	14
FGA	20	23	-	20	22	22	22

Conclusion

Sternum bones are an excellent source of DNA profiling due to their direct access and ease of doing post-mortem activity on cadavers. These bones are common exhibits received in forensic laboratories for identification of unidentified dead bodies or establishment of maternity and paternity. However, care should be taken by the medical staff for the packing of such these bones while sending them to forensic laboratories. They should prefer sternum bones in the plastic jar without preservative or normal saline. The bones can be wrapped with pieces of papers in cloth pieces. They should never use formaldehyde preservative as it causes damage to DNA. DNA profiles generated from sternum bones can be helpful to generate a DNA database at the state and national level.

Acknowledgements

The authors are thankful to the Department of Home, Government of Himachal Pradesh for providing necessary funding to carry out this research work. The funding was helpful in purchasing of consumables and kits for this work.

References

1. Prahlow, J.A., Cameron, T., Arendt, A., Cornelis, K., Bontrager, A., Suth, M.S., Black, L., Tobey, R., Pollock, S., Stur, S., Cotter, K. and Gabrielse, J. (2017). DNA testing in homicide investigations. *Med. Sci. Law*, 57(4), 179-191. doi: 10.1177/0025802417721790.

2. Alonso, A., Martin, P., Albarrán, C., Garcia, P., de Simon, L.F., Iturralde, M.J., Fernández-Rodriguez, A., Atienza, I., Capilla, J., García-Hirschfeld, J., Martinez, P., Vallejo, G., García, O., García, E., Real, P., Alvarez, D., León, A. and Sancho, M. (2005). Challenges of DNA profiling in mass disaster investigations. *Croat. Med. J.*, 46(4), 540-548.

3. Budowle, B., Bieber, F.R. and Eisenberg, A.J. (2005). Forensic aspects of mass disasters: strategic considerations for DNA-based human identification. *Legal Med.*, 7(4), 230-243.

4. Taki, T., Machida, M. and Shimada, R. (2019). Trends of traffic fatalities and DNA analysis in traffic accident investigation. *IATSS Res*., 43(2), 84-89.

5. Artés, T., Oom, D., de Rigo, D., Durrant, T.H., Maianti, P., Libertà, G. and San-Miguel-Ayanz, J. (2019). A global wildfire dataset for the analysis of fire regimes and fire behaviour. *Sci. Data*. 6, 296. https://doi.org/10.1038/s41597-019-0312-2.

6. Dalibor, N. and Vladimir, P. (2020). The challenges of forensic medical expertise in aircraft accidents: A case report. *J. Indian Acad. Forensic Med.*, 42(10), 63-65.

7. Biesecker, L.G., Bailey-Wilson, J.E., Ballantyne, J., Baum, H., Bieber, F.R., Brenner, C., Budowle, B., Butler, J.M., Carmody, G., Conneally, P.M., Duceman, B., Eisenberg, A., Forman, L., Kidd, K.K., Leclair, B., Niezgoda, S., Parsons, T.J., Pugh, E., Shaler, R., Sherry, S.T., Sozer, A. and Walsh, A. (2005). DNA identifications after the 9/11 world trade center attack. *Sci.*, 310(5751), 1122-1123.

8. Roewer, L. (2013). DNA fingerprinting in forensics: past, present, future. *Investig. Genet.*, 4, 22. https://doi.org/10.1186/2041-2223-4-22

9. Prinz, M., Carracedo, A., Mayr, W.R., Morling, N., Parsons, T.J., Sajantila, A., Scheithauerg, R., Schmitterh, H. and Schneideri, P.M. (2007). DNA commission of the international society for forensic genetics (ISFG): Recommendations regarding the role of forensic genetics for disaster victim identification (DVI). *Forensic Sci. Int. Genet.*, 1(1), 3-12.

10. Leena Sakari, S., Jimson, S., Masthan, K.M.K. and Jacobina, J. (2015). Role of DNA profiling in forensic odontology. *J. Pharm. Bioallied Sci.*, 7 (Suppl 1), S138-S141.

11. Siriboonpiputtana, T., Rinthachai, T., Shotivaranon, J., Peonim, V. and Rerkamnuaychoke, B. (2018). Forensic genetic analysis of bone remain samples. *Forensic Sci. Int.*, 284, 167-175.

12. Kumar, N., Maitray, A., Ritika, G., Sharma, D. and Shukla, S.K. (2017). Effect of preservation on DNA and its profiling from sternum bone from unidentified bodies. *J. Punjab Acad. Forensic Med. Toxicol.*, 17(2): 77-79.

13. Abuidrees, A. (2016). A suitable method for DNA extraction from bones for forensic applications: A case study. *Arab J. Forensic Sci. Forensic Med.*, 1(3), 346-352.

14. Kenneth Saladin (2010). Anatomy and physiology: The Unity of form and function. Fifth Edition, McGraw-Hill, New York.

15. Parvathi, S. (2013). Sternal foramen - A case report. *Int. J. Curr. Res. Rev*., 05(6), 80-86.

16. Qiagen (2020). EZ1 DNA Investigator Handbook. https://www.qiagen.com/us/resources/download.aspx?id=46064856-1b88-4b27-a825-d3f616e06c08&lang=en. 08 October 2020.

17. Sahajpal, V., Rajput, S., Sharma, T., Sharma, A. and Thakar, M.K. (2019). Development and evaluation of a novel DNA purification buffer and protocol for blood samples on FTA cards. *Forensic. Sci. Int. Rep.*, 1, 100014. https://doi.org/10.1016/j.fsir.2019.100014

18. Technical Manuals (2020). PowerPlex® 21 System for Use on the Applied Biosystems ® Genetic Analyzers, DC8902 and DC8942. https://www.promega.jp/-/media/files/resources/ protocols/technical-manuals/101/ powerplex-21-system-protocol.pdf?la=en. 08 October 2020.

19. De Donno, A., Mele, F., Baldassarra, S.L., Martini, A., Lauretti, C., Favia, M., Introna, F. and Santoro, V. (2020). DNA extraction from sternum bone for identification of a saponified body: use of a modified protocol. *Anthropol. Anz.*, 77(3), 235-242.