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DNA profiling of unidentified human dead bodies from costal cartilage attached with sternum bone in Himachal Pradesh, India

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Abstract

Sternum bone is one of the most common exhibits received in forensic laboratories for the establishment of the identity of unidentified human dead bodies. However, the extraction of DNA from sternum bone is a multi-step process and time-consuming. The fleshy costal cartilage attached with sternum bone can be used as an alternative for DNA extraction. In the present study, DNA profiling from costal cartilage of one hundred nineteen sternum bones of unidentified dead bodies was done, as a part of casework analysis across seven years between 2014 to 2020.Most of the dead bodies were retrieved in conditions of advanced stages of decomposition and skeletonization. In the present study, the DNA was isolated from costal cartilage with Qiagen EZ1 Advanced XL BioRobotusing magnetic bead based method. The isolated DNA was quantified using agarose gel electrophoresis (0.8%) and subjected to multiplex PCR amplification using Power Plex® 21 System and Global Filer™ kits. The capillary electrophoresis of amplified products was done using 3130, 3500, and 3500 XL genetic analyzers. The data were analyzed using Gene Mapper® ID Software Version 3.2 and Gene Mapper™ ID – X Software v 1.6. Despite advanced stages of decomposition of the bodies, full DNA profiles were obtained from 115 out of 119 sternum bones. Hence, the fleshy costal cartilage of sternum bone can serve as an alternate for quick extraction of DNA as compared to hard sternum bones.

Keywords: Costal Cartilage; DNA Profiling; Sternum bones, Unidentified human dead bodies.

Introduction

Biological evidences play an important role to establish the identity of unidentified dead bodies. The evidences from these dead bodies are received in the forensic laboratories from cases such as homicide[1,2] wildfire[3], traffic accident[4], armed conflicts[5], mass disasters[6,7], disaster victim identification[8], wars[9], terrorist attacks[10], plane accidents[11], etc. The majority of the mremain unnoticed by the public and police hence get decomposed with time. Establishing the identity poses a great challenge due to the onset of decomposition causing loss of soft tissue. The degree of decomposition may range from fresh to skeletonization state. Skeletonization is observed in the final stage of decomposition, due to the breakdown of skin and soft tissues depending on the inter-individual variation in the body mass[12]. While bones are more stable than soft tissues, they can still undergo breakdown via the physical, chemical, or microbial aspects of organic and inorganic components slowly with time[13].

Skeletonisation takes place approximately between 3 weeks to 6 months but can vary greatly due to the impact of taphonomic variables influencing the process of skeletonization[14-16]. The visual identification by clothing and belongings is less reliable. Decomposition is a multistep process happening simultaneously ranging from cellular autolysis to tissue autolysis facilitated by insects and microbial activity[17,18].

Predators ranging from insects to mammals get attracted to the decomposing body as a nutrient source and accelerate the decomposition. The law enforcement agencies, public safety and health officials can play an important role in the identification of unidentified dead bodies from a social and legal perspective[10]. DNA profiling in unidentified bodies is a major challenge due to the complexities arising out from intrinsic and extrinsic factors. While the assessment of identity is accurate with the use of commonly encountered biological evidences such as body fluids or tissues, there have been challenges associated with identification especially when advanced stages of decomposition are encountered[19].

Medical practitioners after the post-mortem of dead bodies send different samples to forensic laboratories such as blood, blood on gauze, blood on Whatman® FTA® Cards, tooth and long bones, however, sternum bone is one of the most frequently received sample. While the age and sex of a person may vary, the sternum bone is a good source of DNA in fresh/active decomposition states to advanced stages[12]. Age estimation to narrow down the identity can be tracked either by dental or skeletal developments or both and is supported by biochemical, morphological, and histological changes[20].

In addition to bones, dental evidences are also collected as a potential source for DNA. The quality and quantity of the yield are variable according to the type of teeth[21].

A dental autopsy to retain the maxillary and mandible arch for assessment of dentition can be done in support of the preservation of skeletal remains for DNA studies[22]. Factors that affect dental DNA depends upon the age of a person, type of tooth[23] whether deciduous or permanent teeth[24] obtained and the ability of DNA to be retained in the root pulp considering the aging process, number of teeth found[25] development and decay, injuries sustained and how well dental DNA was preserved before the extraction process[26,27]. While teeth can be very important in cases of decomposed or burnt remains[28] the process of DNA extraction, yield, and quality is challenging to restore and takes longer processing time in comparison to sternum analysis. In cases where sternum and teeth are sent as evidence, it would be preferable to process sternum faster than teeth and the findings of DNA profiles from both will complement each other.

The sternum is a T-shaped bone within the anterior thoracic portion of the human body. It consists of manu brium, body and xiphoid process[29]. The organic method is most commonly used to extract DNA from these bones but is time-consuming and prone to contamination. The qualitative characteristics of the sternum are useful for personal identification[30]. The costal cartilage attaches the sternum with the ribs and contain abundant cells for DNA extraction. In the last few years, several bones have been recommended with relevant methods for DNA profiling. Despite all available methods, DNA profiling with sternum bones is promising in cases of advanced decomposition. DNA profiling is the new gold standard in forensic science[31] for the identification of dead bodies by Short Tandem Repeats (STR) technology.

Taking into consideration, this study was designed to test the suitability of costal cartilage for DNA profiling. These bones were received from various police stations of the Himachal Pradesh for DNA profiling. The effect of preservation on DNA profiling was also discussed.

Materials and Methods

This study was performed at the DNA Division, State Forensic Science Laboratory, Junga, Shimla, Himachal Pradesh, India. A total of one hundred nineteen (119) sternum bones from unidentified human dead bodies were selected as a routine casework between years 2014 to 2020. The bones were found preserved in plastic jars or wrapped with paper and cloth pieces. The samples were stored at -20ºC for long-term preservation until sample analysis.

DNA Extraction: The DNA extraction from costal cartilage attached with sternum bones was done by magnetic bead based method using Qiagen EZ1 Advanced XL BioRobot[32]. In brief, the costal cartilage was washed with autoclaved ultrapure water and chopped into pieces with sterilized blades and put into the micro vials (1.5 ml). To this, buffer G2 (450 µl) and proteinase K (25 µl) was added. The costal cartilage pieces were vortexed and lysed in an NB 20 water bath (Nuve, Ankara, Turkey) at 56°C for 24 hours. After lysis, the vials were centrifuged at 10000 rpm for 5 minutes in a 5430R refrigerated centrifuge (Eppendorf, Hamburg, Germany) and supernatant was poured into separately labeled sample tubes (2 ml). Elution tubes, tip holders containing filter-tips, and reagent cartridges were inserted in EZ1Advanced XL BioRobot (QIAGEN, Hilden, Germany) as per manual[33]. The "Large-Volume Protocol" was used for DNA isolation without the addition of MTL buffer due to the presence of sufficient lysate. The isolated DNA was stored at -20°C in a refrigerator (Celfrost, India) for further use. The quantification of DNA was done with agarose gel electrophoresis (0.8%) and 1.0 ng DNA was used for PCR amplification.

PCR Amplification: The PCR amplification of extracted DNA was performed using PowerPlex®21 System and Global Filer™ kits[34,35]. The PowerPlex® 21kit amplifies 20 autosomal STR loci and the amelogenin gender determining marker in a single PCR amplification run. The amplification was performed in a reaction volume of 25µl using 5µl of the master mix, 5µl of primer mix, and 15µl of isolated DNA in separately labeled PCR tubes. The contents were mixed thoroughly and spun in SPINWIN micro centrifuge (Tarsons, India). The amplification was done using the GeneAmp PCR System 9700and Veriti Dx thermal cycler (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. 2800 M DNA as positive control and nuclease-free water was used as a negative control to the check quality of the kit.

The GlobalFiler™ kit amplifies 21 autosomal STR loci, the amelogenin, and two male-specific Y-Indel and DYS391 markers. The PCR amplification was performed with 25 µl of reaction volumes using 7.5µl of the master mix, 2.5 µl of primer mix, and 15µl of isolated DNA. The contents were vortexed for 10 seconds and amplification was done with GeneAmp PCR System 9700 and Veriti Dx thermal cyclers (Applied Biosystems, U.S.A.). Control DNA 007 as positive control and nuclease-free water as a negative control was used to evaluate the quality of the kit. The following protocol was set for PCR amplification: 95°C for 1 minute, 94°C for 10 seconds, 59°C for 90 seconds, 60°C for 10 minutes for 29 cycles, then 4°C holds. The amplified products were stored at 4°C in a refrigerator.

DNA Genotyping: The capillary electrophoresis of PCR products from PowerPlex® 21System kit was done with ABI 3130 genetic analyzer (Applied Biosystems, U.S.A.) using 9.5 µl of Hi-Di formamide, 0.5 µl WEN ILS 500size standard, and 1 µl allelic ladder. Besides this, capillary electrophoresis from the GlobalFiler™ PCR kit was done with ABI 3500 and 3500 XL Genetic Analyzers (Applied Biosystems, U.S.A.) using 9.6 µl of Hi-Di formamide, 0.4 µl of GeneScan 600 LIZ size standard, and 1 µl of the allelic ladder.

The 96 well plates were denatured at 95°C for 5 minutes in GeneAmp® PCR 9700 thermal cycler and cooled to 4°C for completion of the denaturation process. The denatured samples were run using POP-4 as a sieving matrix. The genotyping was carried out with GeneMapper® ID Software Version 3.2 for PowerPlex® 21 System kit and GeneMapper™ ID‑X Software v 1.6 for GlobalFiler™ kit.

Results and Discussion

In the present study, a total of one hundred nineteen sternum bones (n=119) were analyzed between 2014-2020. Out of these, 110 were male and 9 were female profiles. The majority of bones were from decomposed dead bodies followed by partially decomposed, fresh, advanced stage of decomposition and partially burnt conditions as seen in Figure-1. Figure-2 indicates various locations where unidentified bodies were reported for forensic analysis. Out of 119 bones, 115 complete DNA profiles were obtained whereas four (04) samples showed partially mixed profiles or no result due to the use of formaldehyde as a preservative. Electropherogram of representative male DNA profile from costal cartilage using PowerPlex® 21 System kit (showing amplification at 21 loci) and GlobalFiler™ kit (showing amplification at 24 loci) is given in Figure-3 and 4.

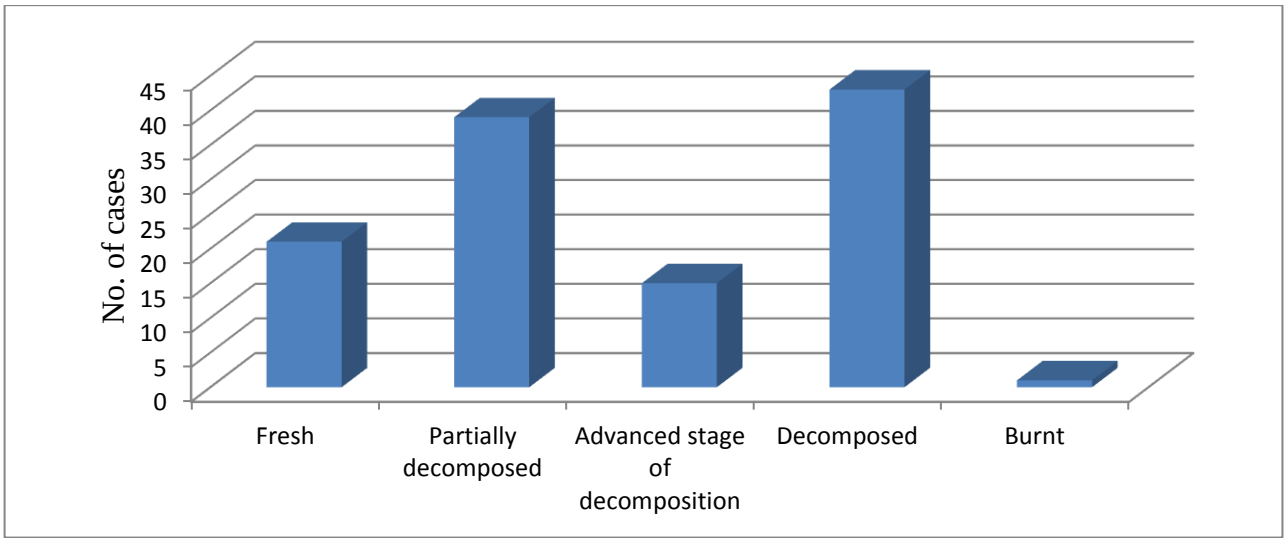

Figure-1: Condition of recovered dead bodies.

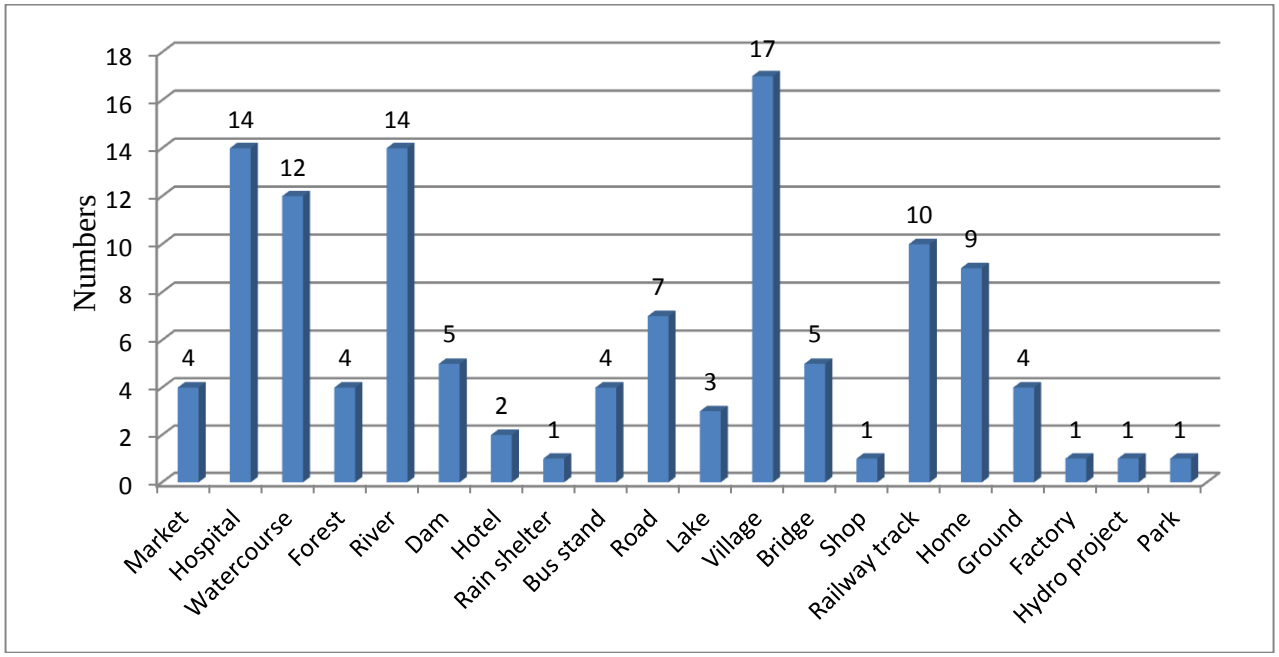

Figure-2: Sites of recovered dead bodies.

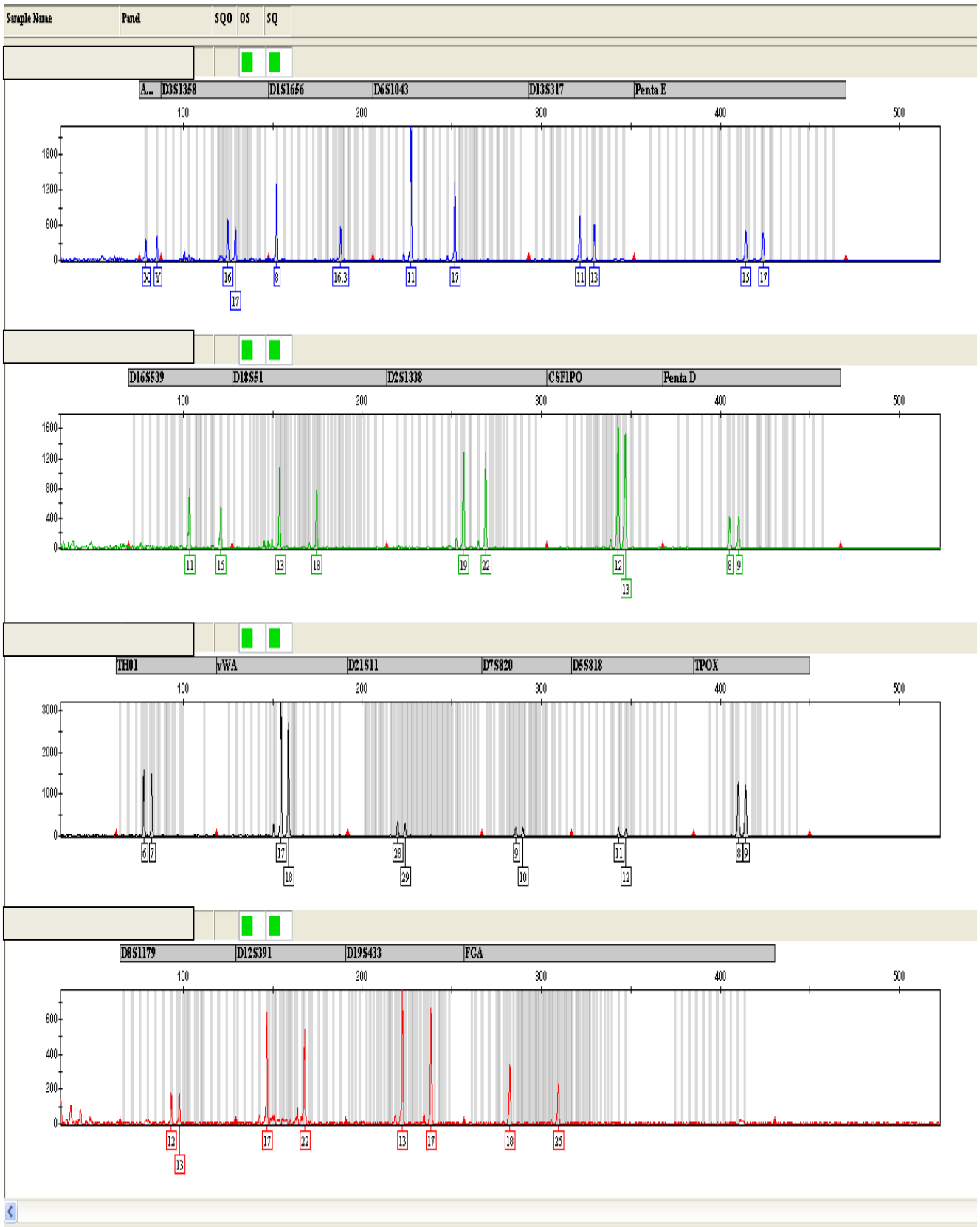

Figure-3: Electropherogram of a representative sample showing the complete DNA profile of a male using PowerPlex® 21 kit.

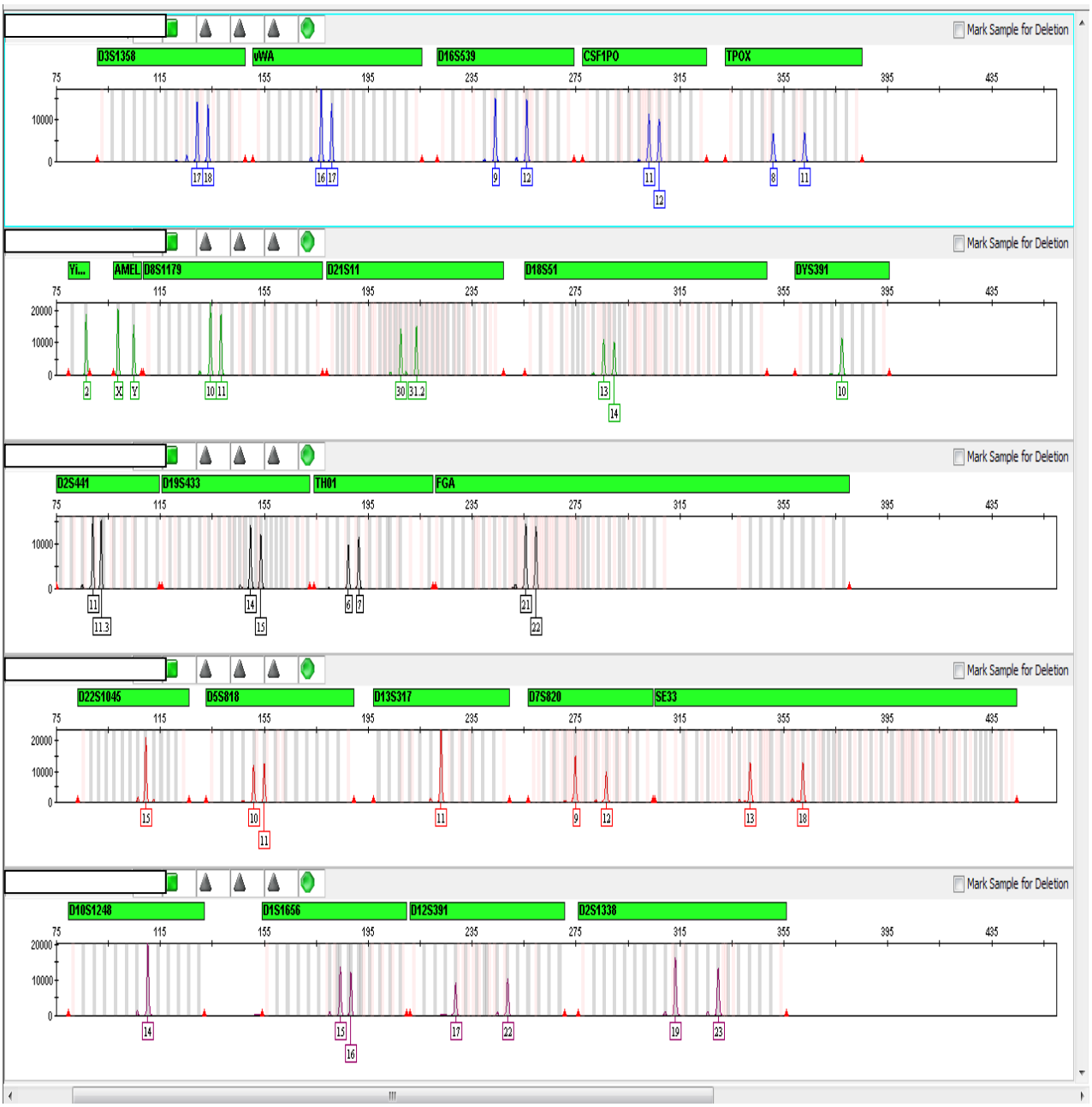

Figure-4: Electropherogram of a representative sample showing the complete DNA profile of a male using GlobalFiler™ kit.

Discussion: Sternum analysis is important in investigating unidentified human dead bodies as they are less prone to environmental factors of degradation as compared to the soft tissues. The analysis of costal cartilage of the sternum show results within 24hours in the entire work flow of DNA profiling, making it faster and efficient as compared to bones or teeth DNA analysis which takes longer than 72 hours. This offers quick results for paternity cases or cases that require quick disposal.

In the present study, most of the unidentified dead bodies were recovered from various locations in decomposed and partially decomposed conditions as shown in Figure-1 and 2. This is because dead bodies remain unnoticed by the public and police because of the tough geography, terrain, and sub-humid-tropical climate of Himachal Pradesh[36]. They are also eaten by animals, worms, etc., and get decomposed with time. The majority of dead bodies were recovered in the village, river, hospital, and water course. This might be due to diseased conditions of persons or lack of adequate health care facilities after accidents. Despite most of the dead bodies were recovered in decomposed conditions, 115 out of 119 bones showed complete DNA profiles.

This suggested that costal cartilage is one of the best parts from sternum bone for DNA isolation within a short time. Also, it is easy to transport, store and process in forensic laboratories as compared to full sternum bone. In comparison, Kumar et al.[37] isolated DNA from the manubrium part of sternum bone within 4-5 days after putting them for decalcification in 0.5 M EDTA.

Besides this, sternum bones preserved in plastic jars and cloth pieces get dried with time which yields a good quantity of DNA, hence showed complete profiles without allelic dropout as seen in Figure-3 and 4. The age of the sample did not affect the stability of the costal cartilage, which is why adequate conservation is most important. A few bone exhibits showed a partially mixed profile and no result. This was because of the preservation of samples in formaldehyde solution.DNA degrades with time in moist conditions[37]. It has been reported that formaldehyde causes single-strand breaks in DNA by forming cross-links between DNA and proteins. Hence, an insufficient quantity of DNA is obtained which results in partial or no DNA profile. There is no literature available on DNA profiling from costal cartilage attached with the sternum, however, there are few studies on DNA profiling from sternum bones. Kumar et al.[37] studied the effect of preservation on DNA isolated from thirty sternum bones of unidentified dead bodies. They observed a good quantity of DNA and complete profiling from dry sternum bones as compared to wet ones.

Recently, De Donno et al.[38] identified a saponified human body without arms and legs from the sea. The dead body was in the advanced stage of putrefaction and they used sternum bone for DNA profiling. A complete DNA profile was obtained and compared with the putative son for a genetic match. It is recommended that medical practitioners should send small pieces of costal cartilage for analysis instead of the whole sternum bone for DNA profiling with ease of handling with minimum sample required. Besides this, costal cartilage should be sent in plastic jars without preservatives. The use of preservatives such as formaldehyde followed by bleaching, maceration, and disarticulation of the tissues and bones is seen while sample preparation is done[39].

Samples preserved using formaldehyde did not give results as it interfered with the stability of the costal cartilages for DNA profiling. Alternatively, normal saline can be used or the bones can simply be wrapped with paper or cloth pieces for better storage and yield of DNA. Hence, this technique can be recommended to law enforcement agencies during search and medical experts during autopsy for better handling and preservation strategies to obtain useful results for casework.

Conclusion

The costal cartilage of the sternum greatly helps in identifying decomposed remains beyond recognition. The current research throws light on less explored areas of analysis of sternal analysis sourced under various decomposing conditions for STR profiling. Their identification by scientific analysis is important for investigation and forensic casework. DNA profiling through short tandem repeats (STR) technology can play a crucial role in their identification. It is time-consuming to isolate DNA from sternum bones hence the costal cartilage attached to the sternum containing abundant cells and can be used for DNA profiling in a short time (<24hours). Formaldehyde as a preservative should never be used. It is suggested that the costal cartilage can be packed in aplastic jar preferably without preservatives. Alternatively, they can be wrapped in paper or cloth during handling, transportation, and preservation until sample processing. In conclusion, findings from the current study showed that costal cartilage attached with sternum bone is useful evidence of obtaining DNA profiles as compared to other biological evidence types routinely used.

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