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Case Report

Identification of a Severely Decomposed Body by Dental DNA STR Analysis: A Case Report

تحديد جسد متحلل بشدة عن طريق تحليل السمات الوراثية STR للحمض النووي المستخلص من الأسنان: تقرير حالة

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Abstract

Of all human DNA sources, teeth are extremely resistant to environmental factors like cremation, drenching, trauma, mutilation and deterioration. Therefore, they constitute an astonishing source of DNA material. The present case study highlights the robustness of dental DNA in identifying a highly decomposed and extremely fragile skeletonized drowned body with severe cranio-facial fractures.

Identification was achieved by paternity testing and positively establishing the car used in the crime as the murder vehicle by isolating DNA from the bloodstains present in the car.

This study also emphasized the dire need to create a DNA database for Indian populations for conclusion of quick results.

Keywords: *Forensic Sciences, Dental DNA Fingerprinting, Personal Identification, Decomposed Body, STR*

المستخلص

من بين جميع مصادر الحمض النووي البشري، تكون الأسنان شديدة المقاومة للعوامل البيئية مثل حرق الجثث، الجثث المغمورة (التنقيع)، الإصابات، التشويه والتلف. ولذلك، فهي تشكل مصدراً مذهلا للحمض النووي.

وتسلط دراسة الحالة الحالية الضوء على الفعالية الكبيرة للحمض النووي للأسنان في تحديد هوية جثة غريقة شديدة التحلل ذات هيكل عظمي هش مع وجود كسور وجهية قحفية خطيرة أيضاً.

وتم تحديد الهوية عن طريق اختبار الأبوة وتعيين السيارة المستخدمة بشكل صحيح في الجريمة كأداة استخدمت في القتل عن طريق عزل وتحديد الحمض النووي المستخلص من بقع الدم الموجودة في السيارة. وأكدت هذه الدراسة أيضا على الحاجة الماسة لإنشاء قاعدة بيانات للحمض النووي لسكان الهند للحصول على نتائج سريعة.

الكلمات المفتاحية: علوم الأدلة الجنائية. بصمة الحمض النووي من الأسنان. تحديد الهوية الشخصية؛ جسم متحلل، *STR*.

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1. Introduction

In India, DNA fingerprinting gained legal validity after four years of its invention, in a benchmark case of paternity solved by Dr. Lalji Singh, father of DNA fingerprinting in India [1]. Other famous Indian cases where identity was established through DNA fingerprinting are the infamous Tandoor murder case (1995), the high-profile assassinations of former Prime Minister Rajiv Gandhi and former Punjab Chief Minister Beant Singh, the Priyadarshini Mattoo case (New Delhi), and the Black Buck killing case [2]. In 2015, DNA fingerprinting was applied in the murder case of Sheena Bora, for the identification of her partially burnt and skeletonized remains after 3 years [3].

Tooth enamel is the hardest substance in the human body, making teeth resistant to adverse conditions such as humidity, heat, high temperature and microbial action. This allows the protected dental DNA to help establish the identity of a deceased person. Dental DNA can be obtained from pulp, dentine, cementum and periodontal ligament fibres. Dental pulp is an excellent protected source of both mitochondrial and nuclear DNA. The result of DNA investigation of teeth is reliant on the amount of DNA extracted, the level of contamination and the proficiency of personnel conducting DNA extraction and examination.

Studies comparing DNA content among four types of human teeth have shown that multi-rooted teeth provide the best source of DNA, due to the presence of more pulp cells in the root [4,5]. The total production of genomic DNA obtained from a dental sample ranged from 6 to 50 µg DNA [6]. Gaytmenn and Sweet [7] demonstrated that even in teeth with pulp present, the DNA yield from the crown is still ten times less than that retrieved from the roots.

Dental diseases also have a negative impact on the DNA content of teeth, which not only reduces the available amount of DNA but also increases the potential for contamination [8]. It has been shown that pulp volume and cellularity decrease over time and pulp becomes more fibrous [9].

2. Case Report

A missing person report was filed with Delhi Police, India. The complaint was registered by the brother of a 40-year-old man, who was last seen in a car with two other men, neither of whom could be traced. After one and a half months, Haryana police found an unidentified, extremely fragile, disfigured male body in the later stage of decomposition. Apparent cause of death seemed to be drowning, as the body was found in a well. But the autopsy report confirmed the cause of death as cranio-facial injuries coupled with throttling. The victim died immediately because of the fatal injuries, and the body was later thrown in a well to get rid of it. The autopsy surgeon preserved one unerupted molar and two premolar teeth for DNA examination. A putative identification of the decomposed and partially skeletonized body as the aforementioned missing man was established by the family members on the basis of the clothes and items recovered from the deceased. However, it became extremely important to establish a positive identification using the latest reliable DNA fingerprinting technique.

During the course of investigation, the car in which the victim was last seen was also found after two months. The

car had been sold twice in a state in North East India (Nagaland) and was being used as a tourist car after thorough washing and cleaning. We were called to examine and collect any possible piece of evidence that could link the car to the homicide. At first sight, it looked new as the seat covers had been changed. The accused confessed that he kept the body in the back seat. At last, the boot of the car was examined. After removing the tools and the spare wheel, a false bottom was discovered in the boot. On lifting it, a car cover and a car mat were recovered (Figure-1). A preliminary test for detection of blood was positive for the stains on the mat and the car cover. Finally, both the exhibits were collected, labelled, sealed and then submitted to the FSL for further analysis to ascertain if the vehicle was indeed involved in this homicide case.

3. DNA analysis

All the exhibits i.e. teeth (2 erupted premolars and 1 unerupted molar) of the deceased, blood stained objects obtained from the car and blood samples of the suspected offspring of the deceased were subjected to DNA analysis.

Tooth DNA extraction initially involved two main steps,

Figure 1- *The interiors of the car used for the commission of the crime (a) the front seat, (b) the rear seat, (c) the black mat recovered from the boot, (d) the car cover recovered from the boot.*

pulverization in liquid nitrogen and incubation with ethylenediaminetetraacetic acid (EDTA). DNA from all samples (including teeth) was isolated using Phenol-Chloroform extraction method. Real-time PCR using Quantifiler® Duo (Thermo Fisher Scientific, Waltham, MA, USA) kit was used for quantification of the isolated DNA. The samples were diluted according to the respective Short Tandem Repeat (STR) amplification kit used, while samples that quantified less than recommended concentration were amplified using the maximum volume of DNA extract.

DNA amplification was performed using AmpFℓSTRIdentifiler Plus PCR Amplification kit.

The DNA Profile for the exhibit was prepared by using AmpFℓSTRIdentifiler. STR analysis was used for the sample. One µL of each PCR product was denatured in 10 µl of loading buffer composed of 9.8 µl HI-DI™ formamide (Applied Biosystems, Warrington, UK) and 0.2 µl LIZ-™-500 (Applied Biosystems) size standard mixture. DNA was separated by capillary electrophoresis using 3500 Genetic Analyzer 8-Capillary Array, 50 cm. Data analysis was undertaken using GeneMapper™ ID-X (Applied Biosystems) software v 1.2. The peak detection threshold was set at 50 RFU. In-house control along with the kit control was also used.

No DNA profile could be obtained from the erupted premolars due to degradation of the DNA but some was generated by the unerupted molar tooth. The genetic profiles obtained from all the other samples showed good quality and balanced peak height ratio (PHR). STR analysis performed on the exhibits was sufficient to conclude that the DNA profile generated from the car cover and the black mat was similar to that generated from the unerupted molar tooth of the deceased (Table-1) which linked the car to the homicide.

Figure-2 shows the electropherograms of the tooth and the car mat. It was also established that the deceased was

Table 1- *Genotyping data of tooth of deceased, car cover and black mat.*

Loci	Tooth of Deceased		Car Cover		Car Mat	
D8S1179	10	14	10	14	10	14
D21S11	31.2	32.2	31.2	32.2	31.2	32.2
D7S820	10	11	10	11	10	11
CSF1PO	11	11	11	11	11	11
D3S1358	14	16	14	16	14	16
THO1	8	9	8	9	8	9
D13S317	10	11	10	11	10	11
D16S539	11	12	11	12	11	12
D2S1338	17	23	17	23	17	23
D19S433	13	15	13	15	13	15
vWA	14	17	14	17	14	17
TPOX	10	11	10	11	10	11
D18S51	14	16	14	16	14	16
D5S818	12	13	12	13	12	13
FGA	23	25	23	25	23	25
AMELOGENIN	X	Y	X	Y	X	Y

the biological father of the two children (whose samples were deposited at the FSL) as both shared one set of the alleles with him (Table-2). Figure-3 shows the electropherograms of the deceased's tooth, daughter and son.

4. Discussion

DNA of a criminal/victim present at the crime scene provides clinching evidence of his participation in a crime. The present study highlights the power of dental DNA in identifying a decomposed body via paternity testing and positively establishing the car used in the crime as the murder vehicle by isolating DNA from the blood stains present in the car. In an outdoor crime scene, the possibility of finding DNA of people other than the involved persons is very high but indoor crime scenes can also be deliberately contaminated or altered by culprits. This is apparent in the present case, where the car (indoor crime scene) was intentionally sold twice after thorough washing and used as tourist vehicle. In such cases, DNA fingerprinting as physical evidence is often the last resort. Hence, it will not be incorrect to state that DNA evidence, in addition to identifying the deceased, also helped in establishing the scene of the crime.

In India, crime is increasing at an alarming rate, particularly rape and sexual assault cases. The conviction rate has dropped from 49% to as low as 29% in the last three years (between 2012 and 2015) in the capital alone, and over 1,37,458 rape cases still stand pending for trial across India [10]. The deficiency of scientific methods, forensic laboratories, experienced forensic personnel and the absence of a DNA databank is hampering justice. The need for DNA casework expansion in India is now more and more critical and imperative to convict criminals in such

Table 2- *Genotype data of tooth of deceased, alleged daughter and alleged son*

Loci	Tooth of deceased		Daughter		Son	
D8S1179	10	14	10	12	13	14
D21S11	31.2	32.2	31.2	32.2	31.2	32.2
D7S820	10	11	8	11	10	12
CSF1PO	11	11	11	11	11	11
D3S1358	14	16	14	15	14	15
THO1	8	9	9	9.3	9	9.3
D13S317	10	11	9	10	9	11
D16S539	11	12	11	11	8	11
D2S1338	17	23	23	25	17	20
D19S433	13	15	14	15	13	15
vWA	14	17	14	18	14	17
TPOX	10	11	8	11	8	10
D18S51	14	16	13	14	13	16
D5S818	12	13	12	13	12	13
FGA	23	25	21	25	21	25
AMELOGENIN	X	Y	X	X	X	Y

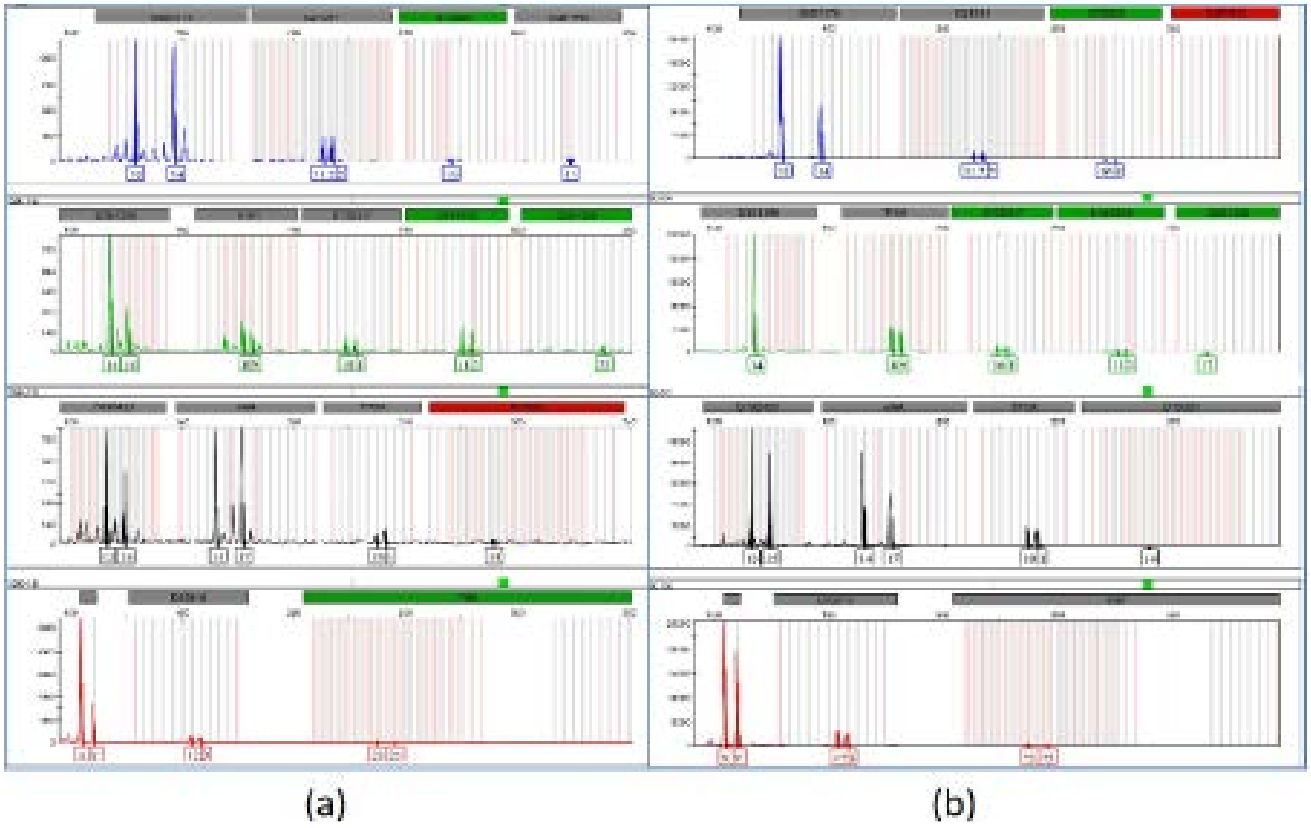

Figure 2- *DNA profile (electropherograms) obtained by (a) unerupted molar of deceased; (b) Car carpet (black mat).*

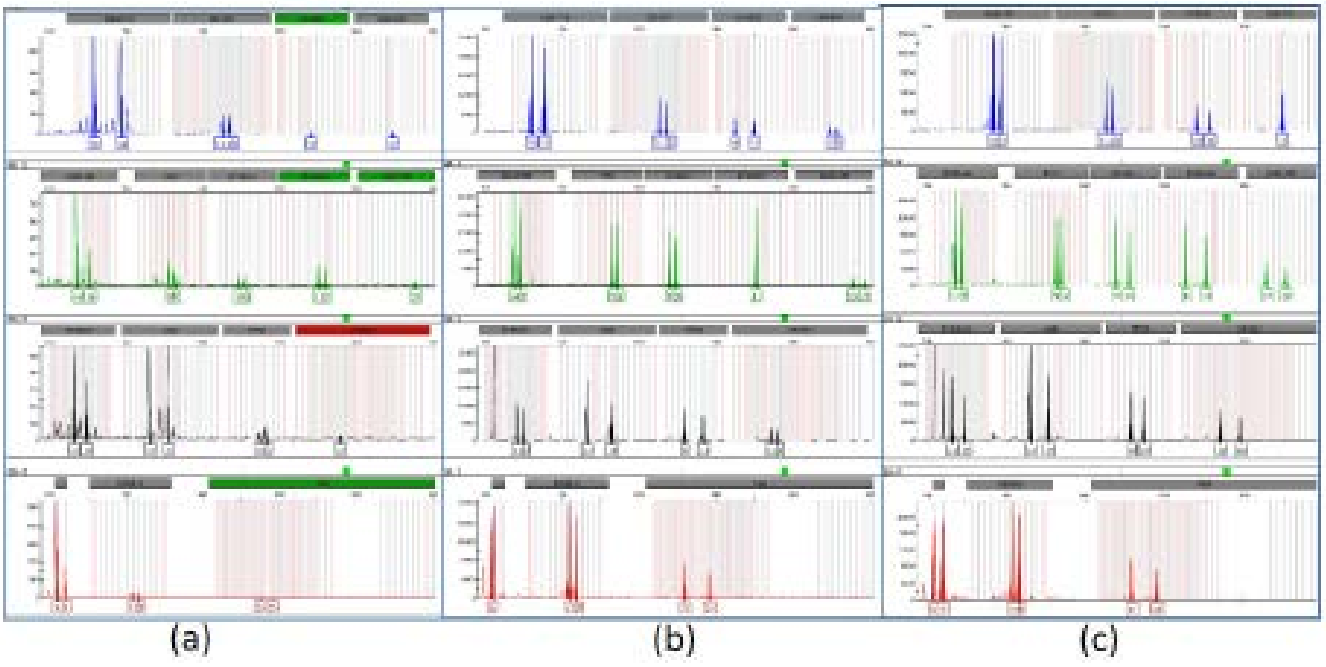

Figure 3- *DNA profile obtained by (a) unerupted molar of deceased (b) Daughter of the deceased (c) Son of the deceased.*

heinous crimes.

Recently, a new human DNA Profiling draft bill for the use and regulation of DNA based technology has been presented by the Law Commission of India for the construction of a DNA Databank [11]. This databank will have six categories including crime scene, suspects, offenders, missing persons, unknown deceased persons along with a volunteers' index [12]. Such a DNA databank will definitely lead to apprehending repeat offenders and exonerating innocent suspects. This will also aid in increasing the conviction rate, which is alarmingly low at present in India [13]. Furthermore, we suggest that the collection of teeth, post-mortem dental records, photographs, identification marks and a list of personal items of the deceased should be a routine process in cases involving unknown dead bodies.

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