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Case Report

DNA Tests Offer Clinching Proof in Awarding Capital Punishment for Rape and Murder of a 7-year-Old Girl

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Abstract

In the present case study, here we report on a rape and murder of 7-year-old girl. We received partially burnt bone for human identification and biological traces of semen on the undergarment of a victim. Total DNA was extracted from bone via organic extraction/automate express and seminal stain using a differential extraction procedure. DNA was quantified through real-time PCR. DNA typing was performed using AmpFISTR® Identifiler® Plus Kit of 15 autosomal STRs loci and Amelogenin locus. 17 Y-STRs loci were analyzed by AmpFISTR® Yfiler™ Kit. Initially identification of deceased girl was confirmed by comparing the STR pattern of the deceased with that of admitted parents. Autosomal and Y-STR DNA profiles obtained from the semen stains of undergarments exactly matched with the suspect of this heinous crime. This case study report once again reiterates the significance of combined autosomal/Y STR analysis in sexual assault cases.

Keywords: Amelogenin; DNA typing; STR pattern; Y-STR

Introduction

Sexual assault is a dark crime and especially a child rape is the most brutal and horrendous offence in nature. High rates of incidence of rape is a big threat to Society. Since the 2012 Delhi rape incident, the laws were strengthened and applied strictly in India, which paved way for speedy trials and stringent punishment in society. The biological snippets of trace material are pivotal in rape cases. Perpetrator or suspect can be fixed based on the biological traces of evidence left at the Scene of The Crime (SOC). This biological evidence is a prime clue and principally essential for the reconstruction of SOC [1]. STR typing has emerged as a vital factor in criminal casework of sexual offences and rape. A Short Tandem Repeat (STR) is a microsatellite, which is highly polymorphic, discriminative and chiefly applied in DNA typing. STR markers are very instructive and practically easy for application [2]. Y-STR typing has been commonly used in forensic science for the identification of male material [3]. This technology of Y STR is useful to detect the male fraction in male-female mixed biological samples. The application of Y-STR has been acknowledged by the International Society of Forensic Genetics [4]. The combination of autosomal and gender-specific Y-STR markers is most widely applied in DNA profiling and is genuinely favorable.

Case Background

A 23 year old man was charged with rape and murder of a seven-year-old girl and booked under sections 363 (kidnapping), 366 (kidnapping, abduction), 354-b (assault or use of criminal force on woman with intent to disrobe), 302 (murder), and 201 (causing disappearance of evidence) of the IPC and section 8 read with 7, 6, 5(m) of POCSO (punishment for aggravated sexual assault). Partially burnt bone and piece of the stained garment were collected from the SOC.

Methods

We received partially burnt bone and teeth for human identification and a portion of victim's undergarment with seminal stain for DNA analysis. DNA was extracted from the bone using Prep filer Express BTA via automate express and differential extraction method for seminal stain. DNA was quantified using the Applied Biosystems 7500 Real-Time PCR System. One ng DNA was amplified with AmpFlSTR® Identifiler® Plus Kit on a Gene Amp PCR System 9700 thermal cycler. Y-STR analysis was performed using AmpFISTR® Yfiler™ Kit. Amplified products were subjected to capillary electrophoresis in POP4 (Performance Optimized Polymer) (Applied Biosystems) using an ABI PRISM® 3130xL Genetic Analyzer (Applied Biosystems) following the manufacturer's protocols. Genotyping was determined using GeneMapperID-software version 1.4 using 100RFU as the analysis threshold. Throughout the procedure, we strictly followed the chain of custody.

Automate express extraction method

Bone/teeth was ground by tissue lyzer. To a tube containing powdered bone/tooth (50mg) were mixed in 250µl of BTA lysis buffer, 3µl of 1M DTT and 7µl of proteinase K were added, vortexed and placed in thermomixer for 2h at 56 °C with shaking speed of 1110 rpm. After incubation, tubes were centrifuged at 10,000 rpm for 10 min at room temperature. The upper aqueous phase was then transferred to a separate prep filer sample tube and the sample volume was made up to 200µl with BTA lysis buffer. Then purification of DNA was done by automate express with final a volume of 100µl. The elute was stored at 4 °C for further use.

Differential organic extraction method

A portion of material from undergarment was finely cut and subjected to Differential Organic Extraction Method. To separate male and female fraction, the seminal cut portions were mixed in 400µl of Tris/EDTA/ NaCl (TNE) buffer, 25µl of 20% Sarcosyl, 75µl MilliQ, 5µl Proteinase K, vortexed and processed on thermomixer at 56 °C for 2 h. After the process, tubes were vortexed, centrifuged and the supernatant was transferred to a new fresh vial and centrifuged at 10,000 rpm for 5 min. The supernatant containing the female fraction was collected in a fresh tube and stored at 4 °C. The pellet containing the male fraction was resuspended in 500µl of TNE buffer, vortexed, centrifuged at 10,000 rpm for 5 min, discarded the supernatant and this step was repeated for 4 times. Then added 150µl TNE solution, 50µl 20% Sarcosyl, 40µl DTT (0.39M), 150µl MilliQ, 10µl Proteinase K to the tube containing male fraction and processed at 37 °C for 2h. Then, both labelled male and female fractions were extracted separately with 400µl of buffered phenol and centrifuged at 10,000 rpm for 10 min. The upper aqueous phase then transferred to a fresh tube and mixed with 400µl of Phenol: Chloroform: Isoamyl alcohol (25:24:1), centrifuged at 10,000 rpm for 10 min. This step was repeated. Then, aqueous phase was transferred to a sample reservoir of amicon placed over a filtrate vial, centrifuged at 14,000 rpm for 3 min. After filtration 30µl Tris EDTA (TE) buffer (pH 8.0) was added and centrifuged at 10,000 rpm for 2 min, the sample was incubated at 56°C for 2h and final elute was stored at 4 °C for further use.

Results and Discussion

The human identification of the deceased girl was achieved through the analysis of a burnt bone and teeth. Simple internal modifications to extraction procedure followed by purification using the Prep Filer® BTA Forensic DNA Extraction Kit, jointly with highly sensitive amplification kits allowed us to obtain typeable STR profile from challenging biological exhibits of partially burnt bone and teeth of the deceased girl. Thus vindicates the reliability of novel approaches [5]. Comparison of the STR pattern of the deceased with that of admitted parents was done with AmpFISTR® Identifiler® Plus Kit. The autosomal STR DNA profile of the diseased girl matched with the admitted parents (father and mother) (Table 1) and thereby the identity of the victim was established.

Table 1: The alleles scored at 15 STR loci and amelogenin with AmpFlSTR Identifier kit in the diseased girl and the admitted mother and father. At each locus, the child received one STR allele from her mother and the other from the father.

STR Loci	Biological Sample Collected from SOC				Comparison of Deceased and Admitted Parents					
	Burnt Bone of Deceased Girl		Teeth of Deceased Girl		Admitted Father		Bone/Teeth		Biological Mother	
D8S1179	14	15	14	15	13	15	14	15	14	16
D21S11	30	31.2	30	31	30	31.2	30	31.2	31.2	31
D7S820	8	8	8	8	7	8	8	8	8	13
CSF1PO	10	12	10	12	10	11	10	12	10	12
D3S1358	15	15	15	15	15	15	15	15	15	15
THO1	7	9	7	9	7	9	7	9	8	9
D13S317	11	11	11	11	11	11	11	11	9	11
D16S539	9	12	9	12	9	12	9	12	9	12
D2S1338	23	25	23	25	22	25	23	25	19	23
D19S433	12	15.2	12	15	14	15.2	12	15.2	12	13

vWA	16	16	16	16	16	20	16	16	14	16
TPOX	9	11	9	11	9	11	9	11	11	11
D18S51	14	19	14	19	11	19	14	19	12	14
D5S818	12	12	12	12	12	12	12	12	11	12
FGA	23	25	23	25	22	25	23	25	23	24
AMELOGENIN	X	X	X	X	X	Y	X	X	X	X

Autosomal 15 STR markers were used for the typing of seminal stains. The analysis of the seminal stain on undergarment revealed a genotype composed of mixed profile of the alleles of the deceased girl and the suspect. STR typing with AmpFISTR® Identifiler® Plus Kit for 15 STR loci revealed the presence of male material in the seminal exhibit. A peak corresponding to the Y-chromosome was detected at the Amelogenin locus (Table 2). Further typing of the Y-STR confirmed the profile belonging only to the male (Table 3). The confirmation of male-specific material of Y STR emerges as prime factor in solving sexual assault cases [6,7]. The forensic exhibits presented for the analysis included the victim's bone and teeth. Seminal stain of the assailant was found on a piece of cloth from the crime scene. The STR typing results guided the clear reconstruction of the scene of crime. The analysis of the exhibit found on the undergarment revealed the presence of a male profile, which precisely matched with the profile of the suspect.

Table 2: The alleles scored at the 15 STR loci in the seminal stain from the undergarment of the deceased girl and the blood sample of the accused.

STR Loci	Seminal Stain from the Undergarment of Victim		Blood Sample of the Accused	
D8S1179	14	15	14	15
D21S11	30	31.2	30	31.2
D7S820	10	11	10	11
CSF1PO	12	13	12	13
D3S1358	16	17	16	17
THO1	9	9	9	9
D13S317	9	12	9	12
D16S539	11	11	11	11
D2S1338	17	18	17	18
D19S433	13	13	13	13
vWA	14	19	14	19
TPOX	9	11	9	11
D18S51	15	17	15	17
D5S818	13	13	13	13
FGA	23	24	23	24
AMELOGENIN	X	Y	X	Y

Table 3: The alleles scored at 17 STR loci by Amp FISTR® Yfiler™ Kit in the undergarment of the diseased girl and the blood sample of the accused.

Y-STR Loci	Profile from the Seminal Stain SOC		Reference Sample of the Accused	
DYS455	15	15	15	15
DYS389I	13	13	13	13
DYS390	25	25	25	25
DYS389II	31	31	31	31
DYS458	15	15	15	15
DYS19	15	15	15	15
DYS385	11	14	11	14
DYS393	13	13	13	13
DYS391	11	11	11	11
DYS439	10	10	10	10
DYS635	23	23	23	23

DYS392	11	11	11	11
GATA- H4	12	12	12	12
DYS437	14	14	14	14
DYS438	11	11	11	11
DYS448	20	20	20	20

Conclusion

In conclusion, our present case report confirmed that combined autosomal/Y chromosomal STR analysis could be very much helpful in analysis of DNA exhibits from rape cases. The DNA based evidence in this case helped the judiciary in awarding capital punishment to the accused for the rape and murder of the seven-year-old girl as the court considered it as 'rarest of rare cases'.

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