

Research Article

DNA Profile of Physical Evidence Help to Establish Hidden Secret

ShashiBala Pahuja^{1*}, Shahjad Ali¹, Anshu Gupta¹ and Md Mustafa²

¹Forensic Science Laboratory, Govt. of NCT of Delhi, India

²Prince Sultan Military Medical City, KSA

*Corresponding author: Shashi Bala Pahuja, Division of Biology, Forensic Science Laboratory, Govt. of NCT of Delhi, Sector-14, Rohini, Delhi, India

Received: July 17, 2018; Accepted: August 17, 2018;

Published: August 24, 2018

Abstract

Introduction: In the forensic science short tandem repeat (STR) analysis helps to resolve the hidden secret of the forensic system work to collect, preserve and understand evidence that can help to prove crime. We reported an alleged case of sexual assault on a three years old girl child who stated she was sexually assaulted by his neighbor.

Material and Method: The genomic DNA was extracted from blood and semen by using organic extraction and differential organic extraction method respectively. DNA was quantified by Nano-drop at 260/280 nm. Real-time PCR was used for expression analysis. Genotyping was done by using AmpFISTR® Identifier® Plus Kit by multiplex PCR reaction for 15 Y-STRs loci. AmpFISTR® Yfiler™ Kit was also used for multiplex PCR of 17 Y-STRs loci.

Results: STR analysis proved that more than one person was involved in committing the crime.

DNA profile of exhibit 'A' (blood sample of victim) matched with the exhibit 'B' (blood on the undergarment of alleged). The DNA profile of exhibits 'C' (micro slide & cotton wool swab of victim) & exhibits 'E' (blood on the gauze of alleged) was matching with exhibit 'D' (bed sheet) on which crime was committed. The mixed DNA profile generated from the bed sheet blood and semen revealed the presence of unknown male alleles along with alleged person alleles confirming the unknown male involvement.

Keywords: Child sexual assault; Exhibits; Organic extraction; DNA profiling; Mixed DNA profile

Introduction

Child sexual abuse is a major problem in India, most of the time sexual abuse offenders are acquainted with the victim, approximately 30% are relatives of the child, 60% may be family friends, baby sitters or neighbors, only 10% of child abuse cases where strangers are involved [1]. The ever increasing rape cases in Delhi particularly the child sexual abuse has no respite even stringent laws like POCSO Act 2012 [2]. The main objective of POSCO was to address effectively the sexual abuse and sexual exploitation of children and also to define different forms of sexual abuse. It includes speedy trial and increased penalties for the offenders. In spite of all the efforts, sexual assault cases are still increasing day by day. In various criminal & civil cases the DNA profiling has become inevitable tool to ascertain the individuality of victim & suspects [3].

Forensic science experts analyze DNA profile which is unique profile of an individual except twins [4]. Hence, DNA profile is used as a valuable evidence for the offence. It will also determine the identity of a person from the body fluids such as blood, saliva, semen, sweat, tissues, teeth, skin, hair roots, bones etc. DNA based identification from biological exhibits in forensic science is considered as most important evidence for legal proof in court of law. The STR DNA profiling is an advanced tool in human identification purposes [5].

It is one of the most valid and useful methods to compare specific

loci on DNA from two or more samples. STR is present in the microsatellite region of the nuclear DNA, consisting of a unit of 2-13 nucleotides repeated hundreds of times in a row which is specific for each individual. STR analysis measures the exact number of repeating units in an individual [6]. The polymorphic nature of the STR regions discriminate between one DNA profiles to another make it valuable for forensic investigation [7]. AmpFISTR Identifier Plus® kit, a globally recognized multiplex PCR kit for highly challenged biological samples [8]. Our genome contains thousands upon thousands of STR markers of which only small core set of loci have been selected for its use in forensic science for the evidence in court of law [9].

Brief sketch of the case

We present here the detailed scientific analysis of the trace evidence of one case pertaining to child sexual abuse from the molecular examination point of view. As per information recorded by the mother of the girl child to the police officer, it was alleged that one neighbor person sexually assaulted her three year old girl child. The case was registered under section 376(2) (i)/377IPC/ & 4/6 POCSO Act. The mother also stated that after taking her child into confidence, the child was able to narrate the unfortunate incidence and told that the neighbor person had done the heinous act with her. After listening the victim's mother saw that blood was present on the clothes of victim. The victim also pointed out the place of occurrence where this offence takes place. The investigating officer collected the

Table 1: Autosomal STR DNA profile and comparative chart of allele distribution of 16 different loci of the tested DNA in the different samples.

S.No	Loci Name	Micro slide of victim		Cotton wool swab of victim		Blood sample of victim		Bedsheet samples	Blood gauze of accused		Blood on accused under wear	
1	D8S1179	12	13	12	13	10	13	12,13,15	13	15	10	13
2	D21S11	28	29	28	29	28	29	28,29,31.2	28	31.2	28	29
3	D7S820	12	12	12	12	8	12	8,12	8	12	8	12
4	CSF1PO	10	12	10	12	11	12	10,11,12,13	11	13	11	12
5	D3S1358	17	18	17	18	15	18	15,17,18	15	18	15	18
6	THO1	9	9.3	9	9.3	9	9.3	8,9,9.3	8	9	9	9.3
7	D13S317	8	9	8	9	8	11	8,9,10	9	10	8	11
8	D16S539	11	11	11	11	10	12	11,11	11	11	10	12
9	D2S1338	18	19	18	19	18	24	18,19,24	24	24	18	24
10	D19S433	14	15	14	15	14	15	13,14,15	13	15	14	15
11	VWA	14	19	14	19	15	17	14,18,19	14	18	15	17
12	TPOX	8	9	8	9	9	11	8,9,11	11	11	9	11
13	D18S51	19	19	19	19	15	21	11,17,19	11	17	15	21
14	D5S818	9	12	9	12	11	11	9,11,12,14	11	14	11	11
15	FGA	24	24	24	24	20	21	23,24,25	23	25	20	21
16	AMELOGENIN	X	Y	X	Y	X	X	X Y	X	Y	X	X

Table 2: Showing allele number and loci name for allelic characterization.

Loci name	Allele number	Loci name	Alleles number	Loci name	Alleles number	Loci name	Alleles number
D8S1179	12	D3S1358	8	D19S433	15	D5S818	10
D21S11	24	THO1	10	VWA	14	FGA	28
D7S820	10	D13S317	8	TPOX	8	Amelogenin	2
CSF1PO	10	D16S539	9	D18S51	23		
		D2S1338	14				

material evidence from the scene of crime as well as exhibits after medical examination of the victim. The Exhibits were forwarded to the Biology Division of Forensic Science Laboratory (FSL) Delhi, Govt. of NCT of Delhi, India. It is requested to look for unidentified traces, bed sheet (form the spot), oral swabs, vaginal swabs, cloths, undergarments, in between fingers, body fluids, nail scrapings, breast swab, cervical mucus collection, rectal swab, oral swab, blood & urine samples of the victim.

Material and Method

Experimental set up

Description of exhibits and samples: Biological samples from victim, accused & scene of crime were forwarded by the investigating officer & deposited in FSL Delhi for Forensic DNA analysis. The samples were stored at 4°C in a refrigerator located in a secured area till the study completed. The exhibits 'A' (blood sample of victim), exhibit 'B' (blood on the undergarment of alleged), exhibit 'C' (micro slide & cotton wool swab of victim), exhibit 'D' (blood and semen on bed sheet) as shown in Figure 1. All the exhibits were subjected to DNA examination.

Chemicals and reagents: The solvents like benzidine, H₂O₂ and acid phosphatase were used for detection of biological fluid as a semen or blood. Forensic buffer, 20% Sodium dodecyl sulfate (SDS), phosphate kinase, phenol, chloroform isomyl, propanol, 3M sodium

acetate, 70% alcohol, 100% alcohol and TE buffer were used for DNA isolation.

Genomic DNA extraction: All exhibits on which blood and semen were detected were subjected to DNA isolation. First, the exhibits were cut in small pieces, soaked in lysis buffer and kept at 56°C for 12 hours. The genomic DNA from blood and blood stains was isolated by using organic phenol-chloroform extraction method. Differential isolation protocol was used for DNA isolation from seminal stains. Isolated DNA was recovered in 30 µl TE buffer and preserved at 4°C for further analysis. The quality of isolated DNA was checked on 1% agarose and quantitative estimation was done using Nanodrop-2000 at 260/280 nm (Thermo scientific, USA). Real Time PCR (RT-qPCR) was performed for DNA quantification as per the recommended protocol. Fluorescent dyes such as blue, green, yellow & red were used in DNA profiles analysis that help in distinguished between different locus (Table 1). There are fifteen (N = 15) autosomal markers (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, FGA) & one sex marker (AMELOGENIN) were analyzed (Table 2) (Figure 2). Capillary electrophoresis of the amplified products was carried out on ABI 3500 XL genetic analyzer (Applied Bio-system, USA). Data was analyzed by using Gene Mapper[®] ID-X 1.4 (Applied Bio-system, USA). STR analysis has been done for comparing specific loci on DNA from two or more samples. In-house control along with the kit



Figure 1: Shows the underwear and blood gauze of known accused claimed by the victims.

control was also used as reference.

Challenges in DNA extraction: The biological samples are source of many PCR inhibitors and considered as leading obstacle in DNA profile generation and analysis. Many PCR inhibitors such as salts and detergent concentration or proteases decrease the DNA polymerase activity. Some other inhibitors like heparin, hemoglobin, proteinase K, phenol, EDTA, excess of magnesium ion can also inhibit PCR amplification. Some chemical such as urea and phenol are known to degrade DNA polymerases thus create nuisance in the PCR reaction. Ions like calcium and other factor such as haematin from blood and dye released from fabrics etc. may inhibit polymerase activity. High concentrations of calcium competes with magnesium by a competitive binding hence leads to competitive inhibition as magnesium is no longer available as a cofactor for the polymerase activity.

Results & Observation

In the present case study successful DNA profile was generated from the blood and semen samples from the exhibits A, exhibit B, exhibit C and exhibit D. STR is used as high relevance molecular markers in the genetic analysis and its interpretation in identifying the actual culprit. In the recent years, forensic science takes advantage of the population's variability in STR lengths in discriminating one DNA sample from another. Boggle mind finding in the case was that the autosomal male DNA profile generated from the vaginal swab stains didn't match with the DNA profile of the known accused. The mix DNA profile was generated on the bed sheet from the scene of crime revealing the unknown person involvement in this crime which was not mentioned in the victims complains. The blood on the underwear of known accused matched with the blood sample of victim (Table 1). As mentioned in the victim complains but the missing information about the involvement of second male in the FIR, this finding startled us. The involvement of second unknown male in this particular case was proved as two different male DNA profile were generated from the exhibits (bed sheet). The unknown male DNA profile of vaginal swab and DNA profile of known accused both are accounted in the alleles of mixed DNA profile generated on the bed sheet from scene of crime. The AmpF ℓ STR Y-STR system for Y-STR profile also confirmed the presence of seminal stains at scene of crime from two different male origins. The Yfiler data supported the identifier data Y-DNA profile of the vaginal swab stains of victim matched with the Y-DNA profile of DNA extracted from bed sheet on which the crime was committed. The Y-DNA profile of known accused also match

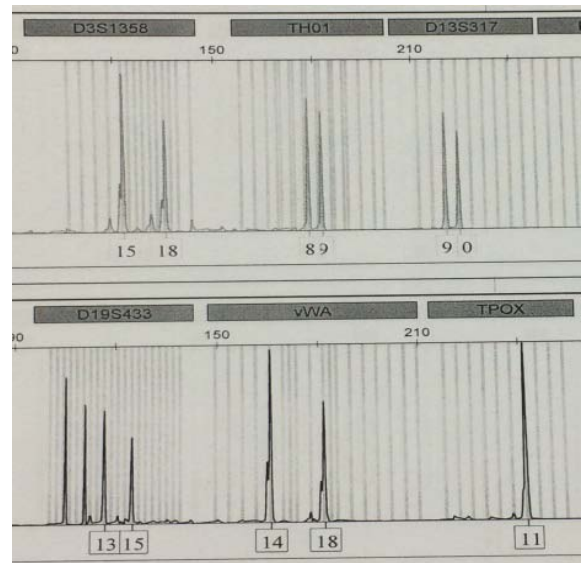


Figure 2: DNA profile of the exhibits.

with the Y-DNA profile seminal stain on the bed sheet. Recently, a similar case study was reported from the State Forensic Science Laboratory Ranchi, Jharkhand, India. They tested DNA by highly sensitive multiplex PCR and quantification by RT-qPCR. They also used the similar genotyping kiti. eAmpFISTR[®] Identifier[®] Plus Kit for both the 15 autosomal STRs loci and a gender locus. The honorable court of the state has recognized the autosomal as well as Y-STR DNA profile as scientific evidence [10].

Conclusion

The above observation of case proved that DNA profiling is a tool that is not only used to apprehend the guilty but also give lead to investing officer for the further investigation in the case. The conventional method in criminal justice system can be tempered with witnesses may turn hostile, but DNA evidence tells the truth. With passage of time if DNA sample properly preserved does not affect and neither does it change on repetition. DNA evidence thus unravels the truth and never lies.

Acknowledgement

Authors are thankful to Forensic Science Laboratory, Govt. of NCT of Delhi, India for their continuous support and scientific facility facilities to conduct the work.

References

1. Child sexual abuse in India.
2. The Protection of children from sexual offences Act, 2012.
3. Magalhães T, Dinis-Oliveira RJ, Silva B, Corte-Real F, Nuno Vieira D. Biological evidence management for DNA analysis in cases of sexual assault. *Scientific World J.* 2015.
4. DNA technology in forensic science. National research council committee. 1992.
5. Bozzo WR, Colussi AG, Ortiz MI, Lojo MM. DNA recovery from different evidences in 300 cases of sexual assault. *Forensic Sci Int.* 2009; 2: 141-142.
6. Vieira ML, Santini L, Diniz AL, Munhoz Cde F. Microsatellite markers: what they mean and why they are so useful. *Genet Mol Biol.* 2016; 39: 312-328.

7. National Commission on the Future of DNA Evidence (July 2002). "Using DNA to Solve Cold Cases". U.S. Department of Justice. Retrieved 2006.
8. Wang DY, Chang CW, Lagace RE, Calandro LM, Hennessy LK. Developmental validation of the AmpFISTR® Identifiler® Plus PCR Amplification Kit: an established multiplex assay with improved performance. J Forensic Sci. 2012; 57: 453-465.
9. Butler JM. Genetics and genomics of core STR loci used in human identity testing. J. Forensic Sci. 2006; 51:253-265.
10. Sumanta, Yadav VK, Bara N, Sinha HK, Singh RS. DNA Profiling of a Rape Case at the State Forensic Science Laboratory Ranchi Jharkhand India. Austin J Forensic Sci Criminol. 2017; 4: 1-4.