Original Article

DNA profiling technique proves the sexual assault convicting the offence of gang rape – A case study

Vaishali B Mahajan[1*], Amulya A Pande[2], Ajit Kokare[3], Kewalram R Gorle[4], Manoj R Bhandarkar[5], Laxmikant B Rewatkar[6]

[1]Assistant Chemical Analyser, Regional Forensic Science Laboratory, Government of Maharashtra, Home Department, Dindori Road, Nashik - 440002, Maharashtra, INDIA.
[2,3]Assistant Chemical Analyser, Regional Forensic Science Laboratory, Government of Maharashtra, Home Department, Irwin Chowk, Amravati – 444602, Maharashtra, INDIA.
[4,5]Assistant Director, [6]Deputy Director, Regional Forensic Science Laboratory, Government of Maharashtra, Home Department, Dhantoli, Nagpur – 440012, Maharashtra, INDIA.
Email: vaishalimahajan.nagpur@gmail.com

Abstract

DNA profiling technique is the tool in forensic science where by biological evidences play important role in providing the source of DNA. DNA is isolated from different sources like Blood, Blood stains, Semen, Tissue, Bones and Hair in different cases like Paternity, Murder, Rape, Missing identity and many more. In this sexual assault case, blood and blood mixed semen stains are source of DNA profiling. A girl aged 13 years was alleged to have been raped by two persons in a wheat field in Amravati district of Maharashtra, India. After filing a complaint by victim's father, garments of victim and both accused worn by them at the time of offence were seized by the police and sent to Forensic laboratory for chemical analysis. DNA was isolated from all blood and semen stains found on clothes and STR Profiles were taken as electropherograms. Reference blood samples of victim and both accused were collected, DNA was isolated from them and STR Profiles were taken as electropherograms. Mixed DNA profiles were obtained from blood mixed semen stains found on undergarments of victim and accused. Similarly, DNA profile was obtained from blood stains on shirt of accused. All above DNA profiles were compared with reference DNA profiles of Victim and Accused confirming presence of semen of one of the accused on the undergarment of victim and presence of blood of victim on the undergarment of other accused. Both the accused were convicted in the case and sentenced for 20 years rigorous imprisonment.

Key Words: DNA, DNA profiling, Polymerase Chain Reaction, Short Tandem Repeat.

[*]**Address for Correspondence:**
Vaishali B. Mahajan, Assistant Chemical Analyser, Regional Forensic Science Laboratory, Government of Maharashtra, Home Department, Dindori Road, Nashik - 440002, Maharashtra, INDIA.
Email: vaishalimahajan.nagpur@gmail.com

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INTRODUCTION

DNA profiling is well known powerful tool to identify criminals which can be done by comparing their DNA profile with DNA profiles obtained from biological evidences that are main sources of DNA[1,2]. In initial days RFLP Technique was used for DNA typing which is now replaced by PCR based STR Genotyping Technique. In criminal cases like Murder, Sexual offences and other offences affecting human body, the different biological sources used to isolate DNA are blood, semen, saliva, tissue, vaginal swab, hair or other body fluids that has nucleated cells. DNA from blood is obtained from white blood cells while DNA from semen is obtained from

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sperm cells which are very useful for connecting accused with the crime in the sexual offence cases. In the instant case, a father from a small village of Amravati district lodged a complaint to police station that his daughter aged 13 year had been raped by two persons aged 21 and 22, in wheat field while returning from the school. Both the accused took her in standing wheat crop field. She shouted but nobody came to rescue her. Both accused committed sexual intercourse against her will and threat to kill her if she discloses the incidence to anybody. They confined the girl overnight with them. In the morning around 4.30 am they dropped her near the village where from she walked to the nearby house and the house owner brought her to her home. The case was lodged under section 376(G), 506 I.P.C. and as the victim was under 18 year old, section 6 POCSO Act 2012 was also added to it[3]. During the course of investigation, the investigating officer submitted clothes of victim as well as both accused worn by them at the time of crime to this laboratory. Along with clothes, vaginal swab of victim and reference blood samples of accused were also submitted. During the course of analysis in Regional Forensic Science Laboratory, Nagpur, mixed DNA profiles obtained from blood mixed semen detected on victim's knicker, underwear of accused and blood on shirt of accused connected both accused with the crime and proved to be vital evidence to convict both the accused convicted for the offence of gang rape punishable under 376 (D) of I.P.C. They are sentenced to suffer rigorous imprisonment for 20 years and fine of 5000/- each. In case of non-payment of fine, rigorous imprisonment will be increased by one month.

MATERIALS AND METHODS

AmpFlSTR PCR Reaction Mix; AmpliTaq Gold DNA Polymerase; AmpFlSTR Primer set; Formamide; Size standard; Allelic Ladder; EZ1 Investigator kit;

Steps used in Analysis:-

Detection of biological stains and collection of blood samples
↓
Preservation of samples
↓
Extraction of DNA
↓
Quantification of DNA
↓
Amplification of DNA using PCR
↓
Electrophoresis
↓
Analysis of STR product
↓
Conclusion

All exhibits submitted to laboratory were checked for the presence of biological material like blood, semen. For detection of blood on clothes Kastle-Mayer (Phenolphthalein) test was employed. For detection of semen stains, Acid phosphatase and Florence test were used. Blood mixed semen was confirmed using Seminal Acid Phosphatase (SAP) agarose gel electrophoresis technique where fluorescence of seminal acid phosphatase is observed under UV light.

Isolation of DNA

DNA was isolated from detected blood, semen, blood mixed semen stains and reference blood samples of victim and both accused using EZ1 investigator kit (Qiagen) and EZ1 Advanced Automatic extraction machine. Briefly, the samples were lysed in 400 µl Lysis buffer and 10 µl Proteinase K. In case of semen and blood mixed semen stains, along with Lysis buffer, 10 µl of 40 mM DTT (Dithriothritol) is added to disrupt the sulphur bonds in coating of sperm cell. Then incubated at 56°C for at least 2 hrs. And after that centrifuged at 14000 g for 2 min and loaded onto the EZ1 Advanced Automatic extractor which has cartridges preloaded with reagents.

Quantification of DNA

Quantity of DNA was checked on gel electrophoresis by comparing DNA of sample in question with DNA of known molecular weight. The accurately quantified DNA was used for downstream application.

PCR based STR Analysis

DNA extracted from blood, semen, blood mixed semen stains detected on clothes and reference blood samples of victim and both accused was amplified by using PCR technique[4,5]. By this process STR's are selectively replicated a million fold or more in about 28 cycles. STR markers also called microsatellite markers are polymorphic DNA loci that contain a replicated nucleotide sequence. Repeat unit can be from 2-7 nucleotides in length. A majority of STR's that have evaluated by the forensic community are composed of 4 nucleotide repeat units[6,7,8]. Polymorphic STR Loci are very useful for human identification[9,10]. During PCR, initially samples were incubated at 95°C for 11 mins. There after DNA was amplified in 28 cycles selecting 94°C, 54°C and 72°C as a temperature of denaturing annealing and extension respectively. Amplified DNA samples were then kept at 60°C for an hour and then at 4°C till separation of STRs. PCR produces millions of DNA fragments of different sizes. Separation of different fragments of DNA molecules on the basis of their size was achieved by capillary electrophoresis[11]. The instrument ABI Prism 3130 Genetic Analyser (Applied Biosystems) was used for carrying capillary electrophoresis. Only single stranded DNA fragments are resolved by this instrument. Therefore amplified DNA

samples were denatured by heating sample at 95°C for 3 min and then snap cooling at 0°C (ice cold). To keep the DNA molecules single stranded throughout the process of electrophoresis, before injecting the samples, they were diluted with Formamide. Voltage employed for separation of DNA fragment was 15000 V. Separated DNA fragments were detected by fluorescence detector. The DNA profiles were interpreted by comparison with each other.

RESULTS AND DISCUSSION

The DNA extracted from blood detected on Kurta, Salwar, Vaginal swab of victim and Full shirt of accused (1), semen detected on Underwear of accused (1), blood mixed semen detected on Knicker of victim and Underwear of accused (1), reference blood samples of victim and both accused were typed at 15 STR Loci and gender specific Amelogenin locus using PCR amplification technique (Table 1).

Table 1: Results of analysis are summarized in table below

STR LOCUS	GENOTYPE										
	Ex.1 Kurta (Victim)	Ex.2 Salwar (Victim)	Ex.4 Knicker (Victim)	Ex.7 Full Shirt (Accused 1)	Ex.8 Underwear (Accused 1)		Ex. 11 Underwear (Accused 2)	Ex. 1 Blood (Victim)	Ex.2 Vaginal Swab (Victim)	Ex.1 Blood (Accused 1)	Ex.1 Blood (Accused 2)
					Blood mixed semen	Semen					
D8S1179	13,15	13,15	13,15,17	13,15	10,13,14,15	10,14	15,17	13,15	13,14	10,14	15,17
D21S11	28,30	28,30	28,30,31.2	28,30	28,29,30	29,30	30,31.2	28,30	28,30	29,30	30,31.2
D7S820	9,11	9,11	8,9,11	9,11	9,10,11	9,10	8,8	9,11	9,11	9,10	8,8
CSF1PO	12,12	12,12	10,11,12	12,12	11,12	11,12	10,11	12,12	12,12	11,12	10,11
D3S1358	15,17	15,17	15,17	15,17	15,16,17	15,16	15,15	15,17	15,17	15,16	15,15
THO1	9,9	9,9	6,7,9	9,9	6,7,9	6,7	6,7	9,9	9,9	6,7	6,7
D13S317	10,12	10,12	10,12	10,12	8,10,12	8,12	12,12	10,12	10,12	8,12	12,12
D16S539	8,11	8,11	8,11,12	8,11	8,11	11,11	11,12	8,11	8,11	11,11	11,12
D2S1338	18,25	18,25	18,20,25	18,25	18,24,25	24,25	18,20	18,25	18,25	24,25	18,20
D19S433	12,12	12,12	12,13,14	12,12	12,13	12,13	13,14	12,12	12,12	12,13	13,14
vWA	15,17	15,17	15,16,17	15,17	15,16,17,18	16,18	16,16	15,17	17,17	16,18	16,16
TPOX	8,12	8,12	8,9,11,12	8,12	8,11,12	11,12	9,11	8,12	8,12	11,12	9,11
D18S51	15,17	15,17	15,17	15,17	13,15,17	13,13	15,17	15,17	15,17	13,13	15,17
AMELOGENIN	X,X	X,X	X,Y	X,X	X,Y	X,Y	X,Y	X,X	X,X	X,Y	X,Y
D5S818	11,11	11,11	11,12	11,11	11,12	12,12	11,12	11,11	11,12	12,12	11,12
FGA	23,24	23,24	21,23,24	23,24	19,23,24	19,24	21,24	23,24	23,24	19,24	21,24

DNA profiles obtained from blood detected on Kurta, Salwar, Vaginal swab of victim and shirt of accused (1) were found to be identical and from one and the same source of female origin and matched with DNA profile obtained from reference blood of victim. Accused (1) was connected with the crime as victim's blood got transfer on his shirt during alleged rape (Table 2). Mixed DNA profile obtained from blood mixed semen stains obtained on Underwear of accused (1) matched with DNA profile of victim and accused (1). As victim's blood got transfer to Underwear of accused (1), it again strongly proved his involvement in crime (Table 2). Mixed DNA profile obtained from blood mixed semen stains obtained on Knicker of victim matched with DNA profiles of victim and accused (2). It proved involvement of accused (2) in the alleged crime (Table 2). In this way, the existence of DNA profile of victim's blood on Underwear of accused (1) proved his involvement and existence of DNA profile of semen on accused (2) on victim's Knicker proved his involvement in the crime.

Table 2: Summarized Table of Analysis

Exhibits	Source	Biological evidence detected	DNA profile matched with
Kurta	Victim	Blood	Victim
Salwar	Victim	Blood	Victim
Knicker	Victim	Blood mixed semen	Victim and Accused (2)
Full shirt	Accused 1	Blood	Victim
Underwear	Accused 1	Blood mixed semen and semen stains	Victim and Accused (1)
Underwear	Accused 2	Semen	Accused (2)
Vaginal swab	Victim	Blood	Victim

CONCLUSION

In the instant case, victim was only 13 year old. Child sexual assault is a serious, pervasive social issue. It is one of the most heinous crimes imaginable. In such cases conviction of accused is necessary so as to avoid repetition of crime in the society. DNA STR Profiling is a powerful tool in crime investigation. On the basis of DNA evidence, Honorable court ordered that both accused convicted for the offence of gang rape punishable under sections 376 (D) of I.P.C. and they are sentenced to suffer rigorous imprisonment for 20 years and fine of

5000/- each. In case of non-payment of fine rigorous imprisonment will be increased by one month.

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