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Research Article

IMPORTANCE OF DNA PROFILING AND BOTANICAL EXAMINATION TO FIX THE OFFENCE AND OFFENCE PLACE

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A B S T R A C T

In sexual offences, specifically semen on victim's clothes or medical samples proves involvement of accused in the crime. But in some cases when semen stains are not detected during investigation, the victim's blood or body fluid on the accused's garments as well as grass or earth from the crime scene adhered to clothes helps to prove the crime. If the blood detected is in very less quantity then it is difficult to prove the evidence by routine ABO grouping. Further, discrimination power of ABO Blood group system is less. Here, DNA profiling technique has created wonders from the time it has been invented. In the present case, a girl aged 7, while going to home from school was abducted by accused on his motorcycle and took her to channel and raped her. Later, as she started crying, he beaten her on stomach to make her quiet and dropped her near home. Her mother lodged complaint to police station. During forensic analysis, DNA profile obtained from few blood stains on shirt of accused matched with DNA profile of victim and grass collected from leggings of victim and jeans full pant of accused after grass comparison proved that both the grass samples belong to same species. Thus, both DNA technique and botanical examination of grass proved the involvement of accused in the crime and fixed the offence place.

INTRODUCTION

In India children below the age of 18 years face staggering challenges from the day they are born. In child sexual abuse, a child is usually maltreated physically or psychologically by a person who is in a position of trust and confidence in relation to the child [(1)]. To solve these problems of children, parliament enacted a special legislation POSCO Act in May 2012 [(2)].Forensic laboratories play important role to solve such type of crimes and provide evidence in the court of law.

The DNA technology used in the forensic laboratory was first developed in England in 1985 by Sir Alec Jeffreys[(3)]. Because of its utility in proving the occurrence of sexual contact and identification of the suspects, biological evidence for DNA studies is nowadays considered the most important evidence for legal proof in the courts of law [(4), (5), (6)]. Preserving DNA evidence is a key requirement for law enforcement's investigation and prosecution of sexual assault case. DNA technology used for identification purposes entails the use of Short Tandem Repeat (STR) markers which are characterized by the high level of polymorphism and are abundant in the human genome [(7).] Methods are in place to carry out multiplex genotyping of STR markers using sensitive and highly reliable fluorescent technologies which are widely used in the field of forensics although other genetic markers are also used for specific applications [(8) (9)].

Forensic botany is also one of the useful application in the criminal investigation[(10)(11)(12)]. It is the study of plants and plant material to relate the crime scene and active crime investigations.It is the use of plants or plant parts including as leaves, pollen, seeds, flowers, fruits and wood in the investigation of criminal cases, legal questions and disputes or in noncriminal cases, to ascertain the location of crime. If the plant is identified from these clues by a plant taxonomist, they may link the crime to a specific place[(13)(14)(15)(16)].Thus, investigators would know the value of locating plant material on a suspect or victim, and then establishing the origin from which that plant came.

Our laboratory had received a case in which a girl aged 7, while going to home from school was abducted by accused aged 27, on his motorcycle and took her to channel and raped

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her. Later, he beaten her on stomach as she started crying and dropped near her home. As the girl was frightened and her clothes were stained with blood, her mother got an idea that something had happened. She interrogated her and came to know the incidence. She lodged complaint to police station under section 376 and POCSO Act[17]. Police arrested the suspect on the basis of doubt and submitted clothes of victim, accused and medical samples of both. Reference blood of victim and accused received in DNA kit. During detection, blood was found on her frock, leggings and few blood stains of about 0.5 to 1 cm on Full shirt of accused. No semen was detected either on clothes or on vaginal, vulval swabs and smears. However vaginal swab and vulval swab were stained with blood as she had genital injuries.

For detection of blood, Kastle-Meyer test was performed and for detection of semen, Acid phosphatase and Florence tests were performed. Later, PCR based STR Genotyping was performed on the blood stains, vaginal swabs, vulval swabs and reference blood samples of the victim and the accused.
Further, few grass was detected on legging of victim and Jeans full pant of accused. Both the grass specimens were collected separately and forwarded to Botany department for grass identification and comparison.

MATERIALS AND METHOD

Detection of blood on the clothes

Blood stains on the clothes of the victim and accused were confirmed by testing with Kastle-Meyer solution (Phenolphthalein solution) and 3% Hydrogen peroxide. Hemoglobin in the blood catalytically decomposes Hydrogen peroxide to release nascent oxygen which reacts with Phenolphthalein to give pink color. Blood was detected on Frock, leggings of victim, Full shirt of accused and vaginal, vulval swab of victim.

Detection of semen on the clothes

***Acid phosphatase test*:** Semen stains on the clothes were checked by testing with Acid Phosphatase reagent (Citrate buffer, Substrate solution of Disodium Phenyl Phosphate, Phenol reagent, Sodium Carbonate). All the exhibits were found to be negative for this test. Hence, no semen detected on any of the exhibit.

After confirming the presence of blood, DNA analysis of blood stains was performed.

Instruments and chemicals used for DNA analysis were as follows

1. Prep Filer Express DNA extraction kit. Lot No. 1807201.
2. Amp FlSTR® Identifiler kit. Lot No. 1807261
3. HiDi Formamide.
4. Liz 600 Size standard.
5. Quantifiler Duo DNA kit. Lot No. 1710101.
6. AutoMateExpress™ Forensic DNA Extraction System. Catlog number: 4441763
7. PCR thermal cycler GeneAmp 9700. Catlog number: 4375786
8. 3500 Genetic Analyzer. Catlog number: 4406017

Isolation of DNA: The PrepFiler™Forensic DNA extraction Kit (Applied Biosystems, Foster City, CA) enables the isolation of DNA from biological samples that contain small quantities of biological material in such a way that it removes the substances interfering with PCR. Additionally, the extracted DNA is having sufficiently high concentration due to which the volume of extract for downstream analysis is minimal [18].

1. Blood stains on all the positive articles were cut into small 1 x 1 mm pieces and were placed in 2ml micro centrifuge tube.
2. For the reference profile, 40 µl blood samples of the accused and victim were taken into another micro centrifuge tubes.
3. 500 µl Lysis buffer from PrepFiler Express F DNA extraction kit [19] was added to all the sample tubes.
4. The sample tubes were kept on thermo shaker at 750 rpm at 70˚C for 40 min.
5. The tubes were then centrifuged at 10,000 rpm for 2 min.
6. Cartridges from PrepFiler Express F DNA extraction kit were loaded to the cartridge rack in AutoMate Express DNA extraction system [20], Sample tubes, elution tubes and tips were loaded as per machine guidelines and the machine program was run as per the recommended machine protocol.
7. After completion of program, elution tubes containing extracted DNA in highly pure form was stored at 4˚C till the next PCR amplification process.

Many different methods are available for extraction of DNA from the forensic samples. Organic extraction using phenol/chloroform is one of the sensitive methods used for extraction ofDNA.However, it is tedious and time consuming [21].

Quantification of the extracted DNA

DNA Extracted from all the blood samples was quantified using Quantifiler® Duo DNA Quantification Kit[22,23] on an Applied Biosystems 7500 Real-Time PCR System according to manufacturer recommended protocols. Quantified DNA was taken for downstream application.

PCR based STR Analysis: The quantified DNA extracted from all the blood stains and reference blood samples of the accused and victim was processed for Polymerase Chain Reaction using the AmpFlSTR® Identifiler PCR Amplification Kit (Applied Biosystems) (Lot No. 1807261) [24] with the help of PCR thermal cycler GeneAmp 9700[25] following the protocols recommended by the manufacturer and described in the studies. This kit contains Reaction mixture, Primer set and Taq Gold Polymerase enzyme. Primer Set contains locus-specific 6-FAM™, VIC™, NED™ and PET™ dye-labeled and unlabeled primers in the buffer. The primers amplify the STR loci CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA and gender marker Amelogenin.
Reaction mixture used for PCR was prepared by adding Reaction mix 10.5 µl, Primer set 5.5 µl and Taq Gold DNA Polymerase 0.55 µl. The extracted DNA sample 10 µl was added to it. The DNA was amplified in 28 cycles using PCR machine selecting 94.0 ºC, 59.0 ºC and 72.0 ºC as temperatures of denaturing, annealing and extension respectively (Table 1).

Table 1 PCR Protocol used for amplification of DNA

Step	AmpliTaq Gold Enzyme Activation	PCR			PCR Final Step	PCR product till separation of STRs
	Hold	CYCLE (28 cycles)			Hold	
		Denaturation	Anneal	Extend		
Temp	95˚C	94˚C	59˚C	72˚C	60˚C	4˚C
Time	11 min	1 min	1 min	1 min	60 min	∞

Table 2 DNA Profiles obtained from the blood stains found on clothes of the victim and accused along with reference blood samples of both of them.

STR Locus	GENOTYPE						
	Frock (Victim) (Blood stain)	Leggings (Victim) (Blood stain)	Full shirt (Accused) (Blood stains)				
			Stain I	Stain II	Stain III	Stain IV	Stain V
D8S1179	15,15	15,15	11,13,15	15,15	11,13,15	15,15	11,13
D21S11	30,30	30,30	30,32.2	30,30	30,32.2	30,30	30,32.2
D7S820	11,12	11,12	10,11,12,13	11,12	10,11,12,13	11,12	10,13
CSF1PO	12,12	12,12	12	12,12	12	12,12	12,12
D3S1358	14,16	14,16	14,15,16	14,16	14,15,16	14,16	15,15
THO1	8,9.3	8,9.3	8,9,9.3	8,9.3	8,9,9.3	8,9.3	8,9
D13S317	11,11	11,11	11,12	11,11	11,12	11,11	11,12
D16S539	8,13	8,13	8,12,13	8,13	8,12,13	8,13	12,13
D2S1338	20,23	20,23	19,20,23	20,23	19,20,23	20,23	19,20
D19S433	13,14	13,14	13,14,15	13,14	13,14,15	13,14	14,15
vWA	16,18	16,18	16,17,18	16,18	16,17,18	16,18	17,18
TPOX	9,10	9,10	9,10,11	9,10	9,10,11	9,10	11,11
D18S51	14,14	14,14	14,15,16	14,14	14,15,16	14,14	15,16
AMELOGENIN	X,X	X,X	X,Y	X,X	X,Y	X,X	X,Y
D5S818	12,13	12,13	11,12,13	12,13	11,12,13	12,13	11,12
FGA	21,23	21,23	21,22,23	21,23	21,22,23	21,23	21,22

Table 2 DNA Profiles obtained from the blood stains found on clothes of the victim and accused along with reference blood samples of both of them.

STR Locus	GENOTYPE			
	Vaginal swab (Victim)	Vulval swab (Victim)	Blood (Victim)	Blood (Accused)
D8S1179	15,15	15,15	15,15	11,13
D21S11	30,30	30,30	30,30	30,32.2
D7S820	11,12	11,12	11,12	10,13
CSF1PO	12,12	12,12	12,12	12,12
D3S1358	14,16	14,16	14,16	15,15
THO1	8,9.3	8,9.3	8,9.3	8,9
D13S317	11,11	11,11	11,11	11,12
D16S539	8,13	8,13	8,13	12,13
D2S1338	20,23	20,23	20,23	19,20
D19S433	13,14	13,14	13,14	14,15
vWA	16,18	16,18	16,18	17,18
TPOX	9,10	9,10	9,10	11,11
D18S51	14,14	14,14	14,14	15,16
AMELOGENIN	X,X	X,X	X,X	X,Y
D5S818	12,13	12,13	12,13	11,12
FGA	21,23	21,23	21,23	21,22

Figure 1

Figure 2

Figure 3

Figure 4

The amplified DNA samples were kept at 60.0 ºC for an hour and then at 4.0 ºC till the separation of STRs (Table 1). PCR produces millions of DNA fragments of different sizes. Amplified products were separated and detected using 3500 Genetic Analyzer [26] and analyzed using Gene Mapper® ID-X Software V 1.5. The separation of different fragments of DNA molecules on the basis of their sizes was achieved by capillary electrophoresis. Simultaneous amplification of 16 STR Loci was completed and analyzed [27], [28]. DNA profiles obtained were interpreted by comparing with each other.

Simultaneously, during detection of the exhibits, grass specimens found on leggings of victim and Jeans full pant of accused. Those specimens were collected separately and sent to botany department for analysis and comparison. They analyzed the grass samples microscopically and given their opinion.

RESULTS AND DISCUSSION

DNA was extracted from blood detected on following exhibits.

1. Frock of victim.
2. Leggings of victim.
3. Full shirt of accused.
4. Vaginal swab of victim.
5. Vulval swab of victim.
6. Reference blood of victim.
7. Reference blood of accused.

The extracted DNA was typed at 15 STR Loci and gender specific Amelogenin locus using PCR amplification technique. The DNA profiles obtained from blood detected on Frock, Leggings, Vaginal swab, Vulval swab were found to be identical and from one and the same source female origin and matched with DNA profile obtained from reference blood sample of Victim.

Five blood stains of 0.5 to 1 cm in diameter were recovered from Full shirt of accused. DNA profiles obtained from two blood stains on Full shirt were identical and from one and the same source of female origin and matched with DNA profile of Victim. One blood stain out of five stains shown DNA profile of male origin and matched with DNA profile of accused and remaining two blood stains on full shirt shown mixed DNA profile. Those mixed DNA profiles were identical and matched with DNA profiles of victim and accused (Table 2).

Even though the case was of sexual offence, there was no semen found either on clothes of victim or in her medical samples. Only blood was found on clothes of both victim and accused as she got injuries in genitals. Further he had beaten her on stomach, she had profuse bleeding. Blood on full shirt of accused found to be important evidence to prove his involvement in the crime. As the crime scene was channel with full of thorny bushes and grass, accused had scratches on his skin from which blood droplets oozed. So, one blood stain shown single DNA profile of himself. Victim's blood transferred to his full shirt that's why two blood stains were having DNA profile of victim. And as both of them having injuries, two blood stains shown mixed DNA profiles of victim and accused. DNA technique is full proof technique to provide the evidence in the court either for convicting the guilty or to exonerate the innocent. It provides capabilities not found in most of the other forensic disciplines. When biological material is transferred between perpetrator and the victim in violent crimes such as murder or rape, DNA recovered from the exhibits has power to potentially identify the perpetrator. While testing sufficient genetic markers, probabilistic

individualization of a DNA profile is achievable. The odds of two people who are not related by blood, having exactly same DNA fingerprint is about 1 in trillion individuals. Hence, it is most useful technique in the field of forensic to give justice.

In support to the DNA technique, grass pieces found on leggings and jeans full pant of accused also played important role to connect the accused with the crime. Both the specimens were sent to botany department for microscopic comparison. According to their findings, both the specimens were grasses, *Setariaverticillata*, both belong to the same species. Barbellate bristles seen clearly and that is unique characteristic of that species, with spikelets, long, ellipsoidal,obtuse and glabrous. Both the specimens found to be similar. This indicates that victim and accused were at the same place as same grass pieces were found on their clothes (Figure 1, Figure 2, Figure 3, Figure 4).

CONCLUSION

Being just 7 years, the victim was under shock and even unable to speak out to elaborate the incidence happened with her. Accused was under influence of alcohol when he committed the crime and repeatedly denying the sexual harassment with the girl.When police was in dilemma to come to the conclusion whether to keep him in the custody or to give him bail, our rapid DNA profiling report played important role to prove the guilt of the accused beyond reasonable doubt. It helped police to file the charge sheet in the court under POCSO Act. Grass comparison report also found important supportive evidence to solve the case.

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