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A Jot of Blood Sends Constable behind the Bars - Justice by DNA Profiling

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Abstract

It is difficult to identify a criminal just by examining the trace of blood on crime site and the garments. In sexual offences, specifically semen on victim's clothes or biological samples proves involvement of accused in the crime. But in some cases where contraceptive devices are used or semen stains are not detected during investigation, the victim's blood or body fluid on the accused's garments helps to prove the crime. It is difficult to prove the evidence by routine ABO grouping in most of the cases because of the less quantity of blood. Further, discrimination power of ABO Blood group system is less. Here, DNA profiling technique has created wonders from the time it has been invented. Once the DNA technique proves involvement of the accused, there is provision of 'Protection of Children from Sexual Offences' (POCSO) Act 2012 in the court to effectively address the heinous crimes. Also, there is provision of Atrocity Act to provide justice to SC/ST communities in order to enable them to live in the society with dignity without suppression from the dominant castes. In the instant case, a girl aged 11, promising to drop to the school on motor cycle was raped by Police Constable aged 41. Just 2 cm blood stain was found on his undergarment and few blood stains were found on his handkerchief at the crime scene. As the DNA profiling proved his involvement in the crime, Hon'ble court convicted him with rigorous imprisonment for 10 years and to pay a fine of Rs. 2000/- under the provision of POCSO Act and Atrocity Act.

Keywords: DNA; Polymerase chain reaction; Short tandem repeats; Genetic analyzer

Introduction

In many sexual offences, a criminal can be identified by comparing his DNA profile with that of the body fluid or hair found at the crime scene as well as on the victim's garments or her biological exhibits like vaginal swab, anal swab, pubic hair etc. In the same way, his involvement can also be proved by comparing the victim's DNA profile with that of the body fluid or hair found at the crime scene as well as on the garments or biological exhibits of the accused. DNA fingerprints are inherited

as simple Mendelian characters. Forensic DNA profiling can make use of any specimen that contains DNA which includes blood, hair with roots, semen, tissue, bones or body fluid that has nucleated cells. In case of blood, DNA is extracted from white blood cells as Red blood cells do not have nucleus.

Earlier, RFLP technique was used for DNA fingerprinting. Now, PCR based amplification of STR Loci has become the method of choice for the purpose of identification in forensic investigations [1,2]. In 1984, Dr. Alec Jeffreys developed DNA fingerprinting technique used to identify the individuals [3]. He found that certain regions of DNA contained repeated DNA sequences. The regions with repeat units that are 2-6 base pair in length are called Short Tandem Repeats. The first STR multiplexes developed was quadraplex created by Forensic Science Services (FSS) that comprises four STR Loci [4].

While alternatives exist, most DNA typing laboratories use commercially available kits to amplify and label STR alleles associated with evidence and reference samples that are the size fractionated with Capillary electrophoresis instruments such as ABI 310 or 3130 Genetic Analyzer and latest 3500 Genetic Analyzers [5-8]. Software GeneMapper® is used to determine the presence or absence of STR alleles associated with the sample.

In this case, the accused who was police constable at Sironcha police station, District Gadchiroli was facing the trial for the offences punishable under Section 376, 377, 506 of Indian Penal Code, Section 6 of Protection of Children from Sexual Offences Act [9] (POCSO Act for short) as well as under Section 3 (1) (12) of Scheduled Caste and Scheduled Tribes (Prevention of Atrocities) Act [10,11].

On 31st March, 2015, at about 9.00 am, the victim and her father were standing on the road in front of their house since victim had to travel to her school at Sironcha. The accused approached and told them that he is too going to Sironcha and hence he shall drop her to the school. The accused being familiar with them, the victim's father helped her sit on the accused's motor bike and allowed him to drop her to the school. However, the accused took her below the bridge on Suryapalli to Sironcha road and committed rape and carnal intercourse with her. The victim was 11 years old and studying in 5th

standard. The accused threatened her to gun down her father if she would disclose the incident to her father. She bore the trauma for about a week and lastly told the incident to her sister on 8th April, 2015. Her parents then learnt about the incidence happened with her. They lodged report with the police station Sironcha and a crime was registered against the accused.

After medical examination of the victim and accused, the police sent the medical samples and clothes to the forensic science laboratory. During detection of clothes, one blood stain of 2 cm was detected on underwear of the accused and few blood stains were detected on the handkerchief of the accused found in the bushes at the crime scene. No semen was detected. The PCR based STR DNA profiling was performed on the blood stains, vaginal swab and rectal swab. This profile was compared with the DNA profile of the victim's reference blood sample and the blood detected on the accused's undergarment and handkerchief. The profiles were matched and this proved him guilty.

Materials and Methods

Detection of blood

The presence of blood stains on clothes found at the crime scene was confirmed by testing with Kastle-Meyer solution (Phenolphthalein solution) and 3% Hydrogen peroxide [12,13]. The principle of this case is that hemoglobin present in the blood catalytically decomposes hydrogen peroxide to release nascent oxygen which reacts with Phenolphthalein to give pink color. Later, using cross over electrophoresis technique, reacting saline extract of blood stains (blood serum) with human anti serum, it was confirmed that the blood was of human origin.

Detection of semen on clothes

Acid phosphatase test: Semen stains were checked on the clothes by testing suspected stains with Acid Phosphatase reagent (Citrate buffer, Substrate solution of Disodium Phenyl Phosphate, Phenol reagent, Sodium Carbonate). The test is based on the principle that when the stain is reacted with a solution of substrate, the enzyme acid phosphatase present in the semen hydrolyses the substrate solution (Disodium phenyl phosphate) to the corresponding phenol and phosphate ion. The phenol formed is simultaneously coupled with a suitable diazonium salt as a chromogen to give a characteristic colored dye stuff which is a positive test for the presence of seminal stain [14]. This test is used as a preliminary test for confirmation of semen detection. It was found to be negative for all the suspected stains.

Isolation of DNA: DNA was isolated from the human blood detected on handkerchief of the accused found at the crime scene, underwear of the accused, reference blood sample of the victim and the vaginal swab and rectal swab of the victim using organic extraction method [15]. Forensic buffer, Proteinase K and 10% Sodium Dodecyl Sulphate were added in appropriate amount to each sample. It was vortexed, quick spun and incubated at 56°C for 3 hours. Then Phenol, Chloroform and Isoamyl Alcohol were added in the proportion 25:24:1, v/v.

Proteins get denatured and collected in the organic phase while nucleic acids remain in the aqueous phase. Then 2M Sodium Acetate and chilled Isopropanol were added to the aqueous phase to precipitate DNA. The precipitated DNA was finally dissolved in Tris EDTA buffer (pH8) (Figure 1).

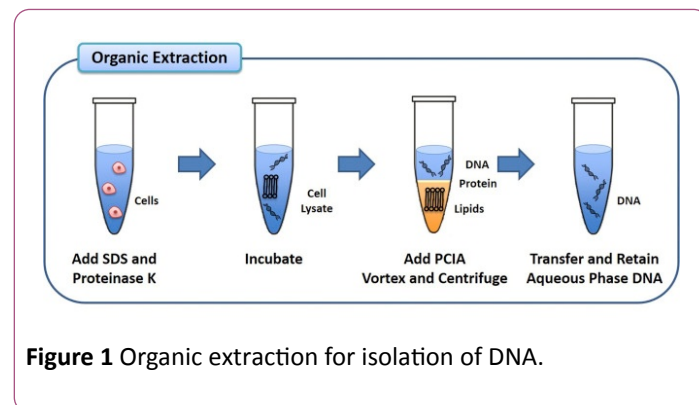


Figure 1 Organic extraction for isolation of DNA.

Quantification: Extracted DNA was quantified using the Quantifiler® Duo DNA Quantification Kit [16] on an Applied Biosystems 7500 Real-Time PCR System according to the manufacturer's recommended procedures. Quantified DNA was taken for downstream application.

PCR based STR analysis: The extracted DNA samples were amplified using AmpFISTR Identifier® kit (Applied Biosystems) (Lot No. 1411179) with the help of PCR thermal cycler GeneAmp 9700 to increase the quantity of DNA [17]. This kit contains Reaction mixture, Primer set and Taq Gold Polymerase enzyme. Primer Set contains locus-specific 6-FAMTM, VICTM, NEDTM and PETTM dye-labeled and unlabeled primers in 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 mixtures used for PCR were Identifier PCR Reaction mix 10.5 µ, Primer set 5.5 µ, DNA polymerase enzyme 0.55 µ and extracted DNA 10 µl. Followed the PCR protocol (Figure 2) on PCR thermal cycler [18] and analyzed using GeneMapper® ID-X Software V 1.5. The separation of different fragments of DNA molecules on the basis of their size was achieved by capillary electrophoresis on 3130 Genetic Analyzer. Simultaneous amplification of 16 STR Loci was completed and analyzed [19]. DNA profiles obtained were interpreted by comparing with each other.

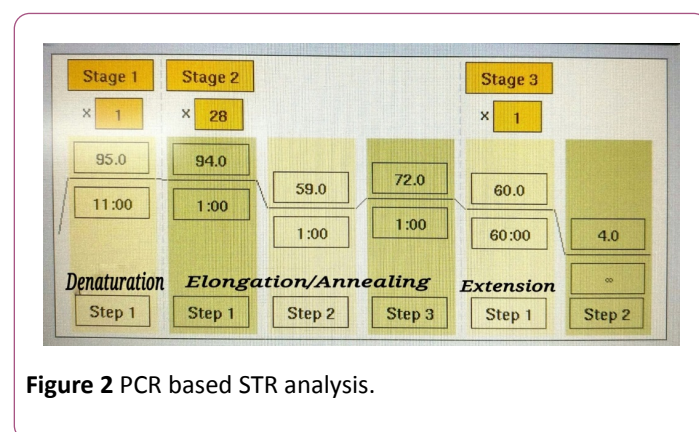


Figure 2 PCR based STR analysis.

Results

The DNA profiles obtained from the blood detected on handkerchief from the crime scene and underwear of the accused were found to be identical and from one and the same source of female origin. They matched with the DNA profile

obtained from reference blood of the victim. As the underwear of the accused was having victim's blood, his involvement was proven in the crime and the blood on handkerchief at the crime scene proved the place of the crime. The incidence was opened by the victim after 8 days of the crime. So her vaginal swab and rectal swab were having DNA profile of only herself (**Table 1**).

Table 1 DNA profiles obtained from the blood stains.

STR Locus	Genotype				
	Handkerchief (Crime Scene)	Underwear (Accused)	Blood (Victim)	Vaginal swab (Victim)	Rectal swab (Victim)
D8S1179	10.15	10.15	10.15	10.15	10.15
D21S11	OL, 32.2	OL, 32.2	OL, 32.2	OL, 32.2	OL, 32.2
D7S820	10.11	10.11	10.11	10.11	10.11
CSF1PO	12.12	12.12	12.12	12.12	12.12
D3S1358	14.15	14.15	14.15	14.15	14.15
THO1	9.9	9.9	9.9	9.9	9.9
D13S317	12.12	12.12	12.12	12.12	12.12
D16S539	8.11	8.11	8.11	8.11	8.11
D2S1338	24.25	24.25	24.25	24.25	24.25
D19S433	15.15	15.15	15.15	15.15	15.15
vWA	15.18	15.18	15.18	15.18	15.18
TPOX	8.11	8.11	8.11	8.11	8.11
D18S51	13.14	13.14	13.14	13.14	13.14
AMELOGENIN	X.X	X.X	X.X	X.X	X.X
D5S818	11.12	11.12	11.12	11.12	11.12
FGA	23.26	23.26	23.26	23.26	23.26

The odds of two people who are not related by blood, having exactly the same DNA fingerprint is about 1 in trillion individuals. Hence, it is most useful technique in the field of forensic science to give justice. It is a reliable and full proof technique to provide evidence in the court either for convicting the guilty or to exonerate the innocent. When biological material is transferred between perpetrator and victim in violent crimes such as murder or rape, DNA recovered from exhibits has power to potentially identify the perpetrator. When testing sufficient genetic markers, probabilistic individualization of a DNA profile is achievable. Once the DNA report shows involvement of accused in the crime, there are different provisions of Act in the court for different cases. The provision of POCSO Act 2012 is for individuals below 18 years and is gender neutral legislation. Definition of child sexual abuse is comprehensive and encompasses (i) Penetrative sexual assault, (ii) Aggravated penetrative sexual assault, (iii) Sexual assault, (iv) Aggravated sexual assault, (v) Sexual harassment, (vi) Using child for pornographic purpose and (vii) Trafficking of children for sexual purposes. The Act prescribes stringent punishment graded as per the gravity of the offence with a maximum term of rigorous imprisonment for life and the fine. In this case, along

with POCSO Act the accused was also under trial of Atrocity Act. The objectives of this Act are to deliver justice to SC/ST communities through affirmative action in order to enable them to live in society with dignity and self-esteem and without fear, violence or suppression from the dominant castes [20-22].

Conclusion

When the police personnel who ought to protect the society from crimes commits such heinous crime, there should be strong punishment so as to avoid such incidences. DNA technology finds useful when the biological evidence is in less quantity. Child sexual abuse is a crime in which an adult or older adolescent uses child for sexual stimulation. It is a kind of mental or physical violation of child with sexual intent. In the instant case, the victim was minor and the accused was a police with a gun. Due to threatening, she couldn't open the incidence for about 8 days under fear. It was DNA report and other medical findings on the basis of which the accused was convicted for rigorous imprisonment of 10 years along with a fine of Rs. 2000/-. Thus, the victim got the justice.

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