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Life Imprisonment for Raping a Toddler- Success of DNA Profiling

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ABSTRACT

In India, according to National Crime Records Bureau Report 2017, a child is sexually abused every 15 minutes. Sexual offences have deep psychological impact on the victims and their families and hence are one of the most heinous offences imaginable. The problem of sexual abuse needs to be addressed through less ambiguous and stringent punishment. The protection of children from sexual offences (POCSO) act 2012 was formulated to effectively address the heinous crimes related to child sexual abuse in India. DNA technology made it possible to give justice by identifying the source of biological samples detected in such criminal cases. In the instant case, an 18 months' toddler who was kidnapped, raped and thrown in the forest was found by one of the villagers. She got justice by DNA analysis which proved involvement of the accused in the crime through semen detected on the victim's clothes and the victim's blood on the accused's clothes.

Keywords: DNA, Polymerase Chain Reaction, Short Tandem Repeats, Genetic Analyzer

INTRODUCTION

India is a country where children below the age of 18 years face staggering challenges from the day they are born. Illiteracy, malnutrition, trafficking, forced labour, sexual abuse, pornography etc. are common among them [1,2]. Child sexual abuse includes physical or psychological maltreatment of a child usually by a person who is in a position of trust and confidence in relation to the child [3]. National study undertaken by the Ministry of Women and Child Development fined 'sexual assault' as making the child fondle with his/her private parts or making the child exhibit private body parts and being photographed in the nude [4]. Application of laws dealing with adults created many problems while applying to cases of children with sexual abuse. Hence, to solve these

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problems, parliament enacted a special legislation POSCO Act in May 2012 [5]. However, to prove this type of sexual harassment cases in the court, forensic science laboratories play important role to solve these cases using DNA technology which is modern, reliable and full proof. The DNA technology was first developed in England in 1985 by Sir Alec Jeffreys [6]. 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 [7-9]. Preserving DNA evidence is a key requirement for law enforcement's investigation and prosecution of sexual assault case. It is used to prove that the sexual assault occurred and to show that the defendant is the source of the biological material left on the victim's body. 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 [10]. 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 [11,12].

In this case, the accused kidnapped an 18 months old victim while she was playing with her two brothers in the courtyard of her house. The brothers of the victim told their father that the accused had taken the victim with him. Therefore, the victim's family searched the victim till night hours. One of the villagers brought the victim to her house in the wee hours and told her father that he found her lying in the open space while he was going to answer the nature's call. There were blood stains on the victim's clothes and blood was oozing from her private part. Thereafter, along with that villager, her father lodged complaint against the accused in the police station. The complaint was lodged under sections 363, 366 A, 376 (2) (i) IPC and section 7 of POSCO Act [13]. Police arrested the accused and forwarded stained clothes and medical samples of the accused and the victim for forensic tests.

During detection of the exhibits, semen stains were found on the victim's knicker and blood stains were found on her frock. The blood stains were also found on the full pant and underwear of the accused. 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, semen stains, and vaginal swabs rectal swabs and reference blood samples of the victim and the accused. The DNA profiles obtained from their clothes were matched with their reference blood samples. The DNA profile of the accuser's semen matched with that found on the victim's clothes. Also, DNA profile of the victim's blood matched with that obtained from the clothes of the accused. On the basis of full proof DNA evidence, the court punished the accused with rigorous imprisonment for life and to pay the fine of Rs. 5000/- to the victim.

MATERIALS AND METHODS

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Steps involved in the analysis

A. 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.

B. Detection of semen on the clothes

i. Acid phosphatase test: Semen stains on the clothes were confirmed by testing with acid phosphatase reagent (Citrate buffer, Substrate solution of Disodium Phenyl Phosphate, Phenol reagent, Sodium Carbonate). This 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 to detect the presence of seminal stain. This test was used as a preliminary test for confirmation of semen.

ii. Florence test: It is necessary to reconfirm the presence of semen using another test since some of the body fluids like vaginal secretions or saliva also contain acid phosphatase enzyme in less amount which may give light color to the reaction. Hence, Florence test was performed to reconfirm the presence of semen. Florence reagent contains Potassium iodide, Iodine and water. Choline present in the semen reacts with Iodine to give choline periodide crystals which are observed microscopically.

These two tests confirmed the presence of semen on the victim's frock.

After confirming the presence of blood and semen on received exhibits, DNA analysis was performed.

C. Extraction of DNA: DNA was extracted from the blood stains, semen stains, vaginal, rectal swabs and reference blood samples using EZ1 DNA Investigator kit (Qiagen) (Lot No. 148049298). This kit reproducibly automates the purification of genomic DNA from the reference and the case work samples in human identity testing. The kit is used with EZ1 Advanced instrument [14]. Purification of DNA is fast and efficient with this kit.

Procedure followed for extraction was as under.

i) Sample was placed into a labeled lyse & spin basket with a 2 mL tube.
ii) Then, 480 µL of Stain extraction buffer, 20 µL of Proteinase K solution, and 1 µL of carrier RNA were added to the sample. Vortexed briefly on low speed.
iii) Samples were incubated for 2 hours at 56 °C in a thermo mixer set to 900 rpm.

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iv) Micro centrifuge tube was spin at high speed for 5 minutes to activate the basket. The fluid was forced to the extraction tube. The spin was repeated to collect the traces of fluid, if remained. The basket was discarded into biohazard waste container.
v) Then, the DNA was purified on the EZ1 Advanced XL using reagent cartridges, tips, elution tubes provided in the EZ1 investigator kit [15] and the running manufacturer recommended program provided by EZ1 Advance machine.

Extracted DNA was quantified and used for PCR based STR analysis.

D. 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 recommended procedures. Quantified DNA was taken for downstream application.

E. PCR based STR Analysis: The quantified DNA was processed for STR profiling using the AmpFlSTR® Identifiler PCR Amplification Kit (Applied Biosystems) (Lot No. 1411179) [17] with the help of PCR thermal cycler Gene Amp 9700 [18] following the protocols recommended by the manufacturer and is 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 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, Taq Gold DNA Polymerase 0.55 µl and 10 µl extracted DNA sample was added to it. DNA was amplified in 28 cycles using PCR machine selecting 94.0 ºC, 59.0 ºC and 72.0 ºC as temperature 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	∞

Amplified DNA samples were kept at 60.0 ºC for an hour and then at 4.0 ºC till separation of STRs (Table 1). PCR produces millions of DNA fragments of different sizes. Amplified products were separated and detected using 3500 Genetic Analyzer [19] and analyzed using Gene Mapper® ID-X

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Software V 1.5. The separation of different fragments of DNA molecules on the basis of their size was achieved by capillary electrophoresis. Simultaneous amplification of 16 STR Loci was completed and analyzed [20,21]. DNA profiles obtained were interpreted by comparing with each other.

RESULTS

DNA was extracted from the below specimens.

a. blood detected on the victim's frock
b. blood detected on the accused's full pant and underwear
c. semen detected on the victim's knicker
d. reference blood samples of both of them
e. vaginal swab and rectal swab of the victim

The extracted DNA was typed at 15 STR Loci and gender specific Amelogenin locus using PCR amplification technique. The DNA profiles obtained from all the blood specimens mentioned above were found to be identical. They were found to be from one and the same source of female origin and matched with DNA profile obtained from reference blood sample of the victim. DNA profile obtained from the semen detected on victim's knicker was found to be of male origin and matched with the DNA profile obtained from reference blood sample of the accused.

DISCUSSION

The study of profiles (Table 2) obtained in this case shows involvement of the accused in the crime in two different ways. One source was his semen found on her knicker and the other source was her blood found on his full pant and underwear. 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.

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

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STR Locus	GENOTYPE							
	Frock (Victim) Blood stain	Knicker (Victim) Semen stain	Full pant (Accused) Blood stain	Underwear (Accused) Blood stain	Blood (Victim)	Vaginal swab (Victim)	Rectal swab (Victim)	**Blood (Accused)**
D8S1179	13,14	11,15	13,14	13,14	13,14	13,14	13,14	**11,15**
D21S11	30,30	28,29	30,30	30,30	30,30	30,30	30,30	**28,29**
D7S820	7,12	12,12	7,12	7,12	7,12	7,12	7,12	**12,12**
CSF1PO	11,11	12,12	11,11	11,11	11,11	11,11	11,11	**12,12**
D3S1358	15,17	15,17	15,17	15,17	15,17	15,17	15,17	**15,17**
THO1	7,9.3	6,9	7,9.3	7,9.3	7,9.3	7,9.3	7,9.3	**6,9**
D13S317	9,12	7,9	9,12	9,12	9,12	9,12	9,12	**7,9**
D16S539	9,13	11,13	9,13	9,13	9,13	9,13	9,13	**11,13**
D2S1338	20,23	18,19	20,23	20,23	20,23	20,23	20,23	**18,19**
D19S433	13,14	13,15	13,14	13,14	13,14	13,14	13,14	**13,15**
vWA	16,19	15,17	16,19	16,19	16,19	16,19	16,19	**15,17**
TPOX	8,11	8,11	8,11	8,11	8,11	8,11	8,11	**8,11**
D18S51	16,16	14,16	16,16	16,16	16,16	16,16	16,16	**14,16**
AMELOGENIN	X,X	X,Y	X,X	X,X	X,X	X,X	X,X	**X,Y**
D5S818	11,12	12,12	11,12	11,12	11,12	11,12	11,12	**12,12**
FGA	**22,25**	**22,23**	**22,25**	**22,25**	**22,25**	**22,25**	**22,25**	**22,23**

Once the DNA report shows the 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

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punishment graded as per the gravity of the offence with a maximum term of rigorous imprisonment for life and fine.

CONCLUSION

Being just 18 months at the time of incidence, the victim was unable to give evidence to the court, DNA profiling report played important role to prove the guilt of the accused beyond reasonable doubt. On the basis of report and evidence provided by witnesses, the Hon'ble court sentenced the accused to Rigorous Imprisonment for life which shall mean imprisonment for the remainder of his natural life and to pay the fine of Rs 5,000/-. The offence is punishable under section 6 of Protection of Children from Sexual Offences Act.

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REFERENCES

1. Seth R, Srivastava RN. Child Sexual Abuse: Management and prevention, and protection of children from Sexual Offences (POCSO) Act. Indian Pediatrics [Internet]. Springer Science and Business Media LLC; 2017 Nov;54(11):949–53. Available from: http://dx.doi.org/10.1007/s13312-017-1189-9
2. Khanna K, Pal V. The Protection of Children from Sexual Offences Act, 2012. Journal of Punjab Academy of Forensic Medicine & Toxicology [Internet]. Diva Enterprises Private Limited; 2019;19(1):185. Available from: http://dx.doi.org/10.5958/0974-083x.2019.00037.2
3. Child abuse Reporting Laws. Legal Issues in Child Abuse and Neglect Practice [Internet]. SAGE Publications, Inc.; 82–101. Available from: http://dx.doi.org/10.4135/9781452225562.n3
4. Study on Child Abuse 2007, Ministry of Women and Child Development, Government of India. Contemporary Education Dialogue [Internet]. SAGE Publications; 2007 Jul;5(1):117–20. Available from: http://dx.doi.org/10.1177/0973184913411162
5. J A, T MK, VTN V. The Protection of Children from Sexual Offences Act (POCSO), 2012' in Clinical Settings. Kerala Journal of Psychiatry [Internet]. Branch of Indian Psychiatric Society (Kerala); 2019 Jul 24;31(2). Available from: http://dx.doi.org/10.30834/kjp.31.2.2019.166
6. Jeffreys AJ, Wilson V, Thein SL. Individual-specific "fingerprints" of human DNA. Nature [Internet]. Springer Science and Business Media LLC; 1985 Jul;316(6023):76–9. Available from: http://dx.doi.org/10.1038/316076a0
7. Newton M. The forensic aspects of sexual violence. Best Practice & Research Clinical Obstetrics & Gynaecology [Internet]. Elsevier BV; 2013 Feb;27(1):77–90. Available from: http://dx.doi.org/10.1016/j.bpobgyn.2012.08.020
8. Raymond JJ, van Oorschot RAH, Gunn PR, Walsh SJ, Roux C. Trace evidence characteristics of DNA: A preliminary investigation of the persistence of DNA at crime scenes. Forensic

ISSN 2047-9093

Science International: Genetics [Internet]. Elsevier BV; 2009 Dec;4(1):26–33. Available from: http://dx.doi.org/10.1016/j.fsigen.2009.04.002

9. Bozzo WR, Colussi AG, Ortiz MI, Lojo MM. DNA recovery from different evidences in 300 cases of sexual assault. Forensic Science International: Genetics Supplement Series [Internet]. Elsevier BV; 2009 Dec;2(1):141–2. Available from: http://dx.doi.org/10.1016/j.fsigss.2009.08.185
10. Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics [Internet]. Elsevier BV; 1992 Feb;12(2):241–53. Available from: http://dx.doi.org/10.1016/0888-7543(92)90371-x
11. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier; 2012; Available from: http://dx.doi.org/10.1016/c2011-0-04189-3
12. Butler JM. Statistical Interpretation Overview. Advanced Topics in Forensic DNA Typing: Interpretation [Internet]. Elsevier; 2015;213–37. Available from: http://dx.doi.org/10.1016/b978-0-12-405213-0.00009-9
13. Mundhe RK, Mali RS, Ghumatkar SV, Malve MK. A Rape on a Minor Victim Under POCSO Act 2012 Investigation Through DNA Analysis Technique—A Case Study. SpringerBriefs in Applied Sciences and Technology [Internet]. Springer Singapore; 2015 Oct 29;7–13. Available from: http://dx.doi.org/10.1007/978-981-287-670-6_2
14. Montpetit SA, Fitch IT, O'Donnell PT. A Simple Automated Instrument for DNA Extraction in Forensic Casework. Journal of Forensic Sciences [Internet]. ASTM International; 2005;50(3):1–9. Available from: http://dx.doi.org/10.1520/jfs2004181
15. Barbaro A, Cormaci P, La Marca A. DNA extraction from soil by EZ1 advanced XL (Qiagen). Forensic Science International [Internet]. Elsevier BV; 2019 Jun;299:161–7. Available from: http://dx.doi.org/10.1016/j.forsciint.2019.04.004
16. Barbisin M, Fang R, O'Shea CE, Calandro LM, Furtado MR, Shewale JG. Developmental Validation of the Quantifiler®Duo DNA Quantification Kit for Simultaneous Quantification of Total Human and Human Male DNA and Detection of PCR Inhibitors in Biological Samples. Journal of Forensic Sciences [Internet]. Wiley; 2009 Mar;54(2):305–19. Available from: http://dx.doi.org/10.1111/j.1556-4029.2008.00951.x
17. Morales JA, Monterrosa JC, Puente J. Population genetic data from El Salvador (Central America) using AmpFlSTR® Identifiler® PCR Amplification Kit. International Congress Series [Internet]. Elsevier BV; 2004 Apr;1261:223–5. Available from: http://dx.doi.org/10.1016/s0531-5131(03)01663-7
18. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific Enzymatic Amplification of DNA In Vitro: The Polymerase Chain Reaction. Cold Spring Harbor Symposia on Quantitative Biology [Internet]. Cold Spring Harbor Laboratory; 1986 Jan 1;51(0):263–73. Available from: http://dx.doi.org/10.1101/sqb.1986.051.01.032
19. Kirkham A, Haley J, Haile Y, Grout A, Kimpton C, Al-Marzouqi A, et al. High-throughput analysis using AmpFlSTR® Identifiler® with the Applied Biosystems 3500xl Genetic Analyser. Forensic Science International: Genetics [Internet]. Elsevier BV; 2013 Jan;7(1):92–7. Available from: http://dx.doi.org/10.1016/j.fsigen.2012.07.003

ISSN 2047-9093

20. Budowle B, Allen RC. Analysis of Amplified Fragment-Length Polymorphisms (VNTRISTR Loci) for Human Identity Testing. Forensic DNA Profiling Protocols [Internet]. Humana Press; 155–72. Available from: http://dx.doi.org/10.1385/0-89603-443-7:155
21. Gill P, Kimpton CP, Urquhart A, Oldroyd N, Millican ES, Watson SK, et al. Automated short tandem repeat (STR) analysis in forensic casework — a strategy for the future. Electrophoresis [Internet]. Wiley; 1995;16(1):1543–52. Available from: http://dx.doi.org/10.1002/elps.11501601257

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