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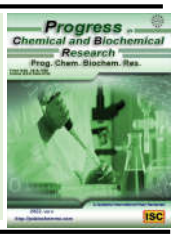

Original Research Article

Importance of forensic techniques in the investigation of rape (sodomy) and murder

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ABSTRACT

The forensic DNA fingerprinting technique play a vital role during the investigation of rape and murder cases and gives strong scientific evidence for linking the suspect with the scene of crime and victim. We present a case along with an analysis technique that will bring about the importance of forensic biology and DNA technique of how forensic science is helpful to give conclusions to the rape and murder case. In forensic biology, blood typing was an important forensic tool but blood typing was not a very discriminating technique. The blood group of victims and suspects may be similar in most cases so the use of DNA profiling has increased. DNA can be extracted from bloodstains and semen proves an act of rape and murder.

HIGHLIGHTES

* Link of suspect with the scene of the crime and proves an act of sodomy.

* Sodomy and murder by the same suspect prove the act of serial killing.

* DNA fingerprinting provides a useful tool for the identification of the suspect

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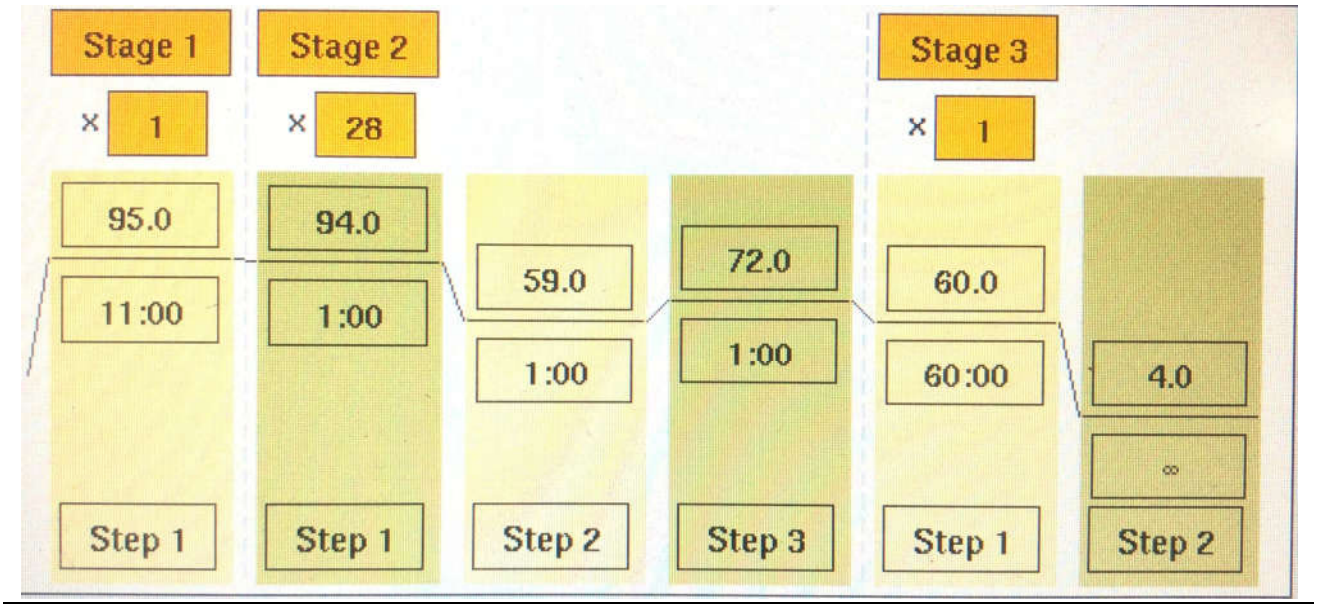
GRAPHICAL ABSTRACT



Introduction

In India, most kidnapping and murder cases of children remain unsolved due to a lack of proper scientific shreds of evidence and clue. Some adults who are sexually attracted to sexual acts with a child are called a paedophile. Some adults repeatedly engage in sexual activities with children and destroy evidence of crime much time followed by murder. In such cases or offences of unnatural sex, penetration is sufficient to conclude the offence. These offences are punishable with imprisonment for life or up to ten years and a fine [1].

Sexual abuse is done in unnatural ways, which is called Sodomy. Sodomy comes under section 377 of I.P.C. It is anal intercourse between two males, or between a male and female. It is called gerontophilia when the passive agent is an adult and pederasty when the passive agent is a child, who is known as catamite. Sodomy is a sexual activity that is found as a strong attack. It is a social as well as a religious crime. It is driven by the disappointment of abounding the pupil of a wide range of sexual cravings [2, 3].

During the investigation of rape and murder cases, the investigation officer and forensic team need to analyze the undisturbed crime scene. Forensic science must collect all the shreds of evidence. This evidence will help to link between the victim, crime scene and suspect [4-6].

DNA profiling has become an important tool for the identification of biological stains found in murder and rape cases. It was first used in a forensic investigation by Alec Jaffrey in 1985 [7]. An analyst from a forensic laboratory used the DNA profiling technique to produce strong scientific evidence and solve complicated crimes like rape, murder, burglary, paternity disputes, etc. DNA analysis puts vital evidence in the Protection of Children from Sexual Offences Act (POCSO), 2012 which followed by murder [8]. DNA evidence in sexual assault cases has become increasingly more important if a prosecutor hopes to secure a [9- 11].

Case History

In this case, three boys of the age group of 9 to 14 years old had kidnapped, raped followed by murder. This brutal act was executed by the habitual homosexual suspect. This crime took place in the same district for one year. The suspect was caught after he committed the third murder. While interrogation confesses the first and second crime. All scene of the crime was

properly examined. The investigation officer collects the physical and biological evidence. From the scene of crime collected articles were deposited in the forensic science laboratory. The victim and suspect medical examination was carried out and necessary articles were collected for examination.

Materials and methods

Phenolphthalein reagent stock solution:

2.0 gm. of Phenolphthalein and 20 gm. Potassium hydroxide was taken and 100 ml distilled water was added after the addition of powdered zinc the reaction mixture was refluxed for two hours until the mixture becomes colourless. Working solution 20 ml Phenolphthalein stock solution taken 80 ml Ethanol added.

Acid phosphatase reagent:

a. **Citrate Buffer** (18.9 gms. of citric acid was dissolved in 500 ml of distilled water 180 ml of 1N NaOH added followed by 100 ml of 0.1 N HCl and made total volume up to 1 litre.

b. Substrate solution (1% disodium phenyl phosphate)

c. Buffered Amino Antipyrine

d. Buffered Potassium ferricyanide

Detection of biological fluid stains on articles sent by investigation officer from the scene of a crime, wearing apparels of victims and suspect and from the weapon. Blood and semen-stained articles stained with blood and semen further analyzed species origin.

In case-I, exhibits 1, 2, 3 and 4 were tested for the blood as mentioned in **Table 1** (Kastle- Meyer test). Exhibits 1, 3 and 4 gives a positive blood test. Exhibit 2 shows a negative blood test. bloodstains on exhibits no 1, 3 and 4 were confirmed for blood by chromatographic test (Thin layer chromatography). To find out the origin of the above bloodstains tested for cross over electrophoresis technique. The origin of bloodstains of 1, 3, and 4 find out by reacting saline extract of stains with anti-human serum, anti foul serum, anti cow serum, anti- buffalo serum and anti-dog serum. Bloodstains extracts of exhibits 1, 3, and 4 give precipitate lines for anti-human serum, indicating exhibits 1, 3 and 4 are of human origin. The toxicological analysis of exhibit 5 (viscera) ruled out the presence of poison and drugs.

In case II, exhibits 1, 2, 3, 4, 5, 6, 7, and 8 were tested for the blood as mentioned in **Table 1** (Kastle- Meyer test). Exhibits1, 3, 4, 5, 6, 7, and 8 gives a positive blood test. Exhibit 2 shows a negative blood test. Bloodstains on exhibits no 1, 3, 4, 5, 6, 7, and 8 were confirmed for blood by chromatographic test (Thin layer chromatography). To find out the origin of the above bloodstains tested for cross over electrophoresis technique. The origin of bloodstains of 1, 3, 4, 5, 6, 7, and 8 find out by reacting saline extract of stains with anti-human serum, anti foul serum, anti cow serum, anti-buffalo serum and anti-dog serum.

Bloodstains extracts of exhibits 1, 3, 4, 5, 6, 7, and 8 give precipitate lines for anti-human serum, indicating exhibits 1, 3, 4, 5, 6, 7, and 8 are of human origin. The toxicological analysis of exhibit 9 (viscera) ruled out the presence of poison and drugs.

In case-III, exhibit (1) was stained with semen, by performing acid phosphatase test details given in **Table 1**. This test was preliminary and confirmed by performing the Florence test. Exhibit 1 shows a negative blood test by performing Kastle –Meyer test. To find out the origin of the above semen stain tested for cross over electrophoresis technique. The origin of semen stain of exhibit 1find out by reacting saline extract of stains with anti-human serum, anti-foul serum, anti-cow serum, anti- buffalo serum and anti-dog serum. Semen stain extract of exhibit 1gives precipitate lines for anti-human serum, indicating exhibit 1 is of human origin.

Table 1-Detection of blood and semen stains of exhibits forwarded by the investigation officer.

Sr.No	Exhibit	Detection of blood and semen	Confirmatory test:	Species of origin
	Case-I 1. Earth mix 2. Earth control 3. Wooden log scene of the crime 4. Slipper 5. Viscera for chemical analysis 6. Sternum bone **Case-II** 1. Earth mix 2. Earth control 3. Full shirt of the victim. 4. Full pant of the victim. 5. Underwear of the victim. 6. T-shirt of the suspect 7. Full night pant of the suspect 8. Pieces of bricks from the scene of the crime. 9. Medical officer preserved viscera for chemical analysis and sternum bone for DNA profiling. **Case-III** 1. Condom, from the scene of the crime. 2. Viscera	(Phenolphthalein test, Kastle Meyer test) -Suspected stains or swabs were placed on the welled tile. Two drops of phenolphthalein solution were placed on the stain followed by 3% hydrogen peroxide **Inference** The intense pink colour was indicative of a positive reaction **Detection of semen-** **Acid phosphatase test** Yellowish white sticky dry sample inside the condom was confirmed by testing against acid phosphatase reagent namely the citrate buffer, substrate solution of disodium phenyl phosphate, phenol reagent and sodium carbonate. The principle of this test is when the suspected semen stain is reacted solution of the substrate, the enzyme acid phosphatase which is present in semen hydrolyses the substrate solution disodium phenyl phosphate with the respective phenol and phosphate ion. The liberated phenol is simultaneously coupled with suitable diazonium salts as a chromogen resulting in a brick red colour dyestuff which shows a positive test for seminal acid phosphate i.e. semen stain. The concentration of acid phosphate enzyme is significantly higher in seminal fluid as compared to other biological fluid-like vaginal fluid, saliva, etc. when seminal fluid stains are exposed to or examined under ultraviolet light, they show fluorescence of a bluish-white colour due to the presence of choline, which is not specifically found in other body fluids. **Inference** A brick red colour dyestuff shows a positive test for seminal acid phosphate i.e. semen stain.	Chromatographic test (Blood): Silica gel paste was spread on the slide and dried. Bloodstain extract was applied on the same. Chromatography Carried out with the solvent system, and then the slide was kept in the oven for 80°c. Sprayed Benzidine solution followed by 3% hydrogen peroxide. **Inference** For blood blue colour haematin spot was developed. **Florence test for semen** Acid phosphatase test which is not confirmatory test hence to reconfirm semen stain to perform Florence test, the stain was extracted in diluted hydrochloric acid and gives dark brown colour crystals of choline iodide appears immediately which was rhombic and needle-shaped crystal using potassium iodide, iodine and water (Florence solutions) treatment.	Cross over electrophoresis Bloodstain extract was placed in the cathodic well and antihuman serum was in the anodic well punched in the closed together along a line of electrophoretic movement. When appropriate reactants meet in the area between the well a precipitate was formed. **Inference** Exhibit stained with blood is of human origin.

The toxicological analysis of exhibit 2 (viscera) ruled out the presence of poison and drugs.

DNA analysis

1. PrepFiler Express F DNA extraction kit. Lot No. 1910243.
2. PrepFiler Express BTA extraction kit. Lot No. 1909203.
3. AmpFlSTR® Identifiler kit. Lot No. 1910269.
4. Dithiothreitol.
5. HiDiFormamide.
6. Liz 600 Size standard.
7. AutoMateExpressTM Forensic DNA Extraction System.
8. Catalog number: 4441763
9. PCR thermal cycler GeneAmp 9700. Catalog number: 4375786 3500
10. Genetic Analyzer. Catalog number: 4406017

Isolation of DNA:

a. DNA was isolated from the sternum bone of the victim and blood of the victim's father in case-I.
b. DNA was isolated from bloodstains found on the suspect's T-shirt and full night pant, the blood of suspect, hair of deceased/ victim, semen found on victim underwear in case II.
c. DNA was isolated from the sternum bone of the victim, the blood of the suspect and the swab from the condom in case III.

Extraction of DNA from bloodstains cuttings:

The bloodstain cuttings from suspect clothes were cut approximately of 0.5 mm sample piece and were taken into 2 ml microcentrifuge sample tubes. The further process was carried out according to the AutoMate Express Forensic DNA extraction system protocol using manual[12, 13].

AutoMate Express Forensic DNA Extraction System Parameters.

Instrument	:	Operating Parameters
Kits designed for this instrument	:	PrepFiler Express and PrepFiler Express BTA
Pipetting range	:	20-250 µl

Extraction of DNA from Condom surface

The surface of the condom was swabbed with a sterilized wet cotton swab using sterile forceps. These swabs were placed in different 2 ml microcentrifuge tubes for analysis. The further process was carried out according to the AutoMate Express Forensic DNA extraction system protocol using manual[13].

AutoMate Express Forensic DNA Extraction System Parameters

Instrument	:	Operating Parameters
Kits designed for this instrument	:	PrepFiler Express and PrepFiler Express BTA

PCR based STR Analysis

The extracted DNA from evidence as well as reference blood samples were subjected to the Polymerase Chain Reaction (PCR) using Amp*f*STR®Identifiler Amplification Kit (Applied Biosystems)AmpF*l*STR®YfilerTMPCR amplification kit on Veriti Thermal Cycler of Applied Biosystems.PCR products were separated and detected using Capillary Electrophoresis (ABI-3500 Genetic Analyzer, Applied Biosystems) and the DNA Profiles were generated and analyzed using GeneMapper® ID-X Software V 1.5. The division of various sections of DNA fragments dependent on their sizes was accomplished by capillary electrophoresis. Synchronous intensification of 16 STR Loci was analysed. After PCR amplification Denaturation was carried out using HiDiFormamide and Liz 600 size Standard. DNA profiles obtained were deciphered by contrasting them to one another.

Quantity requirement for PCR techniques

AmPFlSTR PCR Reaction Mix	:	10.5 µl
AmPFlSTRPrimer Set	:	5.5µl
Taq Gold DNA Polymerase	:	0.5µl
DNA Sample	:	1µl (1ng)

Parameters PCR Thermal Cycler Machine

Instrument	:	**Operarting Paramerers**
Capacity	:	96 wellx0.2ml PCR tubes/one 96 well plate
Heating/cooling	:	Peltier based
Capable of testing temperatures	:	Denaturation, Annealing & Extension steps
Block ramp rate	:	5.0 ºC/Sec.
Sample ramp rate	:	4.4ºC/S
Temperature range	:	4-99ºC/S
Temperature accuracy	:	±0.2 ºC
Customized programming	:	Allows a maximum of 20 steps and 99 cycles
Display	:	LCD touch screen, about 8.5 in.

Parameters of Genetic Analyzer-3500

Instrument	:	**Operating Parameters**
Fragment Size(bp)	:	500bp
No. of Markers	:	16
Polymer	:	POP4
Detector	:	CCD
Oven Temp	:	60 °C
Column Size	:	36cm
Software	:	GeneMapper® ID-X

Results

The finding of the study include statistical analysis and tables, the suspect leave evidence behind like a condom used during anal intercourse against the victim was collected and examined for seminal stains, and in another case, the suspect's clothes were stained with blood. Matching DNA samples from the scene of crime, suspect, victims, becomes a key source of evidence for use in the justice system.

Discussion

In case-I, The DNA profile obtained from the sternum of the victim was matched with the blood of the victim's father, for confirmation of the identity of the victim. As described in **Table 2.** The Y STR DNA profile obtained from the victim's Sternum bone matched with the Y STR DNA profile of the victim's father's blood sample proving them to be from the same paternal progeny.

In case II, The DNA profile obtained from blood detected on the suspect T-shirt and full night pants (Wearing apparel) at the time of the crime and semen stains from the victim underwear were found to be identical and from the same source of male origin. They matched with the DNA profile obtained from the reference hair sample of the victim. This proved the involvement of the suspect in the murder and the presence of the suspect at the scene of the crime. As shown in **Table 3**

In case III, The DNA profile obtained from the swab from the condom which was found at the scene of the crime was found to be identical and from the same source of male origin.

Table 2: STR Genotyping by using Y filler kit in case - 1.

STR Locus	**HAPLO TYPE**	
	Blood Victim's father (Putative Father)	Sternum Victim (Putative Son)
B_DYS456	14	14
B_DYS389 1	14	14
B_DYS390	22	22
B_DYS38911	30	30
G_DYS458	18	18
G_DYS19	16	16
G_DYS385	15.19	15.19
Y_DYS393	12	12
Y_DYS391	10	10
Y_DYS439	11	11
Y_DYS635	23	23
Y_DYS392	11	11
R_Y_GATA_H4	12	12
R_DYS437	15	15
R_DYS438	10	10
R_DYS448	18	18

Table 3: STR Genotyping by using Identifier kit in case - II.

STR LOCUS	**GENOTYPE**				
	Blood of suspect	Hair of Deceased Victim	Semen Stain Under Pant of victim	Blood Stain T-Shirt of suspect	Blood Stain Full night pant of suspect
D8S1179	12,15	13,14	13,14	13,14	13,14
D21S11	31.2,33.2	29,31.2	29,31.2	29,31.2	29,31.2
D7S820	11,11	8,12	8,12	8,12	8,12
CSF1PO	11,12	10,10	10,10	10,10	10,10
D3S1358	16,17	16,16	16,16	16,16	16,16
THO1	8,8	9,9.3	9,9.3	9,9.3	9,9.3
D13S317	11,14	8,11	8,11	8,11	8,11
D16S539	9,11	8,11	8,11	8,11	8,11
D2S1338	18,18	21,25	21,25	21,25	21,25
D19S433	13,15	14,14	14,14	14,14	14,14
vWA	16,18	14,18	14,18	14,18	14,18
TPOX	8,11	8,8	8,8	8,8	8,8
D18S51	14,14	14,14	14,14	14,14	14,14
AMELOGENIN	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	12,12	10,12	10,12	10,12	10,12
FGA	21,26	24,25	24,25	24,25	24,25

Table 4: STR Genotyping by using Identifier kit in case - III.

STR LOCUS	GENOTYPE		
	Blood of suspect	Sternum of victim	Swab from Condom (Crime Scene)
D8S1179	12,15	11,15	12,15
D21S11	31.2,33.2	30,30	31.2,33.2
D7S820	11,11	8,12	11,11
CSF1PO	11,12	11,11	11,12
D3S1358	16,17	17,17	16,17
THO1	8,8	6,9	8,8
D13S317	11,14	8,8	11,14
D16S539	9,11	9,11	9,11
D2S1338	18,18	22,23	18,18
D19S433	13,15	13,14	13,15
vWA	16,18	16,16	16,18
TPOX	8,11	8,10	8,11
D18S51	14,14	16,17	14,14
AMELOGENIN	X,Y	X,Y	X,Y
D5S818	12,12	11,12	12,12
FGA	21,26	21,24	21,26

They matched with the DNA profile obtained from the reference blood sample of the suspect. This proved the involvement of the suspect in the unnatural sexual intercourse with the victim (sodomy) and the presence of the suspect at the scene of the crime as shown in **Table 4**.

Conclusions

Link of suspect with the scene of the crime and proves an act of sodomy. Sodomy and murder by the same suspect prove the act of serial killing and DNA fingerprinting provides a useful tool for the identification of the suspect. The analysis of exhibits collected from wearing apparel of victims, suspect and condom found at the scene of crime proves the act of unnatural sex (sodomy)followed with murder. Multiple crimes of rape and murder by the same suspect prove the act of serial killing.

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