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HEINOUS CRIME OF RAPE WITH MURDER BY UNKNOWN SOLVED BY DNA FINGERPRINTING

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ABSTRACT

DNA fingerprinting is widely used technology to solve various crimes. Rape, murder, unknown body identification, disputed paternity, dacoity, kidnapping, etc. these are the cases which can be solved using this technique. Depending on the nature of the crime, biological samples collected from the victim or deceased and samples from crime scene are used to solve the mystery. Bottles, cigarette or bidi butts, blood, semen on bed sheets and other articles are most common evidences from crime scene that can be left behind by accused. Biological evidence is sometimes the only way to prove the occurrence. Extraction of DNA is a very important task in forensic analysis. In case of rape, swabs collected from victim's private parts are analyzed for traces of male DNA in it. In one of the case swabs from the deceased rape victim and crime scene exhibits were the only evidence to identify the accused. Amplifiable male DNA was extracted from swab samples and crime scene exhibits with the use of optimized and validated DNA extraction protocol which later on matched with the accused. In this investigation, swab samples from the victim and blood mixed semen stain from crime scene are analyzed and compared it with blood of twelve suspects. This approach is useful to solve such a type of cases.

KEY WORDS

DNA, PCR, swabs

INTRODUCTION:

DNA fingerprinting, one of the great discoveries of the late 20 th century has revolutionized forensic investigations. Forensic genetic fingerprinting can be defined as the comparison of the DNA in a person's nucleated cells with that identified in biological matter found at the scene of crime or with the DNA of another person for the purpose of identification or exclusion [1]. DNA fingerprint techniques evolved subtly over the next several years, until the polymerase chain reaction (PCR), developed by Karry Mullis, was introduced into forensic work. By allowing the selective amplification of any desired stretch of DNA, PCR ushered in unprecedented sensitivity in low level DNA detection at crime scenes. All of today's forensic genetic methods are based on PCR [2]. The major application of DNA fingerprinting is in the medico legal cases, such as paternity-maternity disputes, rape cases, murder cases, mass disasters, etc. In most of the forensic cases the main goal is to assign positive identification of the evidentiary material with those of the putative suspects [3]. Number of rape cases is increasing day by day and most of the times it is very difficult to find the real culprit because of lack of strong evidence. But many criminals are not aware that DNA testing has a major advantage that it can be used to identify each, and every portion of the remains collected from victim and scene of crime, to be provided that there is a sufficient intact DNA present to obtain DNA profile and a reference sample is available for comparison. Biological evidence is very important, since it may prove the existence of sexual contact and lead to perpetrator [4]. Samples from accused, victim and samples from crime scene can lead to finding real

culprits. Sexual assault cases are characterized by low rates of disclosure, reporting, prosecution, and conviction. The sensitivity and evidential power of DNA profiling have impacted on the way in which many of the sensitive crimes investigated. Y-chromosomal loci have proven useful in solving investigations where low levels of male DNA are present in a high female DNA background [5]. The direct comparison of DNA results from the swabs of the victim and crime scene samples to DNA obtained from blood of suspects represents the easiest way to obtain a match. In this report mixed profiles were obtained from swabs of the victim and blood mixed semen stain from the crime scene. So AmpFlSTR Yfiler PCR Amplification kit is used to get only male DNA profile from the mixture. Analysis of STRs on the Y chromosome is a robust technique and is widely used in human identification for resolving paternity disputes, male gene flow, etc. Microsatellite loci on the Y chromosome are helpful in identification of a person through paternal lineage as well as in cases of sexual assaults where mixtures of vaginal secretions with semen are found [6]. Simple tandem- repetitive regions of DNA which are dispersed in the human genome frequently shows substantial length polymorphism arising from unequal exchanges which altyer the number of short tandem repeats in a minisatellite [7]. The human genome contains a set of minisatellites, each of which consists of tandem repeats of a DNA segment containing the 'core' sequence, a putative recombination signal in human DNA [8]. Patrilineally transmitted Y-chromosomal markers have been shown to resolve forensic cases under certain scenarios where autosomal markers provide limited or inconclusive evidence [9]. In this report we describe the usefulness of Y-filer technology in rape case.

MATERIALS AND METHODS:

(Refer Table No.1, 2, 3)

1) Prep Filer Express BTA Kit (Applied Biosystems)
2) AmpFlSTR Yfiler PCR Amplification kit (Applied Biosystems)
3) Prep Filer BTA Lysis Buffer
4) Proteinase K
5) DTT(Dithiothreitol)
6) Hipura silica column (Himedia)

Table-1. AutoMate Express Forensic DNA Extraction System Parameters

Instrument	Operarting Parameres
Kits designed for this instrument	PrepFiler Express and PrepFiler Express BTA
Pipetting range	20-250 µl
Protocols/main application on this instrument	Purification of DNA, mRNA, total RNA, and viral RNA and DNA
Samples per run; throughput	1 to 13 samples per run
Technical data of the instrument	Weight 55 kg, 100–240 V AC, 50–60 Hz
Technology	Magnetic-particle technology

Table-2. PCR Thermal Cycler Machine

Instrument	Operating Parameters
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	5.0ºC/Sec.
Sample ramp rate	4.4ºC/S
Temperature range	4-99ºC/S
Temperature accuracy	±0.2ºC
Customized programming	Allows a maximum of 20 steps and 99 cycles
Display	LCD touch screen, about 8.5 in

Table-3 Genetic Analyser-3500xL

Instrument	Operating Parameters
Fragment Size(bp)	500bp
No. of Markers	17
Polymer	POP4
Detector	CCD
Oven Temp	60°c
Column Size	36cm
Software	Gene Mapper®

A) DNA extraction from swabs

Piece from swab and blood mixed semen stain were taken into 2ml micro centrifuge tube, 230µL of Prep Filer BTA Lysis Buffer, 7µL of Proteinase K and 3 µL of DTT were added. Tubes were kept at 56^{0C} with shaking overnight. Next day supernatant was filtered, and filtrate obtained was loaded with Prep Filer BTA cartridge in the magnetic bead based liquid handling system of Automate Express (Applied Biosystem) instrument, BTA protocol of Automate Express instrument was applied to extract amplifiable DNA.

B) DNA extraction from blood

Extraction of DNA from control blood samples is carried out by using silica column of Himedia.

200 µL of blood was taken into 2ml micro centrifuge tube. 20 µL of Proteinase K, 200 µL of Himedia Lysis Solution (C1) were added. Tubes were incubated at 56^{0C} with shaking for 10 mins. After incubation chilled ethanol was added and transferred the lysate to the column. After washing twice using wash solution elution buffer was added to elute the DNA.

PCR PROTOCOL: - (Refer Fig.1)

The DNA extracted from blood samples of all twelve suspects and swab samples from the victim and blood mixed semen stain from crime scene was typed at 17 Y-STR loci using PCR amplification technique. STR genotyping was carried out using the AmpFlSTR Yfiler PCR Amplification kit (Applied Biosystems).

AmpFlSTR PCR reaction mix: 10 µL

AmpFlSTR Primer set: 5 µL

DNA Sample: 10µL

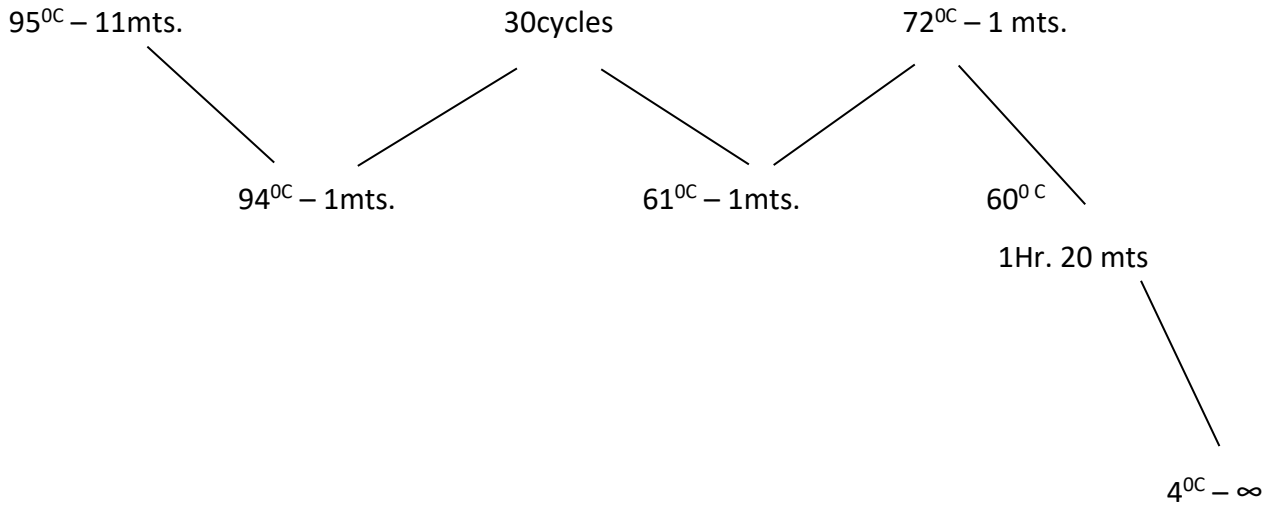

Fig.1

Genotyping: -

Y-STR genotyping is detected and analysed on 3500xL Genetic Analyser (Applied Biosystems) instrument by capillary electrophoresis of single stranded amplified DNA fragments includes following steps.

- Denaturation of PCR product.
- Load the mixture in auto sampler tray of genetic analyzer.
- Electrophoresis was done through fine glass capillary filled with polymeric gel.

- DNA fragments travel through capillary according to their size & reach the window which coincides with the Laser device in the instrument.
- Laser excites the fluorescently labeled DNA fragments.
- CCD Camera behind the window records the excitation peaks.
- Excitation peaks for 16 different loci are obtained.
- For each set of sample standard allelic ladder is run.
- DATA COLLECTION software collects the data of these excitation peaks

RESULTS AND DISCUSSION:

A 25-year-old physiotherapist who was living with her family in a chawl, raped and murdered by unknown accused in Dec 2016. A school dropout accused came to Mumbai six years ago and was working in a jewelry shop 400 meters from the victim. On the unfortunate night, between 2-2.30 a.m., accused entered the victim's mezzanine floor house as she was fast asleep and had forgotten to lock the door. Accused raped and murdered the victim. He covered victim's body with her clothes and books and lit a fire. After post mortem swab samples of victim and blood samples of twelve suspects were submitted to FSL, Kalina, Mumbai for DNA profiling. Along with that victim's cloth articles and crime scene articles were submitted to FSL, Kalina, Mumbai.

Male DNA profile obtained from swab samples from private parts of victim matched with one of the suspects. Similarly, DNA profile of blood mixed semen stain detected on bed sheet from crime scene matched with DNA profile of the same suspect. Thus, swabs from the victim and exhibits found on crime scene proved to be very useful to solve the case.

Use of Yfiler kit in investigation of this case helped to zero on accurate suspect out of twelve suspects. Thus, with the use of DNA fingerprinting strong evidence against accused is obtained, which can help the victim and her reltives for justice.

After the complete study of the case, DNA was extracted from Swab of perianal area, Swab of vaginal and high cervix area, Swab from perivaginal area and blood mixed semen detected on chaddar from crime scene by using AutoMate Express Forensic DNA Extraction System.This is a magnetic bead based liquid handling system. Blood of tweleve suspects was extracted by using Column based blood extraction kit (Hi-Media). Extracted DNA was amplified by using PCR amplification technique. Electropherograms (DNA profiles) of above samples of deceased victim and accused were generated by capillary electrophoresis (3500xL Gene Analyser System) with the help of 17 Yfiler STR technique established by Applied Bio-systems.

The DNA extracted from swab of perianal area, Swab of vaginal and high cervix area and Swab from perivaginal area of deceased victim was typed at 17Y STR LOCI using Y- filer PCR amplification technique.

The DNA extracted from blood mixed semen detected on ex.4 chaddar and blood sample of 12 suspects was typed at 17Y STR LOCI using Y- filer PCR amplification technique. (Refer Table No.4)

Table 4-The results of DNA typing is summarized below: -

STR LOCUS	GENOTYPE				
	Swab of perianal area	Swab of vaginal and high cervix area	Swab from perivaginal area	Blood mixed semen detected on chaddar from crime scene	Suspect No.12
BDYS456	12	12	12	12	12
BDYS389I	14	14	14	14	14
BDYS390	21	21	21	21	21
BDYS389II	32	32	32	32	32
GDYS458	17	17	17	17	17
GDYS19	16	16	16	16	16
GDYS385	13,18	13,18	13,18	13,18	13,18
YDYS393	13	13	13	13	13
YDYS391	10	10	10	10	10
YDYS439	13	13	13	13	13
YDYS635	22	22	22	22	22

YDYS392	-	11	11	11	11
RYGATAH4	11	11	11	11	11
RDYS437	15	15	15	15	15
RDYS438	11	11	11	11	11
RDYS448	18	18	18	18	18

Table No.4 clearly shows that Male haplotypes obtained from Swab of perianal area, Swab of vaginal and high cervix area, Swab from perivaginal area and blood mixed semen detected on chaddar from crime scene matched with the male haplotypes of blood sample of Suspect no. 12. This shows that male haplotypes obtained from Swab of perianal area, Swab of vaginal and high cervix area, Swab from perivaginal area and blood mixed semen detected on chaddar from crime scene and male haplotypes of blood sample of Suspect no. 12 are from the same paternal progeny.

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