STR TYPING: BIOLOGICAL PROBE FOR CONVICTION OF DACOITY & SEXUAL ASSAULT CASE IN RUNNING TRAIN

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Abstract -

Short tandem repeat (STR) typing continues to be the primary workhorse in forensic DNA profiling. Therefore, the present review discusses the prominent role of STR marker in criminal justice system. All over the world, deoxyribonucleic acid (DNA) profiling provides evidence that may be used to convict criminals, as an irrefutable proof of wrongful convictions, invaluable links to the actual perpetrators of crimes, and could also deter some offenders from committing more serious offences. Clearly, DNA profiling tools have also aided forensic scientists to re- evaluate old cases that were considered closed as a result of inadequate evidence [1].

Use of Short Tandem repeats in forensic casework has become an important tool to reach the real culprit in criminal cases like rape, murder, robbery etc. Most of the time due to the lack of circumstantial evidence judicial system becomes a strong benefit to the accused. In Maharashtra day by day rate of heinous crimes and lot of rape crimes have increased. In rape case medical examination sampling by medical officer play an important role to avoid the loss of trace biological evidence. In sexual offences, specifically semen on victim‟s clothes or biological samples prove involvement of accused in the crime. But in some sexual offence 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 or vice versa helps to prove the rape crime.

As per Railway ministry records, about 20 percent of the 2.3 crore people who travel daily by Indian railways are women [2]. Several incidents of crime against women in trains and railway premises have been recorded in the past decades. In order to mitigate this, Indian Railways has come up with some steps, said an official statement issued by the Ministry of Railways. Women passengers are especially vulnerable in long-distance trains. We need well-armed police in the bogies. Besides pulling chains, there is a need for alarms that can be connected straight to train drivers‟ cabin and can ring in the train itself.

In Oct 2021 a 20-year-old women was molested by eight men in the running train, four people have been arrested. Taking advantage of the darkness, the dacoits robbed, but even more shamefully assaulted women. The molestation incident took place when the express train was running between Igatpuri and Kasara, Maharashtra around 8 pm on Friday, according to Government Railway Police (GRP).

“The train had just left from the Igatpuri station and was slowing down before entering a tunnel, when the accused boarded the sleeper bogie. They were armed with knives and leather belts, and started threatening the

passengers," said a GRP officer, who did not want to be identified. The accused then allegedly dragged the survivor to a corner and molested her, the officer said. According to report, there were eight men who started behaving aggressively on the train and they looked like they were under the influence of alcohol or some drug. The accused persons had weapons like fighters and knives, which they used to threaten and rob passengers.

"The victim has been taken for a medical examination and Police have booked the four accused under sections 395 (dacoity), 397 (robbery or dacoity), 376(D) , 354 (assault or use of criminal force to any woman, intending to outrage her modesty), 354(B) (assault or use of criminal force to any woman or abetment of such act with the intention of disrobing or compelling her to be naked of the Indian Penal Code (IPC), and the Railways Act.

In the instant case, victim woman"s husband (Injured 1) and a witness (Injured 2) blood got transferred on accused clothes and seized weapons (Fighters) from Accused. As the DNA profiling proved their involvement in the crime.

Introduction

Recently sexual offences against women and number of minor victims have been increased day by day. Census data from 2011 shows that in India 472 million children are below age of eighteen and out of them 225 million are girls [3]. Due to pressure and lack of knowledge, these children fear to talk about atrocious crime. India has taken lot of precautions since the „Nirbhaya" case happened and made legal provision of new act "Protection of Children from Sexual Offences Act 2012 (POCSO Act 2012)" and continuously monitoring these cases [4] . It is difficult to identify a criminal just by examining the trace of blood on crime scene and the garments. In sexual offences, a specifically biological fluid on victim"s clothes 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. 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 [5]. While performing forensic analysis of exhibits seized in sexual offences, though semen is absent in medical samples like vaginal swab, pubic hair or on her clothes, if blood of accused is found on her exhibits, it can play a vital evidence to prove the crime.

DNA profiling in forensic science is on the analysis of short tandem repeat (STR) loci using PCR. It is the technique of choice for the national strategy to create criminal intelligence databases. Apart from the increased sensitivity inherent with any PCR technique, with STRs there is also the advantage of definitive allelic identification. This is a consequence of lower measurement errors associated with the use of polyacrylamide gel electrophoresis to detect DNA fragments ranging between 200-400 bp in size [6-8] .

Materials and Methods:

Short tandem repeat (STR) typing methods are widely used today for human identity testingapplications including forensic DNA analysis. Following multiplex PCR amplification, DNAsamples containing the length-variant STR alleles are typically separated by capillaryelectrophoresis and genotyped by comparison to an allelic ladder supplied with a commercial kit.This work provides a brief perspective on the technologies and issues involved in STR typing [9]. **Materials-**

Table 1: Reagents and Chemical Reagents

Reagents and Chemical Reagents	Parameters
Lysis Buffer	1 ml Tris HCL-100ml 0.5ml 0.5ml EDTA Buffer -10ml 5M, Nacl- 10mlMake up volume
Proteinase K	Appearance- Colorless solution in 50% glycerol, cont.20mM Tris., 1mM CaCl2, PH ca.7.4Concentration 20mg solid/ml
Investigator kit Identifier®	Buffer G2, Prot. K, Carrier RNA,Amp FSTR
PCR amplification Kit	Allelic Ladder, Ampli Taq Gold® DNApolymerase, Primers,
Hi-DiTM Formamide	CAS 75-12-7, CAS 60-00-4
Size Standard	GeneScanTM-500, LIZ TM

Table-2:EZ1 Automate DNA Extraction System

Instrument Operating	Parameters
Kits designed for this instrument	QIAGEN EZ1 KitsPipeting
range	50-1000 µl
Protocols/main application on this instrument	Purification of DNA, mRNA, total RNA, and viralRNA and DNA
DNA Samples per run	Throughput 14 samples per run
Technology	Magnetic-particle technology

Table-3: Polymerase Chain Reaction Thermal Cycler Machine

Instrument Operating	Parameters
Capacity	96 well x 0.2ml PCR tubes/one 96 well plate
Heating/cooling	Peltier based Capable of testing temperaturesDenaturation, Annealing & Extension steps
Block ramp rate	5.0ºC/Sec. Sample ramp rate 4.4ºC/S
Temperature range	4-99º C/S
Temperature accuracy	± 0.2º C
Customized programming	Allows a maximum of 20 steps and 99 cycles
Display	LCD touch screen, about 8.5 in

Steps used in analysis: - Detection of Biological fluids: -

In this case, we received different cloth articles of accused and injured collected by investigation officer, reference blood samples of injured and accused. While analysis, the blood was detected on injured as well as accused‟s cloth, Routine Kastle -Meyer solution was used for detection of blood.

Extraction of DNA: -

DNA was extracted from blood detected on cloth articles of accused, injured , reference blood sample of injured and reference blood sample of accused. The DNA extraction was done using Automate Express machine using Prep Filer™ Express DNA extraction kit. The Prep Filer ™ Forensic DNA extraction Kit (Applied Biosystems, Foster City, CA) is efficient for isolation of DNA from a variety of biological samples that contain small quantities of biological material so that if traces of undetected are present in blood on cloths or medical samples.

The protocol used for extraction was as follows: -

- Blood stains on all the positive articles were cut into small 1 x 1 mm pieces and were placed in 2ml micro centrifuge tube. 500 µl Lysis buffer from Prep Filer Express F DNA extraction kit (19) was added to all the sample tubes.
- The sample tubes were kept on thermo shaker at 750 rpm at 70 ˚C for 40 min [10-11].
- The tubes were then centrifuged at 10,000 rpm for 2 min .
- Cartridges from Prep Filer 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.
- After completion of program, elution tubes containing extracted DNA in highly pure form were stored at 4˚C till the next PCR amplification process.

- Different methods are available for extraction of DNA. This organic extraction method was employed for extraction of DNA from reference blood samples of accused and injured in both the cases. In organic extraction method, samples were lysed using Forensic Buffer (pH 8), Proteinase K, and Sodium Dodecyl Sulphate. Further samples were incubated for 2 hrs at 56˚C and Phenol: Chloroform: Isoamyl alcohol previously prepared solution was added.
- The aqueous layer containing DNA separated and treated with 2 M Sodium Acetate and the DNA was precipitated using chilled Isopropanol.
- Finally extracted DNA dissolved in TE buffer (pH 7).

Quantification of DNA:-

Extracted DNA was quantified using Quantifiler human DNA kit on 7500 Real Time PCR System (Applied Biosystems) according to the protocol. Proper diluted DNA sample was used for further PCR reaction. Master mix used for Polymerase Chain Reaction was AmpFISTR PCR reaction mix: 10.5 µl AmpFISTR Primer Set: 5.5 µl Polymerase: 0.55 µl Volume of Master mix used: 15

HiDi Formamide and Liz 600 size Standard.

PCR PROTOCOL:-STR genotyping was carried out using the Amp FISTR identifier PCR Amplification kit (Applied Biosystems, Foster City)

Amp FISTR PCR reaction mix:	10.5ul
AmpliTaq Gold DNA polymerase:	0.5ul
AmpFISTR Primer set:	5.5ul
DNA Sample:	10ul

95^0 C– 11mins. 28cycles 60^0 C– 60mts.

94^0 C– 1min. 59^0 C– 1min. 72^0 C– 1mts. 4^0 C– ∞

STR Genotyping: -

After completion of PCR amplification of DNA, amplified DNA products were analyzed on 3500 Genetic Analyzer and processed using Gene Mapper® ID-X Software V 1.5 according to manufacturer recommended procedure. Simultaneous amplification of 16 STR Loci was achieved. DNA profiles obtained from above samples in both cases were interpreted and compared with each other.

RESULTS OF ANALYSIS-

After Detection and conformation of bloods on cloths of injured 1 & 2 and 8 accused, we proceed for DNA analysis as follows-

The DNA extracted from ex.4 Blood stain cuttings from Full Shirt, ex.5 Blood stain cuttings from Full Pant , ex.1 Prepared Blood stain from blood of Accused 1, ex.1 Prepared Blood stain from blood of Accused 2, ex.1 Prepared Blood stain from blood of Injured 1, ex.1 Prepared Blood stain from blood of Injured 2, ex.1 Prepared Blood stain from blood of Accused 3, ex.1 Prepared Blood stain from blood of Accused 4, ex.1 Prepared Blood stain from blood of Accused 5, ex.1 Prepared Blood stain from blood of Accused 6, ex.1 Prepared Blood stain from blood of Accused 7, ex.1 Prepared Blood stain from blood of Accused 8 and ex.1 Blood stain cuttings from Full Shirt, ex.3 Blood stain cuttings from Full T-Shirt, ex.7 Blood stain cuttings from Full Jacket, ex.15 Blood stain collected from Fighter 1, ex.16 Blood stain collected from Fighter 2 of this case was typed at 15 STR LOCI and gender specific Amelogenin locus using PCR Amplification technique..

The results of DNA typing are summarized as follows: ---

STR LOCUS	GENOTYPE																
	Accused 2		Accused 1	Accused 2	Injured 1	Injured 2	Accused 3	Accused 4	Accused 5	Accused 6	Accused 7	Accused 8	Injured 1	Accused 8	Accused 4	Weapon 1	Weapon 2
	Ex.4 Blood Stain Cuttings from Full Shirt	Ex.5 Blood Stain Cuttings from Full Pant	Ex.1 Prepared Blood Stain from Blood of A1	Ex.1 Prepared Blood Stain from Blood of A2	Ex.1 Prepared Blood Stain from Blood of Injured 1	Ex.1 Prepared Blood Stain from Blood of Injured 2	Ex.1 Prepared Blood Stain from Blood of A3	Ex.1 Prepared Blood Stain from Blood of A4	Ex.1 Prepared Blood Stain from Blood of A5	Ex.1 Prepared Blood Stain from Blood Of A6	Ex.1 Prepared Blood Stain from Blood of A7	Ex.1 Prepared Blood Stain from Blood of A8	Ex.1 Blood Stain Cuttings from Full Shirt	Ex.3 Blood Stain Cuttings from Full T-Shirt	Ex.7 Blood Stain Cuttings from Full Jacket	Ex.15 Blood Stain Collected from Fighter 1	Ex.16 Blood Stain Collected from Fighter 2
D8S1179	14,17	13,15	12,14	14,17	13,15	14,14	10,12	13,14	14,15	10,10	8,15	13,14	13,15	13,14	13,15	14,14	13,15
D21S11	29,31.2	28,28	30,31.2	29,31.2	28,28	28,31.2	29,32.2	31.2,32.2	31.2,31.2	29,30	29,30	29,30	28,28	29,30	28,28	28,31.2	28,28
D7S820	10,11	10,11	10,11	10,11	10,11	8,10	8,11	12,12	11,11	11,12	8,8	8,12	10,11	8,12	10,11	8,10	10,11
CSF1PO	11,12	11,11	11,12	11,12	11,11	12,13	11,12	11,12	10,12	11,12	10,12	11,11	11,11	11,11	11,11	12,13	11,11
D3S1358	15,17	15,18	16,16	15,17	15,18	16,17	15,16	16,16	15,18	15,16	16,16	15,17	15,18	15,17	15,18	16,17	15,18
THO1	8,9	8,9.3	8,9	8,9	8,9.3	6,9	9,9.3	6,6	6,9	6,9.3	6,7	6,9	8,9.3	6,9	8,9.3	6,9	8,9.3
D13S317	8,8	11,12	8,11	8,8	11,12	8,12	11,12	12,13	11,14	9,13	11,12	10,11	11,12	10,11	11,12	8,12	11,12
D16S539	9,11	8,11	10,12	9,11	8,11	11,12	13,13	11,12	10,13	8,11	11,12	12,14	8,11	12,14	8,11	11,12	8,11
D2S1338	23,25	18,19	21,23	23,25	18,19	21,24	18,19	23,23	18,18	19,23	18,18	20,21	18,19	20,21	18,19	21,24	18,19
D19S433	14,15.2	14,14.2	13,14	14,15.2	14,14.2	12,13	14.2,16	13,13	13,16.2	13,13	13,15.2	12,13	14,14.2	12,13	14,14.2	12,13	14,14.2
vWA	16,19	16,17	17,18	16,19	16,17	16,19	17,17	18,18	15,18	16,17	17,17	16,16	16,17	16,16	16,17	16,19	16,17
TPOX	8,10	8,8	8,9	8,10	8,8	8,8	9,11	10,11	9,9	10,12	8,11	8,11	8,8	8,11	8,8	8,8	8,8
D18S51	14,14	14,14	13,16	14,14	14,14	13,14	13,13	14,14	14,14	13,15	13,14	14,17	14,14	14,17	14,14	13,14	14,14
AMEL	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	10,11	10,12	10,11	10,11	10,12	11,12	11,12	10,13	9,12	11,13	9,11	11,12	10,12	11,12	10,12	11,12	10,12
FGA	22,24	23,24	20,23	22,24	23,24	22,23	22,24	24,24.2	22,22.2	23,27	20,23	22,24.2	23,24	22,24.2	23,24	22,23	23,24

Interpretation:-

1.) DNA profile obtained from ex.4 Blood stain cuttings from Full Shirt is of male origin and **matched** with DNA profile obtained from ex.1 Prepared Blood stain from blood of accused 2. DNA profiles obtained from ex.5 Blood stain cuttings from Full Pant, ex.1 Blood stain cutting from Full Shirt, ex.7 Blood stain cutting from Full Jacket, ex.16 Blood stain collected from Fighter 2 are identical and from one and same source of male origin and **matched** with DNA profile obtained from ex.1 Prepared Blood stain from blood of Injured 1. DNA profile obtained from ex.3 Blood stain cuttings from Full T-Shirt is of male origin and **matched** with DNA profile obtained from ex.1 Prepared Blood stain from blood of accused 8.DNA profile obtained from ex.15 Blood stain collected from Fighter 1 is of male origin and **matched** with DNA profile obtained from ex.1 Prepared Blood stain from blood of Injured 2.

2) Control DNA profiles are obtained from ex.1 Prepared Blood stain from blood of accused 1, ex.1 Prepared Blood stain from blood of accused 3, ex.1 Prepared Blood stain from blood of accused 4, ex.1 Prepared Blood stain from blood of accused 5, ex.1 Prepared Blood stain from blood of accused 6 and ex.1 Prepared Blood stain from blood of accused 7 **did not match with** DNA profiles obtained from ex.4 Blood stain cuttings from Full Shirt, ex.5 Blood stain cuttings from Full Pant and ex.1 Blood stain cutting from Full Shirt, ex.3 Blood stain cutting from Full T-Shirt, ex.7 Blood stain cutting from Full Jacket, ex.15 Blood stain collected from Fighter 1, ex.16 Blood stain collected from Fighter 2 respectively.

Conclusion -

The amount of DNA required for analysis can be obtained from even a miniscule biological sample like blood, saliva and semen, which allows investigating agencies to match crime scene evidence with suspects. However, because forensics is a science largely rooted in probabilities, even a confirmed "match" does not supply concrete proof of guilt. In addition, DNA databases designed to simplify the process of connecting past offenders to recent crimes are fraught with concerns involving individual genetic rights, as well as problems related to delayed sample entry, both of which hinder the ultimate usefulness of these databases. As a result, even though forensics is undeniably important to the modern justice system, its personal ramifications and ethical questions are topics of continuing discussion within the scientific, law enforcement, and legal communities. It is definitely a sufficient evidence to prove the guilt of accused in the court.

Here, DNA profiles obtained from full pant (Accused 2), Blood stain cuttings from Full Shirt (Injured 1), Blood stain cuttings from Full Jacket (Accused 4), Blood stain collected from Fighter 2 are identical and from one and same source of male origin and **matched** with DNA profile obtained from Prepared Blood stain from blood of Injured 1.

From above DNA interpretation results it is revealed that victim women‟s husband got injured during dacoity and molestation attempt to victim in running train. The police are still on the lookout for other perpetrators while continuing to investigate the case. It is also being reported that the woman‟s husband and

a co-passenger got injured while trying to prevent the crime.

The running train incident is enough to bring the bile up in persons. The point is what we learn and do after this, that will be a mirror about just how serious we are about women‟s safety. The case has test again raised questions about the safety of women in the country.

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