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ORIGINAL ARTICLE

Genetic Profiling of Short Tandem Repeat (STR) in forensic science to Crackdown complex cases of sexual abuses

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ABSTRACT
Application of Y-STR in forensic science lab is common. It provides sustainable evidence which has huge impact in court of law mostly in complex cases. Sexual assault case is one of the most underreported criminal offences in India. Social structure of shaming has reduced the anticipated reaction from victims, and overreacted laws have been misused by section of oppressed. Therefore, evidence based charge sheets can have larger impact on the degree of justice. In this study, we investigated a sexual assault case with both autosomal and Y chromosome STRs analysis. A total of 23 Y-STR loci, 20 autosomal STR loci and one amelogenin were investigated in various exhibits collected from crime scene, victim and accused. In the present case both the suspects were found as a perpetrator of male DNA on the undergarments of victim. We found that all exhibits except for exhibit 22 contained XY sex chromosomes. All distinctive locus present on the suspect's Y chromosomes, were found in exhibit 1 and 2 collected from victim. Locus for markers such as DYS389II, DYS19, DYS385a/b, DYS439, DYS438, DYS635, DYS456, DYS533, DYS576, and DYS570, confirmed definitively that both prime suspects were involved in this crime.
Key Words: *Y-STR, Autosomal STRs, Forensic science, Sexual assault*

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INTRODUCTION

Sexual assaults are most under reported crime in the world creating significant gap in justice, thus, causing ever growing incidents around the world. It is not only under reported but also lack of conviction during court trail. Jewkes and Abrahams [11] reported that among age of 17-48 there are more than 2 incidents of forced sex out of 100 occur in a year, which was further explained that reports are registered at police stations. Authors strongly believe that the data is only the tip of the iceberg, our statement is further supported by World Health Organization [23].

According to National Crime Record Bureau (NCRB) in India, the number of reported rapes have increased by more than 700% since 1971. For a long period of time after independence most of the rape cases were unfortunately not recorded [16, 4]. A study report that most of the cases of forced sex goes unregistered due to culturally male dominated society, leading to as much as 84% cases go unreported [8].

Initially, rape cases were regarded as crime against property but not against a person. Through evolution in criminalization set up it was considered as patriarchal inheritance right and female reproductive capacity thus stringent laws were only for unmarried virgin [20]. It is quite difficult to prove rape cases in court of law even with valid witnesses. As per most of the jurisprudence in the world, the basic elements of rape are use of force and in absence of mutual consent [1].

A method for identification and definition of forced sex varies throughout countries, creating suggestive loopholes in cases which become advantageous to rape culprits. The scientific evidences play vital role in cases of forced sex, mainly in reconstructing the crime scene [7].

In past two decades, forensic science has revolutionized criminal investigations and prosecutions [19]. In case of rape forensic science based on recommendation by agencies have revealed sexual assault kits. These kits include biological samples such as; blood, saliva, semen etc. along with statements of medical experts, photographs of injuries, victim's statement [17, 8].

It is prerequisite in case of rape that samples are tested for semen, in absence of which case weaken substantially. In case of low quantity of semen techniques such as acid phosphatase test is conducted to identify stains, which can further be processed for DNA profiling [14]. DNA profiling can instantly distinguish perpetrator's profile from victim's profile. For cases in which delayed medical examination were conducted are often fail to provide robust scientific evidence. Through PCR based STR techniques, objects of epithelial cells containing semen profile can be examined for its identity and therefore electropherograms are matched with the potential perpetrator/s. Autosomal STRs(through autosomes) are inherited by both the parents while Y-STRs are inherited by paterilineal side. Evidence material in sexual assault cases consists stains of body fluids that may be contributed by both the victim and suspect. As a part of this both autosomal STR as well as Y-STR study proved helpful in solving complex mixed samples in sexual assault cases. This kit co-amplifies 17 Y-STRs, including the markers defined as the "European minimal haplotype" viz. DYS19, DYS385 a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393 [6]. Additionally, two separate loci viz. DYS438 and DYS439 were added according to the methods explained by SWGDAM (Scientific Working Group on DNA Analysis Methods) [13]. Further to this highly polymorphic loci such as; DYS437, DYS448, DYS456, DYS458, DYS635 and Y_GATA_H4 were also added to the list of STRs [15]. STRs such as DYS481, DYS570, DYS576 and DYS643 were also added. These STRs are considered as minimal or extended haplotype of Y-STRs [5]. Apart from these two new highly discriminating Y-STR loci were also investigated, labelled as; DYS549 and DYS533 [2]. Therefore, DNA profiling provides an edge over conventional differential DNA extraction, specifically in mixed a sample [19].

In this study, we examined a child sexual abuse case where limited information was available and multiple perpetrators were involved. This study was conducted to affirm that the Y-STR markers are best tool in collecting scientific evidences against complex forced sexual assaults. A total of 23 Y-STR loci, 20 autosomal STR loci and one amelogenin were investigated in various exhibits of victim and suspects.

CASE PRESENTATION

Brief about the case

In this case study, we conducted a series of scientific examination on the biological evidences collected from the crime scene, victim and suspects. A case registered under sections IPC- 363, 366(cb), 342, 376(D), 504 IPC, 5/6 POCSO act 2012, 66(E), 67(cb), IT act 2000, for child sexual assault was expected for DNA examination. As per the registered complaint, two suspects kidnapped a minor girl from outside a Computer Academy. Thereafter, took her to alone place and sexually assaulted.

MATERIAL AND METHODS

Collection of samples

Samples of victim and both the suspects were collected during medical examination as per guide line of Cr.P.C. All the samples/exhibits collected during investigation of the case are listed in table-1.

Table-1: Samples collected from the crime scene, victim, and alleged accused

S.NO.	PACKET	EXHIBIT	EXHIBIT NAME
1	A	1	Underwear of victim
		2	Underwear of victim
2	D	3	Underwear of suspect Jitendra
3	E	4	Underwear of suspect Mahesh
4	B	5	Vaginal Swab of victim
5	C	6	Cervical Swab of victim
6	A	7	Pubic hair of victim
7	ABCD	8	Glans Penis (Smegma) Slide of suspect Jitendra
		9	Glans Penis (Smegma) Swab of suspect Jitendra
		10	Public hair of suspect Jitendra
		11	Blood of suspect Jitendra
		12	Blood of suspect Jitendra on FTA Card

		13	Saliva Swab of suspect Jitendra
		14	Saliva slide of suspect Jitendra
8	ABCD	15	Glans Penis (Smegma) Slide of suspect Mahesh
		16	Glans Penis (Smegma) Swab of suspect Mahesh
		17	Public hair of suspect Mahesh
		18	Saliva Swab of suspect Mahesh
		19	Saliva slide of suspect Mahesh
		20	Blood of suspect Mahesh
		21	Blood of suspect Mahesh on FTA Card
9	D	-	Saliva sample of victim
10	E	-	Venous blood of victim
11	F	22	Blood of victim on FTA card

DNA EXTRACTION

Forensic samples were processed for biological examination. During biological examination human sperm cells were detected on underwear of victim as well as vaginal samples of victim. The samples which were found positive for semen, along with control blood sample of victim and both the suspects were subjected to DNA isolation. DNA was extracted using Auto Mate Express™ forensic DNA Extraction system (Thermo Fisher Scientific, CA, USA) with the PrepFiler™ Express DNA extraction kits as per recommended protocol of the manufacturer. Blood samples of victim and both the suspects on FTA cards (Whatman, GE healthcare) were processed for direct PCR amplification as recommended protocol of the manufacturer.

DNA Quantitation

Extracted DNA was quantified using Quantifiler duo kit (ThermoFisher scientific, USA) on RT-PCR 7500 (ThermoFisher scientific, USA) as per recommended protocol.

PCR AMPLIFICATION

Quantified DNA was processed for PCR amplification kits. A total of 20 autosomal markers *viz*. D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, FGA, D1S1656, D6S1043, PENTA-E, PENTA-D, D12S391 along with sex determining marker Amelogenin were amplified using PowerPlex® 21 system kit (Promega, USA) as per recommended protocol except half reaction volume.

A total of 23 Y chromosomal STR loci *viz*. DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385a, DYS385b, DYS456 and Y_GATA_H4 were amplified using PowerPlex® Y-23 system kit (Promega, USA) as per recommended protocol except half reaction volume.

Genotyping and Data analysis

Amplified PCR amplicon were separated on Genetic Analyzer 3130 using POP™-4 (Performance Optimized Polymer), 36cm Capillary array. Each amplified samples mixed with 0.5 µL WEN ILS 500 standard and 9.5 µL Hi-Di Formamide. Samples were injected at 1.2 kV for 5s. Electrophoresis results were analyzed with GeneMapper ID-X v1.1 software. Alleles were named according to the contained number of repeat units under the recommendations of the DNA Commission of the International Society for Forensic Genetics [6, 10].

RESULTS

The alleles of Autosomal DNA profiles obtained from control blood sample of both the suspect are accounted in the Mixed DNA profile obtained from semen stains detected on exhibit no. 1 (Underwear of victim). The Autosomal DNA profiles obtained from Underwear of both the suspect were matching with their respective control blood samples. At Amelogenin locus both the X and Y alleles were amplified on exhibit no. 5(vaginal swab of victim) and 6(cervical swab of victim). Autosomal DNA profile was not obtained from exhibit no. 2(underwear of victim). Interestingly, most of the markers in exhibit 1 contained at least one of the locus from exhibit 12 and 21 which was not present in exhibit 22.

Mixed male DNA profile was obtained from exhibit no. 1 (Underwear of victim) and 2 (underwear of victim). The alleles of Male DNA profiles of both the suspects are accounted in the Mixed male DNA profile obtained from exhibit no 1 (Underwear of victim) and 2 (underwear of victim). The Y marker DNA profiles

obtained from underwear of both the suspects is consistent with male DNA profile of their own control blood samples.

DISCUSSION

Over the last few years, significant advancements were made in laboratory practices, software systems and mathematical algorithm model for the practical analysis of mixed DNA samples. The methods for the analysis of mixed DNA samples often yield low detection rates, reducing their practical usefulness in criminal investigations due to failure to meet out the relevant legal standards provided by court systems. However, improvement in the amplification and sensitivity of testing have facilitated the use of DNA testing in more criminal cases, such as rape, where very small amount of suspect DNA may be mixed with victim's DNA.

Y-STR analysis is a strong and effective way to produce evidences in the court of law, specifically in case of rape incidents where victims are compromised, hostile or dead. Initial autosomal STRs evidences are robust and confirmatory to results observed form Y-STR markers. In this case study, Autosomal and Y STR confirmed the involvement of both the main suspects. Our study also revealed that loci for all Y markers (both suspects) were present in the samples collected from victim's undergarment which are affirmed by Autosomal STRs (Table-2).

This case study indicated that the mixed DNA profile of two perpetrators can be deciphered with the use of Y STR markers. Surprisingly instead of amplification of Autosomal markers on exhibit no.2(underwear of victim), mixed male DNA profile was obtained from both the underwear's of victim by using male specific Y STR markers which shows its high sensitivity indicting that both prime suspects were involved in this crime.

Mathematical evaluation of male DNA profile showed that, 32% differentiating alleles of Y markers such as DYS389I, DYS448, DYS391, DYS481, DYS549, DYS437, DYS390, DYS392, DYS643, DYS393, DYS458, and Y_GATA_H4, were found on both the underwear's of victim (Table-3).

Table-2: Autosomal allelic distribution of STRs loci in collected samples along with its corresponding sex chromosome

Locus	Exhibit No.1	Exhibit No. 3	Exhibit No. 4	Exhibit No. 5	Exhibit No.6	Exhibit No. 12	Exhibit No. 21	Exhibit No. 22
AMELOGENIN	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,X
D3S1358	16,17,18	17,18	16,16	-	-	17,18	16,16	14,17
D1S1656	10,11,12,16	11,12	10,16	-	-	11,12	10,16	11,16
D6S1043	11,12,13	11,13	11,12	-	-	11,13	11,12	11,19
D13S317	11,12,13	13,13	11,12	-	-	13,13	11,12	9,13
PENTA-E	7,11,13	7,11	13,13	-	-	7,11	13,13	13,15
D16S539	10,11,12	10,11	12,12	-	-	10,11	12,12	9,13
D18S51	14,16	14,14	14,16	-	-	14,14	14,16	11,19
D2S1338	19,20,22	22,22	19,20	-	-	22,22	19,20	18,23
CSF1PO	10,12	10,12	10,12	-	-	10,12	10,12	10,11
PENTA-D	9,11,13	9,11	13,13	-	-	9,11	13,13	10,10
TH01	8,9	8,9	8,8	-	-	8,9	8,8	9,9
vWA	14,16,17,19	16,17	14,19	-	-	16,17	14,19	16,17
D21S11	28,29,30,32,2	30,32.2	28,29	-	-	30,32.2	28,29	28,32.2
D7S820	8,12	8,8	8,12	-	-	8,8	8,12	11,12
D5S818	11,12,13	12,13	11,12	-	-	12,13	11,12	9,10
TPOX	8,11	8,11	8,8	-	-	8,11	8,8	8,9
D8S1179	13,14,15,16	13,15	14,16	-	-	13,15	14,16	14,14
D12S391	19,20,22,24,	20,22	19,24	-	-	20,22	19,24	18,18
D19S433	12,15,15.2	12,15	15,15.2	-	-	12,15	15,15.2	13,13
FGA	22,24	22,24	22,22	-	-	22,24	22,22	20,20

Table-3: Specific STR locus distribution on Y chromosome in the collected samples

LOCUS	Exhibit No.1	Exhibit No.2	Exhibit No. 3	Exhibit No. 4	Exhibit No. 12	Exhibit No. 21
DYS576	17,19	17,19	17	19	17	19
DYS389I	13,14	13,14	13	13	13	13
DYS448	19	19	19	19	19	19
DYS389II	29,30	29,30	30	29	30	29
DYS19	14,15	14,15	14	15	14	15
DYS391	10	10	10	10	10	10
DYS481	23	23	23	23	23	23
DYS549	13	13	13	13	13	13
DYS533	11,12	11,12	11	12	11	12
DYS438	9,10	9,10	10	9	10	9
DYS437	14	14	14	14	14	14
DYS570	17,18	17,18	17	18	17	18
DYS635	17,20	17,20	17	20	17	20
DYS390	22	22	22	22	22	22
DYS439	10,11	10,11	10	11	10	11
DYS392	11	11	11	11	11	11
DYS643	9	9	9	9	9	9
DYS393	12	12	12	12	12	12
DYS458	17	17	17	17	17	17
DYS385a/b	15,17,18	15,17,18	15,18	15,17	15,18	15,17
DYS456	15,16	15,16	16	15	16	15
Y_GATA_H4	12	12	12	12	12	12

CONCLUSION

Our study confirms that aggressive sexual struggle might have occurred by both accused leading to traces of biological fluids of suspects like semen on the undergarment of victim as well as both the suspects. However, results do not show complete Autosomal DNA profile in vaginal samples and exhibit no.2 (underwear of victim), but due to higher sensitive Y marker, mixed male DNA profile was obtained from both the underwear's of victim which indicate that both the prime suspects are the contributor of male DNA on the underwear's of victim

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