

Y Chromosome STR Typing: A Distinguishing Tool for Exclusion in a Casework of Sexual Assault

Suminder Kaur^{1*}, Monica Lamba¹ and Ritika Gupta²

¹Regional Forensic Science Laboratory, DNA Fingerprinting Unit, Biology Division, New Delhi-110021, India

²Forensic Science Laboratory, DNA Fingerprinting Unit, Biology Division, Delhi-110085, India

Abstract

The sexual assault cases are on a rise in India's capital Delhi (approximate fivefold increase in last 10 years) and so are the cases where men are falsely implicated for the same. Many cases go unreported owing to the social stigma attached. There have been cases where women misused these laws for personal gains [1]. Hence, any sexual assault case should be investigated carefully and impartially so as not to miss a single piece of evidence. We present a similar case study where the crime scene showed signs of violence. The presence of Y-peak on Amelogenin locus in Identifiler STRs in vaginal swabs of victim raised doubt of case being positive but Y-Filer STR helped in distinguishing the male contributor from the alleged accused. This conclusion strongly indicates the power of Y-STRs in forensic DNA analysis –it not only helps in identification of perpetrator but also in exclusion of the innocent.

Keywords: Forensic science; DNA typing; Sexual assault; Identifiler; Y-Short tandem repeats

Introduction

The analysis of STR loci has become a routine procedure in forensic laboratories [1,2]. A Short Tandem Repeat (STR) is a microsatellite, which is polymorphic in nature and is used for genetic fingerprinting. STR markers are highly informative and relatively easy to use [3]. According to the section 376 of the Indian Penal Code (IPC), "Whoever, except in the cases provided for in sub-section, commits rape, shall be punished with rigorous imprisonment of either description for a term which shall not be less than seven years, but which may extend to imprisonment for life, and shall also be liable to fine."

Sexual assault is a crime that disproportionately affects adolescent and young adult women [4]. In the 1980s, forensic biologists began to establish DNA as a pillar of the investigative process. There has since been prolific growth in the application of DNA to forensic cases, and today DNA is one of the most highly regarded tools available to the forensic scientists [5].

Autosomal STR analysis may not be possible if the sample contains an admixture of body fluids other than semen, such as in saliva/saliva mixtures, or fingernail scrapings comprising cells from the victim and cells from the perpetrator [5,6]. It is because autosomal STRs allow the detection of minor components only if they account for more than 5% of the mixture, as the rule of thumb [7,8]. Sex-typing based on amelogenin is an integral part of most identifiler PCR multiplex kits widely used for gender determination and plays an important role in forensic casework and creating DNA database [9].

Y-STRs are routinely used in certain forensic cases [10-16]. The unique biology of the Y chromosome has led to the extensive use in forensic studies in determining identity of male individuals and patrilineal relationships [1,3,6,13,17-21]. Their intended use is not to supersede the current battery of autosomal STR loci but to apply them to certain defined casework situations whereby the traditional autosomal loci would not be expected to yield sufficient probative information [6,20,15,21]. Y-STR multiplexes are being used to enhance the ability to deconvolute complex female-male-male mixed profiles often found in sexual assault cases [22]. Honda reported the application of Y chromosomal DNA analysis in a retrial request case where DNA

was extracted from mixed seminal/vaginal secretion stains collected 25 years ago [12].

Recently we investigated a sexual assault case reported by the Delhi Police team where they found a half-naked woman (aged 32 years) and the accused cab driver (aged 36 years) at the scene of crime. According to FIR (first information report) "She had boarded the cab when there was one other passenger. Once she was alone in the cab, the driver moved to the back seat and tried to force himself upon her. When she protested, he started slapping and punching her. He also threatened to murder her if she shouted. He removed her salwar (a form of baggy trousers) and raped her. Then as the cab moved forward, it was stopped by a police team and the cab driver tried to run away and was caught and arrested". The cab was meticulously examined for every piece of evidence (Figure 1). The cab exhibited signs of struggle (broken hair clip and crushed water bottle).

Material and Method

The exhibits from crime scene and at hospital i.e. vaginal swab, clothes, swab of bite mark of the victim; and blood in gauze, nail clippings and pubic hair of the accused were deposited at Delhi Forensic Science Laboratory (DFSL) by the police team for further processing. All the exhibits were subjected to DNA examination

DNA was isolated using organic Phenol-Chloroform extraction method for blood and saliva stains. Differential isolation protocol was used for seminal stains. Isolated DNA (pellet) obtained was dissolved in 30 µl TE buffer and preserved at 4°C. Dissolved DNA was used for

***Corresponding author:** Dr. Suminder Kaur, Regional Forensic Science Laboratory, DNA Fingerprinting Unit, Biology Division, New Delhi-110021, India, E-mail: ksuminder@gmail.com

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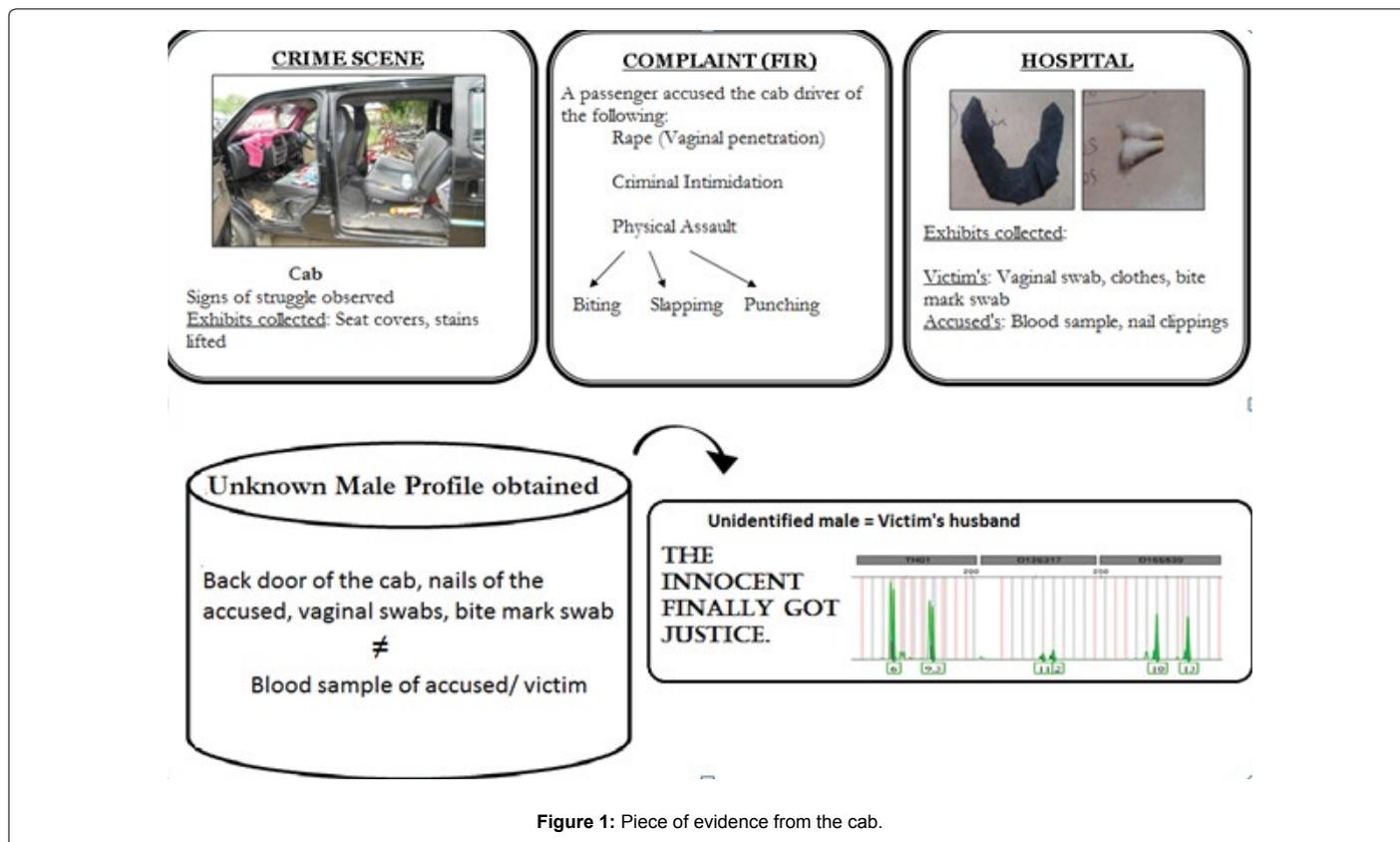


Figure 1: Piece of evidence from the cab.

DNA profiling. Applied Biosystems® 7500 Real-Time PCR was used for quantification of the isolated DNA as per the recommended protocol by the manufacturer. DNA fragments were amplified using AmpFℓSTR® Identifier kit (one Amelogenin locus and 15 autolocus markers) and AmpFℓSTR® Yfiler® PCR Amplification Kit. Capillary electrophoresis of the amplified products was carried out on ABI 3500 XL Genetic Analyzer (PE). Data was analyzed by using Gene Mapper ID-X software (Applied Biosystems, Foster City, CA, USA). In-house control along with the kit control was also used.

The swab (from the bite mark) was amylase positive. The backseat cutting and door taken from the cab were detected to be positive for blood. The vaginal swab and the salwar of the victim (having whitish stains) were detected negative for blood (Benzidine test). They were further processed for DNA examination. Cuttings from four different places (where maximum whitish stains were present) were taken and independently subjected to differential isolation.

Result and Discussion

An interesting finding in the case was that the autosomal male DNA profile generated from the door of cab and the nails of the accused did not match with the DNA profile of the accused. Since the complainant and the police personnel did not mention about the involvement of second male in FIR, this finding startled us. Was it just a coincidence? Did he have nothing to do with the assault? Or was this unidentified male somehow linked to this case? The involvement of the man in this particular case was proved as his profile was also generated from the vaginal swab and bite mark swab, which negated any chances of it being a coincidence.

Table 1 represented the AmpFℓSTR® Identifier® data (autosomal

STR analysis) for the exhibits collected at the crime scene, at hospital and the blood sample of the complainant's husband. The DNA profile of seat cuttings matched with the DNA profile of accused whereas the DNA profile generated from the cab door, nails of the accused and bite mark swab matched with the complainant's husband.

In case of vaginal swabs, the AmpFℓSTR® Identifier® data (autosomal STR analysis) failed to detect the autosomal DNA profile of the semen contributor although the existence of Y-peak at amelogenin locus revealed the presence of minimal male DNA template in the vaginal swab. This proportion of vaginal swab was further evaluated by AmpFℓSTR® Y-STR system.

During interrogation, the taxi driver insisted on having a fight with the victim's husband and further probe was ordered by Hon'ble Court to investigate the role of husband and his blood sample was sent to DFSL.

Table 2 represented the Y-STR profile of the exhibits collected from the scene of crime, collected at hospital and blood sample of the complainant's husband. The Y filer data supported the Identifier data. In this table, Y- DNA profile of the seat cuttings is similar with the Y-DNA profile of accused whereas the Y-DNA profile generated from the cab door, vaginal swab, the bite mark swab and the nails of the accused is similar with the Y-DNA profile generated from the complainant's husband.

Fiction has crept its way into reality in the form of DNA technology. DNA profiling can give a strong indication of guilt or innocence but the corroborative evidence may not always be supportive. Improper collection and preservation can weaken or destroy a potential source of facts in a case [23]. The application of DNA profiling in the criminal investigations is an important aspect of criminal justice system today.

Loci	Exhibits collected from the crime scene				Exhibits collected at hospital						Exhibit collected during further probe as directed by the court	
	Seat cutting		Gauze prepared from the cab door		Cotton wool swab (Bite mark)		Nails of accused		Blood sample of accused		Blood sample of husband of complainant	
D8S1179	8	10	11	14	11	14	11	14	8	10	11	14
D21S11	30	31.2	29	29	29	29	29	29	30	31.2	29	29
D7S820	12	13	10	10	10	10	10	10	12	13	10	10
CSF1PO	10	11	10	10	10	10	10	10	10	11	10	10
D3S1358	16	17	16	17	16	17	16	17	16	17	16	17
THO1	6	7	6	9.3	6	9.3	6	9.3	6	7	6	9.3
D13S317	9	12	11	12	11	12	11	12	9	12	11	12
D16S539	9	13	10	13	10	13	10	13	9	13	10	13
D2S1338	20	24	19	24	19	24	19	24	20	24	19	24
D19S433	14	15	15.2	15.2	15.2	15.2	15.2	15.2	14	15	15.2	15.2
vWA	15	19	16	17	16	17	16	17	15	19	16	17
TPOX	11	11	9	11	9	11	9	11	11	11	9	11
D18S51	12	13	13	15	13	15	13	15	12	13	13	15
D5S818	10	12	10	12	10	12	10	12	10	12	10	12
FGA	20	24	25	27	25	27	25	27	20	24	25	27
AMELOGENIN	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y

Table 1: Autosomal short tandem repeat analysis using AmpFtSTR® Identifier kit.

Loci	Exhibits collected from the crime scene		Exhibits collected at hospital				Exhibit collected during further probe as directed by the court
	Seat cutting	Gauze prepared from the cab door	Cotton wool swab (Bite mark)	Nails of accused	Vaginal Swab	Blood sample of accused	Blood sample of husband of complainant
DYS455	15	15	15	15	15	15	15
DYS389I	13	13	13	13	13	13	13
DYS390	22	24	24	24	24	22	24
DYS389II	29	31	31	31	31	29	31
DYS458	15	16	16	16	16	15	16
DYS19	15	16	16	16	16	15	16
DYS385	11	11	11	11	11	11	11
DYS393	13	13	13	13	13	13	13
DYS391	10	11	11	11	11	10	11
DYS439	10	10	10	10	10	10	10
DYS635	24	23	23	23	23	24	23
DYS392	11	8	8	8	8	11	8
GATA- H4	12	12	12	12	12	12	12
DYS437	14	14	14	14	14	14	14
DYS438	11	11	11	11	11	11	11
DYS448	20	19	19	19	19	20	19

Table 2: Analysis of the samples with Y-chromosome short tandem repeats (Y-STRs) using AmpFtSTR® Yfiler® PCR Amplification Kit.

The forensic use of DNA Profiling (Identifiler coupled with Y-filer) is a major contribution to a technology which can help not only in including the culprit but also to exclude the innocent [24]. The minor DNA component is generally undetectable below a ratio of 1:25–1:50 for autosomal mixtures [19,25]. This diagnostic gap can be closed or at least reduced by Y-STR typing in an alleged sexual assault [19].

Shortly after the characterization and evaluation of the first Y-chromosomal STR polymorphism its usefulness in crime casework was demonstrated by Roewer [19,26,27] where a mixed stain from a vaginal swab of a raped and murdered female victim was resolved by Y-STR analysis and a falsely convicted male was excluded.

In the present case, the first spark of doubt about the authenticity of the case flashed with the absence of accused DNA profile in any of the victim's exhibits (vaginal swabs, bite mark and clothes of the victim) indicated the fabrication of the sexual assault charges. Moreover, the DNA profile generated from the blood stains found in the car was

similar to the DNA profile generated from the blood sample of the alleged accused, which proved that the cab driver was also injured in the scuffle that took place inside the cab [28].

None of the stains in the cab generated a female profile hinted the injuries of being self-inflicted or fabricated. Interestingly, the involvement of another male in the scuffle was proved as his DNA profile was also generated from the nails of the accused and cab door. His presence was also confirmed in the private parts and bite mark of the victim. This person was later identified as the complainant's husband. The bite mark was aggravated by the husband to ensure that the cab driver got punished for his 'violent behavior'. In the present case, DNA fingerprinting technology was successfully applied in solving the criminal case in our laboratory.

The above illustrative case proved that DNA profiling is a tool that is not only used to apprehend the guilty but also to exonerate the innocent. As it often happens in the justice delivery system, conventional evidence

can be tempered with, witnesses may turn hostile, but DNA evidence remains true. The passage of time does not affect it and neither does it change on repetition. DNA evidence thus unravels the truth- it never lies [25].

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