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Case Study: "Substantiate the Offender of Sexual Assault Case Through Their Source Exhibits"

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Abstract

We analyzed forensic DNA samples of a sexual assaulted minor girl, using Y-chromosome-specific DNA short tandem repeats (STR) markers and a panel of autosomal STRs markers. The primary effort was done on the exhibits seized from the source of victim to perceive seminal stain. The possibility of presence of male DNA detection was carried out using Y-STRs kit but male profile could not be detected as their very low concentration compared to female DNA. Another attempt was also done on different exhibits of suspect using autosomal STRs kit to find the presence of victim's blood. Obtained result revealed that a complete autosomal STR DNA profile of the victim was found on the exhibit of the suspect linked the involvement of suspect in the crime. Our experience shows that the main advantage of the using of Y-STR kit to detect Y chromosomal STRs DNA profile in female mixed DNA but when semen present at very low concentration then Y STR DNA profile could not get completely. In a case of sexual assault of minor, excessive bleeding causes dilution of the seminal stain hinder the generation of kit based autosomal as well as Y STR DNA profile. The detection of presence of victim autosomal STR DNA profile on the exhibits of suspect was another approach to confirm the involvement of suspect in committing of crime.

Keywords: DNA fingerprinting; Autosomal; Y-Chromosome; Sexual assault

Introduction

Practice of DNA typing within the criminal justice system in the present scenario rationalized the field of forensic science [1]. With the enhancement of DNA analysis techniques in forensic laboratories, linking of a suspect to a crime and crime scene has become easier. Sexual assault is one of the atrocious crimes, required prominent approach not only to confirm to presence of seminal stain on the source of victim but also prove that allegation imposed by victim on suspect has true [2]. At present, a variety of commercial kit based on Y and autosomal chromosome are using to solve sexual assault case [3,4]. In most of the rape cases, exhibits seized from the source of victim send to the laboratory for finding of spermatozoa. Because exhibits contain both male and female DNA therefore, Y-STR multiplexes are being used to emerge male DNA profile. The value of Y-chromosome in forensic DNA testing is that it is obligate for male individuals. The sex determining region of the Y (SRY) gene determines maleness [5].

With Y chromosome tests, interpretable results can be obtained in some cases where autosomal tests are limited when high concentration of female DNA found with trace quantity of male DNA [6]. Sometimes when the ratio of male- female DNA is as high that is unable to detected by Y- STR kits. Under this condition autosomal loci would not be also expected to yield feasible information regarding the male . In minor rape case, excess bleeding take place that diluted the seminal stain and create the low possibility of detection of male stain. Overindulgence of blood may also get transfer on the cloths/body part of accused also make enable to link the culprit through another direction. By proper collection of exhibits from the source of accused and their careful examination scientifically prove the guilty of accused.

Present study was based upon a sexual assault of minor girl. The DNA was extracted from the source of victim as well as suspect also. Both Y STR and autosomal kits were used on the extracted DNA and result was analyzed.

Case History

A minor girl aged about 8 years old brought in hospital by her parents. After medical examination it was confirmed by doctor that she was sexually assaulted. A case was registered near police station. During medical examination vaginal smear slide, swab, nails and under garments were seized (Figure 1). After registration of case, police started investigation and booked suspect. Pubic hair, underwear and blood sample were seized from the suspect (Figure 2). Samples were sent to the DNA finger printing unit, State Forensic Science Laboratory Sagar (M.P.) for DNA examination.

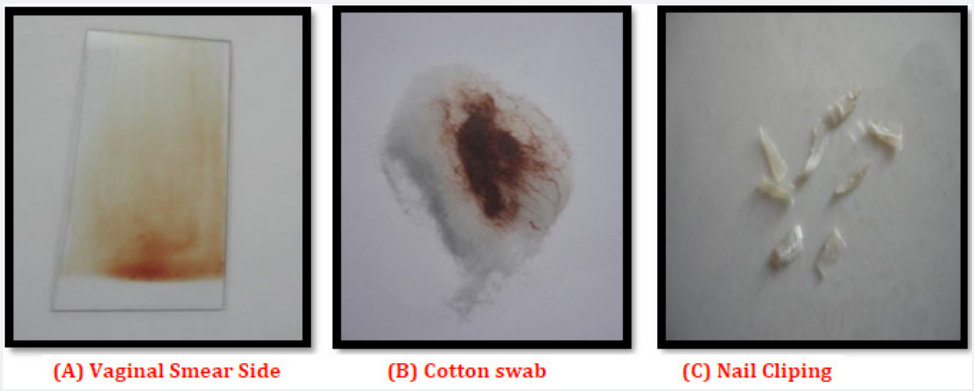

Figure 1: Different exhibits seized from victim.

Figure 2: Different exhibits seized from suspect.

DNA extraction

Different exhibits of both victim as well as suspect were collected with written informed consent. The seized exhibits of victim were found moist with excessive blood. Appropriate stain containing sample was selected and taken adequately into the 1.5 ml tube. Genomic DNA was extracted by using differential extraction protocol that separates sperm and non-sperm cells (Gill et al.,1985) [7]. Samples were incubated for two hours at 37ºC with 500 µl forensic buffer (50 mM NaCl, 100 mM Tris-HCl, pH 8.0, 5 mM EDTA),50µl SDS (20%) and 10 µl Proteinase K (0.1 mg/mL). Incubated sample then centrifuged at 10,000 RPM for 10 min to separate possible spermatic fraction from lysed epithelial cell. The obtained pellet was washed with phosphate buffer twice to remove traces of epithelial cell. Washed pellet further incubated at 56ºC with 500 µl forensic buffer, 50µl SDS, 10 µl Proteinase K and 25 µl DTT (39 mM). Both sperm and non spermatic sample were performed extraction of DNA by phenol chloroform method. At the last stage of phenol chloroform method, the upper aqueous layer was removed and subjected to spin filtration using a Microcon 30 kD concentrator (Millipore, Bedford, MA) according to the manufacturer's instructions. Samples were stored at 4ºC until analysis.

DNA quantification

The extracted DNA was quantified by the Real-Time polymerase chain reaction (RT-PCR) using the Trio DNA Quantification Kits (Thermo Fisher Scientific) [8]. Quantification process was done with kit components containing PCR reaction mixture (dNTPs, buffer, enzyme, Mustang Purple™ Passive Reference Standard, and stabilizers), primer mix (Target-specific primers, ABY™,JUN™, VIC™, and FAM™ dye-labeled probes, and Internal PCR Control (IPC) template),DNA dilution buffer and DNA standard (100 ng/µl).

PCR amplification

DNA quantification was followed by the dilution of template DNA to obtained final concentration in the range of 1–2 ng/µl. This final concentration was used for both type of PCR amplification process. PCR amplification was carried out by using Verity Thermal Cycler (Applied Biosystems, USA). Cycle and temperature programs were used according to the instructions of manufacturer regarding the PCR kit. A total of 16 autosomal STRs (D13S317, D7S820, D5S818, CSF1PO, D18S51, FGA, D21S11, D8S1179, vWA, D16S539, TH01, D3S1358, D2S1338,D19S433,TPOX) were amplified by using AmpFlSTR® Identifiler® Plus (Applied Biosystems, USA) kit and 23 non-autosomal STRs (DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385a & b, DYS456 and Y-GATA-H4) (Y-chromosome) analysis was carried out by using Power Plex® 23 System (Promega, USA).

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DNA analysis and STR typing

The PCR amplified products were separated and typed by employing capillary electrophoresis technique on ABI 3500xL Genetic Analyzer (Applied Biosystems, USA) using POP-4 polymer and Data Collection Software v1.0. Peak sizing and genotype assignments were performed by Gene Mapper ID-X v1.2.

Result

In the first phase of routine examination, Y STR kit was applied on the DNA of male fraction but we could not detect Y STR DNA profile. We also applied Y chromosomal kit on the DNA of female fraction, but we got same result similar to male fraction. We putted autosomal PCR for extracted DNA of male and female fraction and got only female autosomal STR DNA profile (Figure 3). Medical history was indicating that there was brutal injury in vaginal region and excessive bleeding also suggested the washing or dilution of seminal stain. Presence of high female-male ratio was tumbling the efficacy of Y STR kit to detect the Y chromosomal DNA. We started our examination on the DNA, extracted from the source of suspect. We hypothesized that under excessive bleeding it may be chance of transfer of blood of victim on the source of suspect. We putted autosomal PCR for the extracted DNA of suspect source and got mixed autosomal STR DNA profile from the underwear (Figure 4). It was found that all the alleles of each genetic marker of the autosomal STR DNA profile obtained from the source of victim were included in the mix autosomal STR DNA profile. Result also revealed that all the alleles of autosomal STR DNA profile obtained from the blood of suspect (Figure 5) were also present in mixed DNA profile. The obtained result strongly supported the hypothesis of Lockard's principle of exchange that the victim was the female contributor in the mixed stain found on the source of suspect.

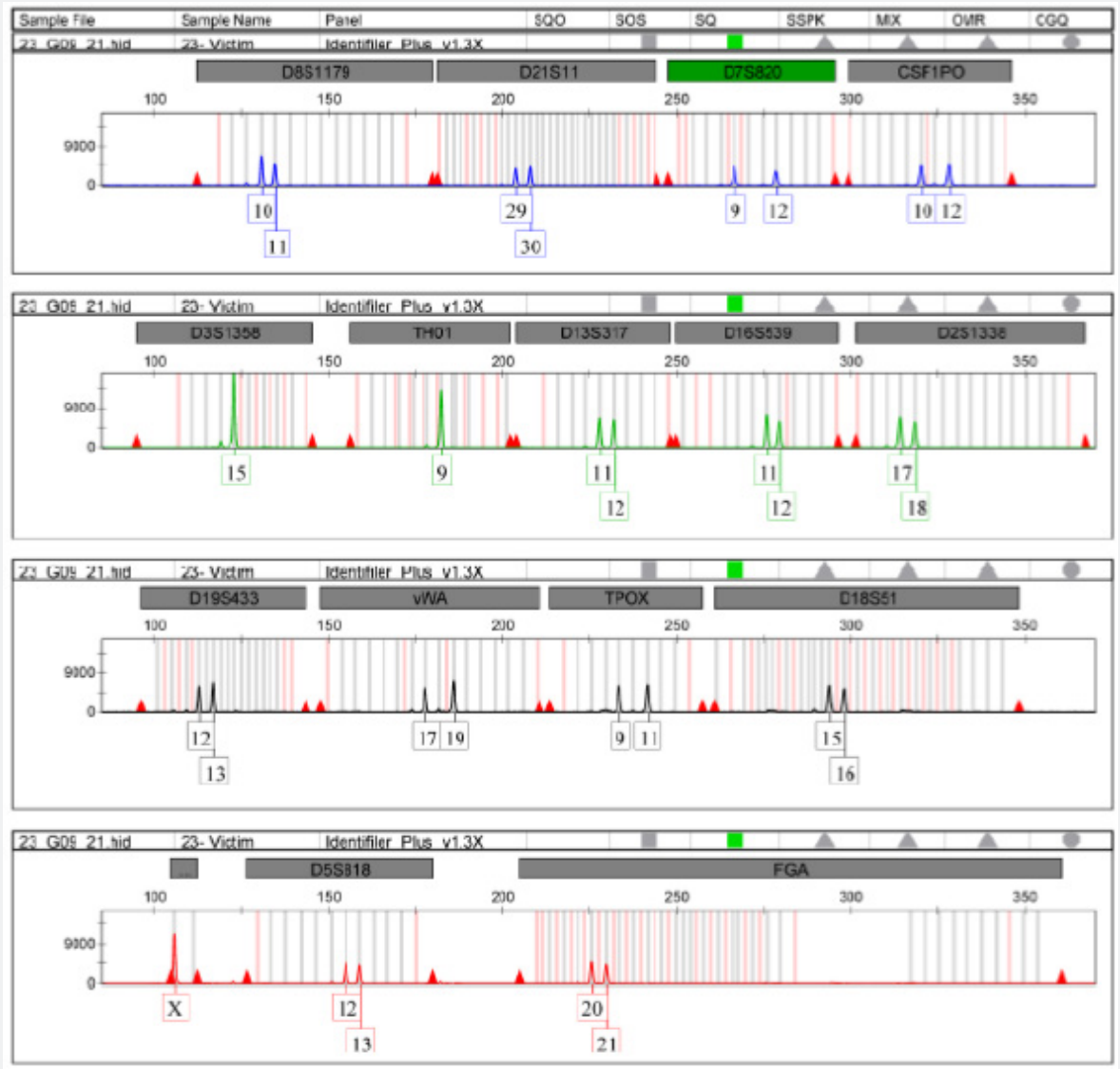

Figure 3: Autosomal STR DNA profile obtained from source of victim.

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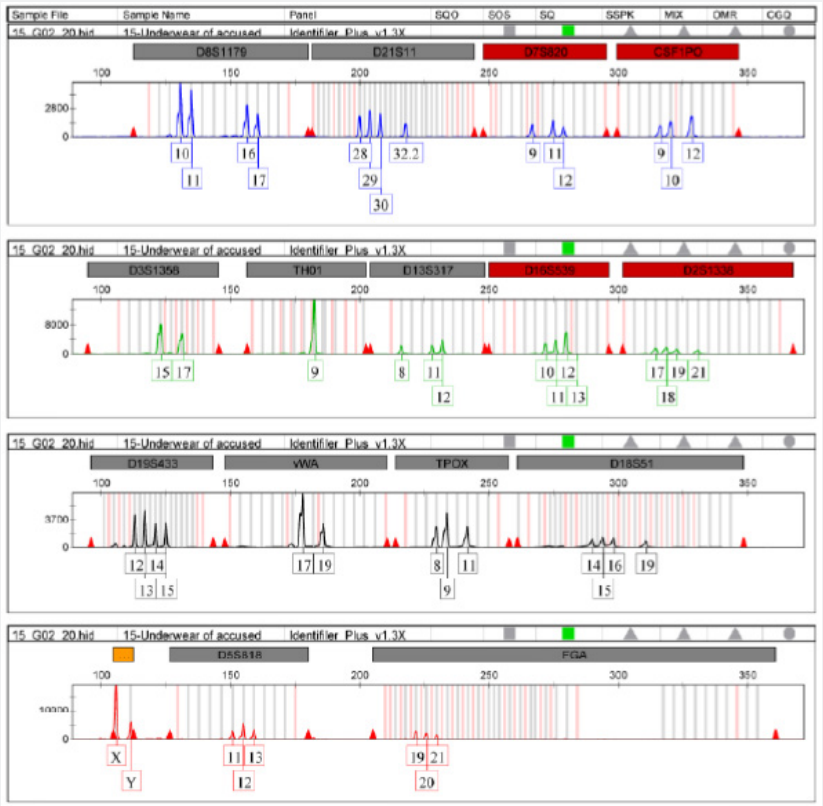

Figure 4: Mixed Autosomal STR DNA profile obtained from source underwear of Suspect/Accused.

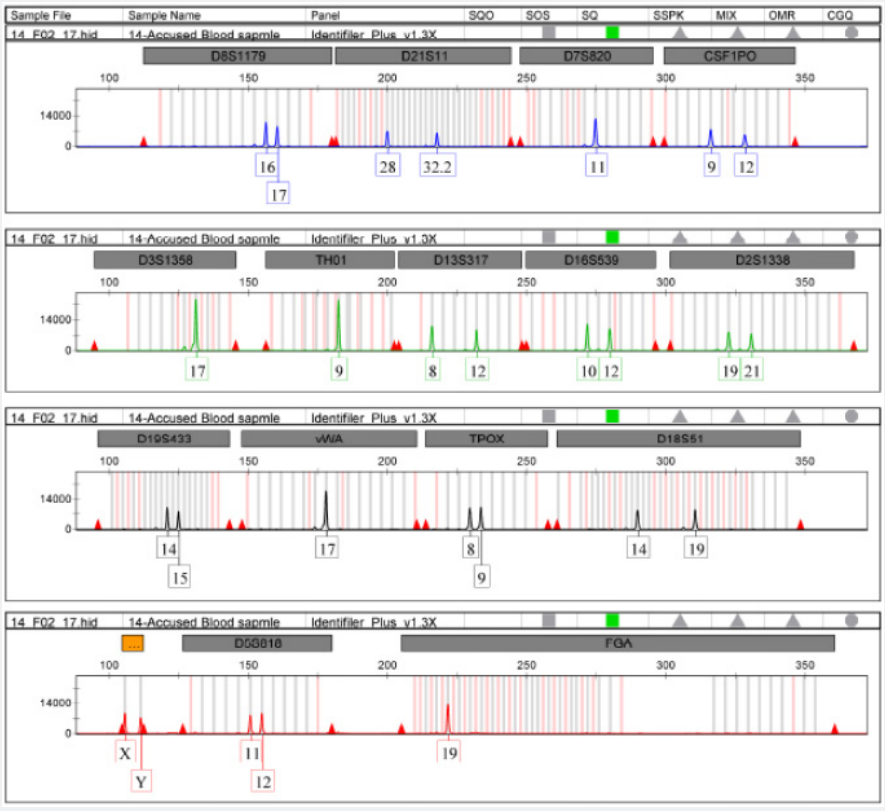

Figure 5: Male autosomal STR DNA profile obtained from blood sample of accused.

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Discussion

DNA typing is a result of amplifiable product obtained through multiplex PCR using commercial kits. The result of the DNA typing obtained in the form of electropherogram which illustrate the alleles in the form of peak designate with numerical number. In the electropherogram, peak numbers are unique for each entity that compare with reference sample. In the present case, we got sample from the source of victim containing excess blood with minute occurrence of male fraction. Presence of male fraction (spermatozoa) confirmed through microscopic examination. The high concentration of female fraction with very tiny portion of spermatozoa restricted the success of differential extraction process as a result we did not get male profile even though using Y specific STR kit Very high Female/male DNA mixture of two contributors always affects the amplification of the DNA of low contributor weather we are using sex specific marker kit (Y-STR kit). This is so because in PCR template DNA used in limited quantity that is about 0.5–2.0 ng.

At this restricted sample size of the input DNA, the extent of lower contributor is not enough to attain the lower sensitivity range of commercially available kit. As a consequence, there are very less chance to generate male (Y) chromosomal DNA profile. carried out study of different proportion of male/female mixture by Power Plex-Y and was obtained full profile with the 1:1000 male: female DNA ratio and was totally lost at 1:5000. In our finding of case work, DNA extracted from victim exhibits detected ratio of male female was more than 1:5000 therefore we could not be getting complete Y STR DNA profile. It has also been found that different proportion of male/female also affects the amplification of Y STR markers. Current PCR technique is extremely effective for amplification of mixer DNA samples, but they often fail to amplify when applied to forensic specimens that harbor unequal proportion of two different DNA.

We also performed autosomal PCR of the DNA extracted from the underwear of suspect as well as exhibits from the source of victim. We got mixed autosomal STR DNA profile from the underwear of suspect but got only female DNA profile from the source of victim. Usually, autosomal PCR fragment analysis is not a deep sequenced therefore it allows the detection of a minor DNA component in a mixture only when minor DNA represents more than 10% of the total DNA; however, the complete autosomal STR DNA profile of minor contributor DNA requires at least 1:5 ratio [9]. In our case study, we found that complete female autosomal STR DNA profile was included in the mixed autosomal STR DNA profile of suspect underwear.

The main focus of DNA examination in sexual assault cases to detect and obtained male DNA profile from the source of victim. In mainly rape cases, involvement of suspect with crime link through examination of exhibits of victim. But due to complicated condition like excessive bleeding hamper the possible finding regarding the male profile from the source of victim. Careful examinations of exhibit of the suspect also make the result conclusive and enable us to establish guilty of suspect [10-16].

Our finding also suggested that the use of amplification systems of autosomal STRs could be very important in the analysis of the mixed profiles. We also think that the performance of Y-STRs was not necessary in this case, because we were able to confirm that the evidentiary sample represented a DNA mixture of the victim and the suspect only by using autosomal STRs. In conclusion, our study confirmed that Y-STRs could be useful in analysis of DNA mixtures from rape cases. It is important to keep in mind the limits of this method, which depend on the type of material that has to be analyzed.

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