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Case Study

Significance of autosomal STR Markers Kits to determine sexual assault case involving brother in law

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Abstract

Sexual harassment of women and children is an unforgivable crime that results from the distorted mentality of wrong men. A crime of this nature becomes very tragic and complicated when a close relative or family member of the victim is involved in such a crime. In many cases the event is hidden under family pressure due to fear of desecration. Once such a crime is reported, it is necessary to detect the incident by scientific method. DNA fingerprinting plays an important role in detecting a criminal's involvement in a crime. Currently the Y STR marker kit is available which provides information about the presence of male material on exhibits received from the complainant. But in some circumstances in which the woman is raped by her husband's spouse, the Y STR marker kit is not sufficient for the conclusive outcome. The present study is based on a similar incident in which a married woman was raped by her brother-in-law. The presence of male DNA was confirmed by Y STR markers when DNA fingerprinting of the exhibits from the source of the victim was done. A complete Y STR profile was obtained from the victim's source, corresponding to the accused's Y STR profile. But this profile was also similar to the victim's husband's Y STR DNA profile. In this case, the results obtained using the autosomal STR marker kit were clearly stating that the male DNA profile obtained from the victim's source was of the accused rather than the victim's husband. The current case study suggests that the main advantage of using the Y-STR approach is the ability to detect male DNA at the source of the victim, but when the woman is married and her husband's paternal relatives are involved in incidents share same Y Lineages, so only such autosomal STR markers can resolve such cases.

Keywords: Sexual assault, paternal relative, Power plex Y-STR, Global filer autosomal STR, DNA typing, Multiplex PCR.

Introduction

Sexual violence represents a serious social problem that requires most attention to avert it[1]. In mostly rape cases, known person of the victim is culprit oftenly. In rape case, detection of spermatozoa on the source of victim is a primary examination to intimate regarding the incidence. But when a paternal relative of spouse of victim is involved in sexual assault then it requires prominent approach to confirm the detected spermatozoa source[2]. Recently using of DNA typing technique is well grounded upon in forensic examination of samples as it generates confirmatory results for the identity of the person[3]. At the developing phase, DNA typing was done based upon large size fragment (mini satellite) generated by using restriction endo-nuclease enzyme called Restriction Field Length Polymorphism (RFLP). Now it has been shifted into very small sized fragment (micro satellite) amplified by PCR called short tandem repeat (STR) and now using NGS which is SNP based[4]. Currently there are so many different categories of STR based kits are using in routine forensic case work viz. mitochondrial STR, X-STR, Y-STR, autosomal STR etc[5]. In the rape case, Y-STR kit plays an important role to prove the allegation of victim[6].

The chief intend of this examination to detect presence of male content on the source of victim. The Y-chromosomal short tandem repeat (Y-STR) loci have also been extensively employ in forensic science for identification of male persons[7] . Moreover, the DNA Commission of the International Society of Forensic Genetics has published a series of recommendations concerning the applications of DNA polymorphisms for the Y-chromosome[8]. It has also found that Y-STR kit is very sensitive and specific to perceive presence of male content in the blend of biological material of female- male mixture[9]. Because of unique identity of the Y chromosome, it is widely used in forensic studies in determining individuality of male persons[10,11]. The Y-STR analysis plays an important role in dispute type of paternity case with male offspring. It is also helpful in paternal relationship testing, including ancestral analysis, to establish identity of missing person with their paternal relative and as well as in special cases of missing person and any calamity victim identification involving men[12]. Although Y-STR analysis is being useful in several cases including sexual assault but it is not conclusive when same Y lineage persons are suspected in sexual offence and only single is involved in committed the crime.

The condition is more typical when married women sexually assaulted by paternal relative of her spouse. Such case required autosomal STR kit as a discriminating tool.

Present work done on a sexual assault case reported by 25 year old married women who raped by her brother in law at her house. She narrated her incidence to their father in law that when she was alone at her house, accused grabbed her hair snatched to a room and forcefully raped with her .After this incidence victim filed FIR against accused. During medical examination exhibits sized from the source of victim and sent to the DNA lab for test. After arrest of accused their blood sample also retrieved during medical examination and also sent to the DNA lab.

Materials and methods

Exhibits received: Exhibits of victim, her husband as well as accused were collected with their written consent and sent to our DNA unit, State Forensic Laboratory, Sagar Madhya Pradesh. We received underwear and vaginal smear slide from the source of victim while blood sample in EDTA vial from the source of victim's husband and from accused.

DNA extraction from blood sample: The DNA extraction from blood sample was done by organic extraction method. The 500 µl blood sample of received article was suspended in 500µl lysis buffer-I (30mM Tris HCl{Himedia} pH 8.0, 5mM EDTA {Himedia}, and 50 mM Sodium Chloride {Sigma}), and keep it at - 80ºC for 12 hours in a deep freezer {Sanyo}. The blood sample than kept in water bath (Thermo Scientific) at 65ºC for 10 minutes. Then the sample allow to centrifuge at 10,000 RPM for 10 min. Centrifugation created separation of cells in palate form while liquid phase obtained as a supernatant. After discarding the supernatant, 500µL Lysis buffer-II (75 mM EDTA, and 2mM Sodium Chloride) were added into tube to suspended the pallet along with 50µl 20% SDS (Sigma) and 10µl 20mg/ml proteinase K (Himedia). The samples were incubated at 56°C for 4 hours. Then the DNA was extracted by phenol chloroform method.

Digested blood sample was treated with phenol-Chloroform-Isoamyl alcohol to remove cell debris containing protein, carbohydrates and lipid followed by precipitation of genomic DNA. There was equal volume of tris saturated phenol solution (Himedia) added into digested blood sample then mixed by inverting the tube up and down in rotator (Neuaction technology) at 50 RPM for 10 min. This inverting step followed by centrifugation (Eppendorf) at 10,000 RPM to got two separate layers. The upper aqueous layer was transfer into separate tube for next step. In the next step 25:25 mixture of Tris saturated phenol and Chloroform-Isoamyl alcohol (Himedia) (24:1) were added into aqueous phase followed by 10-10 minute inverting and centrifugation steps. After centrifugation again two layers separated, out of which upper aqueous layer transferred into another tube. In this aqueous phase, 50% volume of mixture of Chloroform-Isoamyl alcohol (24:1) was added followed by 10-10 minute inverting and centrifugation step (Figure-1). The supernatant aqueous layer was then transferred into another tube. Then 3M Sodium acetate (1/30 volume of final aqueous phase) was added into aqueous phase and vortex slightly. The genomic DNA was precipitated with propanol (Himedia) (1X) by inverting the tube up and down for 2 min. The tubes were centrifuged at 14,000 RPM for 10 min. Due to centrifugation, The pellet of genomic DNA was obtain at the bottom which was washed with 70% ethanol two times to remove remnant salts followed by washing with absolute alcohol two times. After washing, pellet was allowed to dry at room temperature. Then it dissolve in desired volume of Low TE buffer (0.1mM EDTA, pH 8.0, 10mM Tris-Cl,) and stored at 4°C[13].

DNA Extraction from forensic sample: DNA Extraction from underwear and vaginal smear slide of the victim's source was done by differential extraction method (Figure-2). In this method, sample was taken from exhibits of victim and inoculated into forensic buffer (100mM Tris, 5mM EDTA,50 mM Sodium chloride) along with 20% Sodium dodicyle sulphate and Protenase K enzyme in 1.5ml conical micro-centrifuge tube (Genaxy).

These Samples were incubated at 37ºC for 3 hours in water bath. After fraction incubation, pieces of underwear were squeezed than taken liquid. The liquid of both squeezed underwear and vaginal smear allow to centrifuge at 10,000 RPM for 10 minute. Supernatant was separated into another tube as a female fraction. Pellet was washed with saline solution in order to remove traces of female fraction then it suspended in forensic buffer with 20% Sodium dodicyle sulphate, 0.1 M Dithiothritol (Thermo Fisher scientific) and Protenase K enzyme .This was male fraction which kept at 56º C for 12 hours for lysis of sperm cells. Next day phenol chloroform method was done for both male and female fraction to extract the DNA. The method was similar as mentioned in extraction of DNA from blood but at last stage, aqueous phase was taken into Amicon Ultra-0.5 ml Centrifugal Filter (Merck millipore) and centrifuged at 5000 ×g for 10 min. Filtrate was discarded. Then added 500µl of milli Q water for removing of salt traces adhere on the DNA present at the filter pad. Filter tube again centrifuged at 5000x g for 10min. This process repeated three times. After completing of washing we got very less volume (20-30µl) at the bottom of filter then this filter reverted into collector tube and centrifuged at 5000×g for three min. The tubes contained concentrated DNA, were stored at 4ºC until the use (Figure-2a).

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)[14]. 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). Ten-fold dilution series with five concentration points were prepared (Figure-3). For per sample 8µl primer mix and 10 µl of reaction mix was taken. There were 18µl volume of mixture transferred into each well of semi skirted 96 micro-titer plate (Tarson). There were 2µl of sample, standard and control added to the each appropriate wells and run the plate for quantitation using Real-Time PCR machine (Quanta studio, Applied Bio-systems).

Amplification of DNA: After quantification, extracted DNA was diluted to the appropriate concentration (1ng/µl) and amplified by using two multiplex kit systems available commercially. These kits were Power plex Y-23 (Promega) and Global filer (Applied biosystem). Power plex Y-23 containing 23 STR loci (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) was used to detect presence of male content on the source of victim as well as also got Y STR DNA profile from blood source of accused and husband of victim. Y STR multiplexing was done with master mix (5.0µl), primer (2.5µl) and amplification grade water (15.5µl) per sample reaction volume of 23µl and added 2µl quantitated DNA template (1ng/µl). For autosomal STR DNA profile, Global filer kit was used containing twenty one autosomal STR markers (D13S317, D7S820, D5S818, CSF1PO, D1S1656, D12S391, D2S441, D10S1248, D18S51, FGA, D21S11, D8S1179, vWA, D16S539, TH01, D3S1358, D2S1338, D19S433, DYS391, TPOX, D22S1045, SE33 a), two male STR marker (Y Indel and DYS391) and one amylogenin marker(X/Y). This autosomal multiplexing was done with supplied kit content master mix (10.0µl), primer (5.0µl) and milli Q water (8.0µl) per sample reaction volume of 23µl and added 2µl quantitated DNA template (1ng/µl).Both amplification were done on ABI thermal cycler Veriti (ABI/Thermo/LT) as per recommend protocol given by manufacturers.

DNA typing: Amplified PCR product subjected to capillary electrophoresis for DNA typing. Process was done on genetic analyzer 3500xL (Applied biosystem) with twenty four capillary. Capillary electrophoresis required single strand DNA labeled with fluorance dye. Therefore Hi-Di formamide (Hi-Di) (Thermo fisher scientific) was used as a chemical denaturant. There were 10µl per well Hi-Di taken and added with suitable size standard supplied in respective kit. For Power plex Y-23 STR DNA typing, WIN ILS 500 size standard was added (4.5µl) in 240µl volume of Hi-Di (For 24 wells) while for autosomal DNA typing (Global filer kit), LIZ 600 size standard was added (2.5µl) in 240µl volume of Hi-Di (For 24 wells). The quantity of size standard of both STR kits were optimized by internal validation of laboratory under guide line provided by SWGDAM. The optimized volume of size standard was added into Hi-Di and after slightly vortex –mini spine, mixture was dispense 10-10µl into each well of micro titer plate. There were about 0.3µl amplified PCR product added into well for separation of fragment. The one micro litter of allelic ladder was added separately into one of the well of the plate. Each run had taken 45 min to complete the process. The result was analyzed using software version ID-X 1.4/1.5 version. For Y-STR analysis, analysis method: Y-23 (select with RFU), Panel: Power plex Y-23_IDX_v2.0 and size standard: WEN Internal Lane Standard 500 were select while for autosomal STR, analysis method: Global filer (select with RFU), Panel: Globalfiler_Panal_v1 and Size standard GS600_LIZ_(60–460) were select.

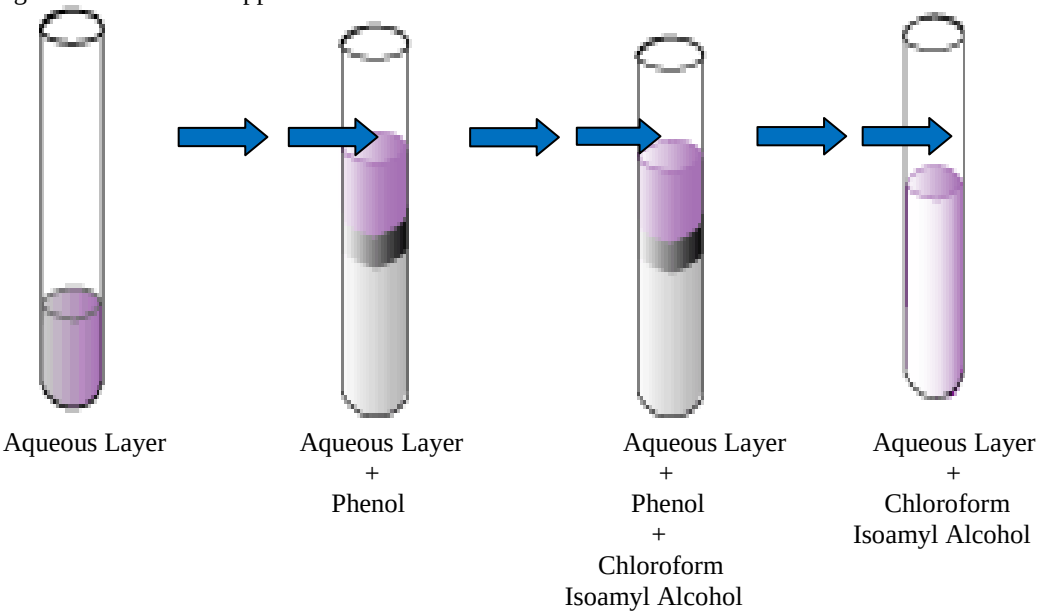

Figure-1: DNA extraction by Phenol Chloroform Isoamyl Alcohol method.

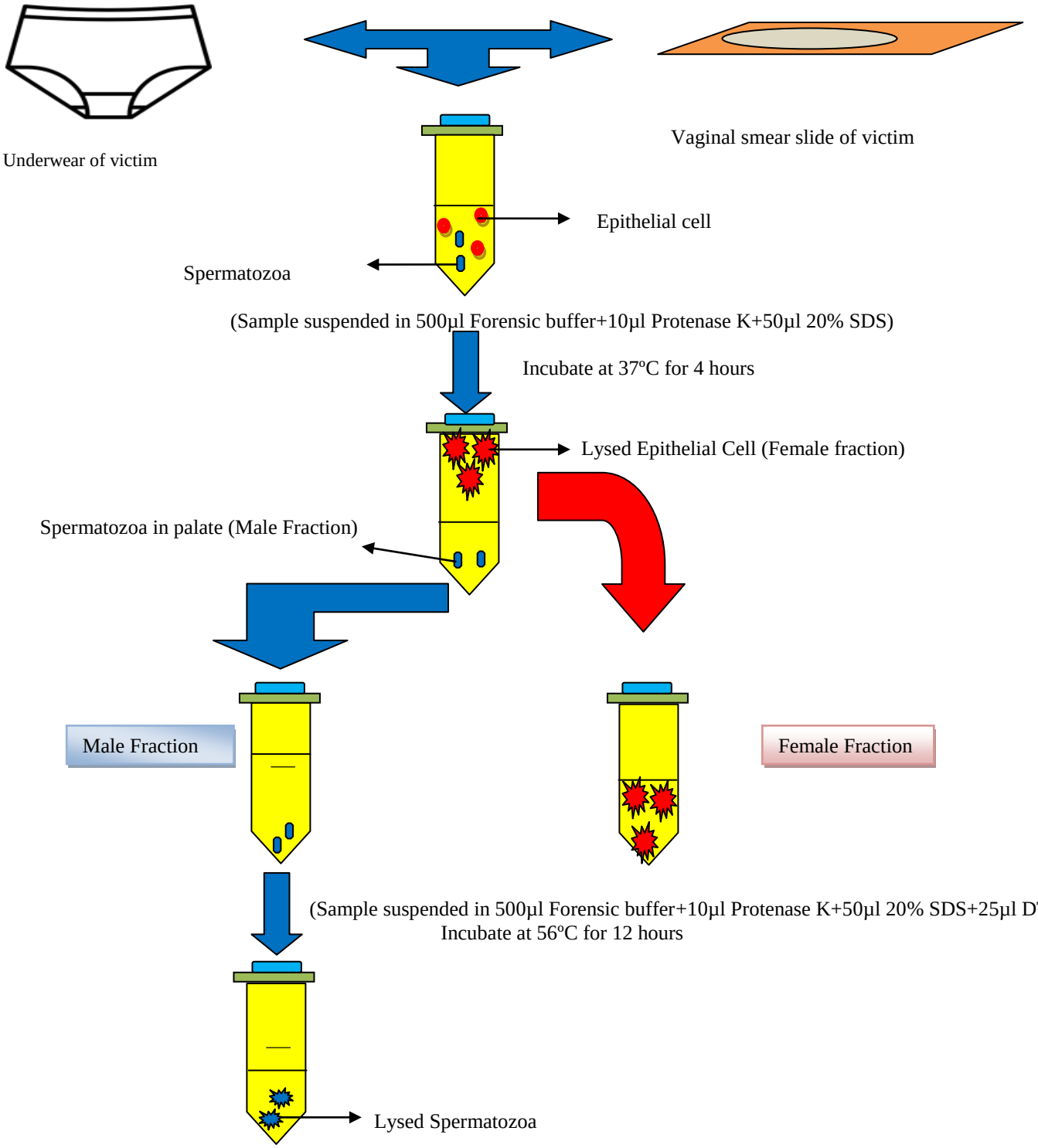

Figure-2: DNA extraction by Differential extraction method.

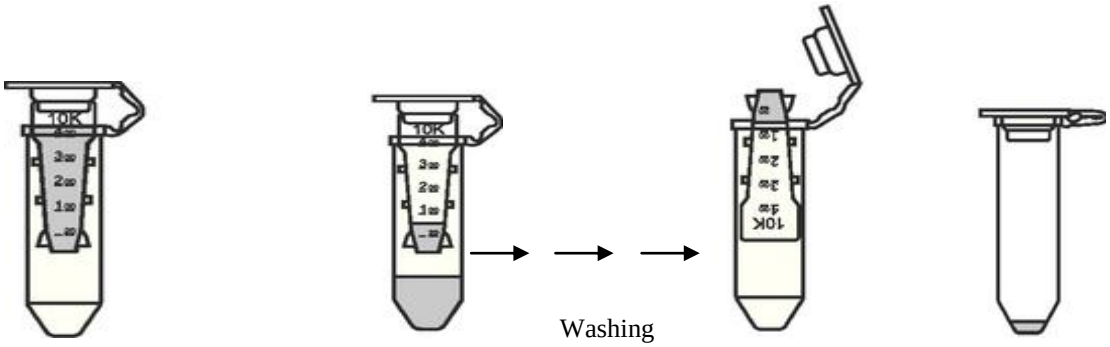

Figure-2a: Filtration of aqueous layer through filter.

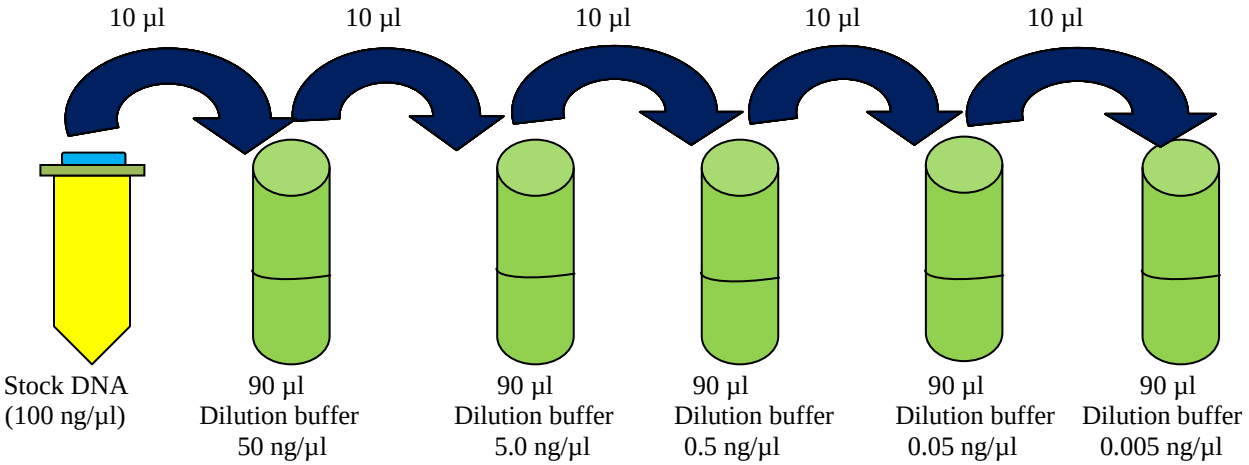

Figure-3: Standards dilution series of Stock DNA.

Results and discussion

We analyzed Y chromosomal STR based result and got Y STR DNA profile from both underwear and vaginal smear slide of the source of victim. The alleles of all STR markers of the DNA profile are depicted in Table-1. It was also found that both profiles were similar. Present result was indicating the presence of male content on the source of victim. We also got Y STR DNA profile from the blood source of accused and found that all alleles of each genetic marker were similar to the all alleles of each genetic marker of victim's Y STR profile. Because victim was married and accused was her brother in law (Dewar) therefore Y-STR DNA profile detected from the source of victim could have been either her husband or accused. In order to clarify the doubt, we generated Y STR DNA profile from blood source of husband of victim and found same Y STR DNA profile like profile of accused. Present finding was not giving conclusive opinion that male content which was detected on the source of victim was of accused or was of her husband.

Consequently we generated autosomal profile from the DNA source of victim, her husband and accused. We got male mixed autosomal STR DNA profile from the source of both underwear and vaginal smear slide of victim which depicted in Table-2. This result shows that all allelic pairs of each genetic marker of the autosomal STR DNA profile of accused's source were included in the male mix autosomal STR DNA profile obtained from the source of victim. It was also found that all allelic pairs of each genetic marker of the autosomal STR DNA profile obtained from victim's husband did not present in the male mix autosomal STR DNA profile of the victim's source. The autosomal finding supported the outcome of Y STR obtained from the source of victim elucidate that male content was of accused. The relevance of DNA typing in the forensic case investigation is an imperative part of present criminal justice system[15]. This technology not only assists in including the offender but also to exonerate the innocent[16]. In rape case, the basic important thing is to detect the presence of male content on the source of victim. But it is very tough when the presence of male content is very low and could not detect in autosomal profile[10]. After emerging of Y-chromosomal STR polymorphism in crime casework it has possible to detect presence of seminal fluid mixed with vaginal secretion[17].

Table-1: Allelic number of each genetic marker of Y STR DNA profile.

Genetic Markers	Blood Sample of Husband of victim	Underwear of victim	V.S. Slide of Victim	Blood Sample of Accused
DYS576	18	18	18	18
DYS389I	14	14	14	14
DYS448	21	21	21	21
DYS389II	30	30	30	30
DYS19	19	19	19	19
DYS391	10	10	10	10
DYS481	16	16	16	16
DYS549	15	15	15	15
DYS533	12	12	12	12
DYS438	19	19	19	19
DYS437	11	11	11	11
DYS570	16	16	16	16
DYS635	24	24	24	24
DYS390	9	9	9	9
DYS439	11	11	11	11
DYS392	39	39	39	39
DYS643	17	17	17	17
DYS393	14	14	14	14
DYS458	15,17	15,17	15,17	15,17
DYS385	32	32	32	32
DYS456	12	12	12	12
YGATAH4	11	11	11	11

Table-2: Autosomal STR DNA profile.

Genetic Markers	Blood Sample of Husband of victim	V.S. slide and Underwear of victim	Blood Sample of Accused
D3S1358	15,17	17,18	17
vWA	12,17	6,9,14,17	14,17
D16S539	11,13	11,12,29,31.2	11,12
CSFIPO	10,12	10,12,14	10
TPOX	8,11	5,8,10,11	8,11
D8S1179	14,17	11,14,15	14,15
D21S11	30.2,31.2	12,30.2,31.2	30.2,31.2
D18S51	16,18	10,11,15	15
D2S441	10,11	8,10,11,12	10,11
D19S433	13,14.2	12,13,14.2	13,14.2
TH01	10,13	9,12	9
FGA	23,25	18,20,23,25	23,25
D22S1045	11,15	11,15,16	11,15
D5S818	11	8,11	11
D13S317	11,12	11,12,20,22	11,12
D7S820	10,14	10,11,17,18	10,11
SE33	19,30.2	6,9,19,30.2	19,30.2
D10S1248	13.15	13,15,29,31.2	13.15
D1S1656	16	12,14,16	16
D12S391	18.2	5,10,18.2	18.2
D2S1338	22.26	11,22.24	22.24
DYS391	11	11	11
Y-Indel	2	2	2
Amylogenin	XY	XY	XY

With the advantage of Y STR DNA typing in rape case, there are some limitations also with those cases in which one is real culprit among number of suspects having similar Y lineage. In the present case study, the first spark of doubt about the presence of male DNA found on the source of victim was of her husband who was real brother of accused but autosomal DNA typing result provided confirmatory view to this case.

Conclusion

Using of STR kit in forensic cases play vital role to link the crime with real culprit. Existence of different type of kits resolves the complexicity of crime. Rape case is one of the most heinous types of crime and detection of male content on female source proves the allegation. The Y STR kit is widely using in sexual assault cases but always it is not give conclusive finding when suspects having same Y lineage. Such sexual assault cases can be concluded by combined analysis of autosomal and Y STR. From this study it is concluded that employing of autosomal STR is the best when paternal relatives are involved who share same Y lineage.

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