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Research Article

DNA Profiling of a Rape Case at the State Forensic Science Laboratory Ranchi Jharkhand India

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Abstract

In the forensic, profiling of DNA resolves the hidden secrets but it is not always a simple task. In the present case study, here we report on a rape of 27 years old lady in a jungle (Jharkhand, India) by more than one. DNA profile of exhibit marked-A (Blood positive under garment cuttings of rape victim) matched with the DNA profile of the exhibit marked-B1 (Blood and Semen positive under garment Cuttings of suspect) i.e. DNA profile generated from the blood stains of female on the undergarment of one of the suspect. DNA was extracted from blood and semen using Organic Extraction and Differential Organic Extraction Method respectively. DNA was tested by highly sensitive multiplex PCR and quantified via real-time PCR. DNA typing was executed using AmpFISTR® Identifiler® Plus Kit by multiplex PCR reaction (co-amplification) of 15 autosomal STRs loci and a gender locus. AmpFISTR® Yfiler™ Kit was also used for multiplex PCR reaction (co-amplification) of 17 Y-STRs loci. Autosomal as well as Y-STR DNA profile was generated from the biological evidences such as blood and semen sample (termed as exhibit) received in the SFSL Jharkhand from the crime scene through court.

Keywords: DNA extraction; Multiplex PCR; Blood and semen; DNA profiling; Rape case

Introduction

The brutal gang rape of a young student on a bus in Delhi (India) in 2012 led to major reform of India's rape laws, including speeding-up of trials and increasing penalties for offenders, still sexual assaults cases persist. The crimes against women, particularly rape, are rising in Jharkhand. Till November 2015, sexual rape cases in Jharkhand state were found around 1,124. In various criminal and civil cases, requirement of DNA profiling has become inevitable to ascertain the individuality of victim and suspects. Recently, DNA profiling is one of the most important tools in the sexual assault and rape cases where biological evidence (exhibit) plays key role for identification of the perpetrator or suspects [1]. DNA based identification from the biological exhibits in the forensic considered as the most important evidence for legal proof in courts of law [2,3]. STR DNA profiling or typing is advanced tools in the human identification purposes, when DNA extracted from challenging biological evidences. Commercially available AmpFISTR Identifiler Plus® kit has been accepted globally for multiplex PCR for highly challenged biological samples [4].

Challenges of DNA extraction

Inhibitors come from challenging evidences in DNA samples that creates major obstacle in generation of DNA profile and analysis processes. The DNA polymerase activity is directly or indirectly hampered by many PCR inhibitors. Salts and detergent concentration or proteases present in the reaction cause decline in the enzyme activity. Some other inhibitors like humic acid present in the reaction comes from soil during extraction of DNA even at low concentration prevent the enzymatic reaction. Some chemical such as urea and phenol are known to degrade DNA polymerases thus create nuisance in the PCR reaction. Ions like calcium and other factors such as haematin from blood and dye released from fabrics etc. may inhibit polymerase activity. High concentrations of calcium competes with magnesium by a competitive binding hence leads to competitive inhibition as magnesium is no longer available as a cofactor for the polymerase activity thus polymerase activity decreased [5]. The aim of the present work was to resolve the sexual rape case from challenged samples whether biological samples having PCR inhibitors hampered in the generation of DNA profile.

Case history

At the police station an FIR launch by police personhood under sections: 376(D)/323/341/307/34 of Indian Penal Code on the basis of information by a victim (female), namely XYZ Devi (identity has been concealed), age 27 years raped on a secret place in a jungle by more than one including her husband and tried to kill her. The investigation agency collected the under garments and genital hairs of victim and accused (two in number). These exhibits were received at State Forensic Science Laboratory Jharkhand.

Materials and Methods

Exhibits and sample preparation

Exhibits which were received in the SFSL, Ranchi Jharkhand through the court are marked-A (Source: Blood Positive Saya-undergarment) of victim, exhibit marked-B1 (Source: Blood and semen Positive Janghiya- undergarment) of suspect and exhibit marked-C1 (Source: Blood and semen Positive Janghiya-undergarment) of another suspect. The exhibits taken for DNA extraction from these sources are listed in the Table 1. These exhibits (Blood and Semen positive) were cut in small pieces, soaked in lysis buffer following the organic and differential extraction methods.

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Table 1: Exhibits taken for DNA extraction and mean DNA concentrations of blood and semen sample; used software: 7500 SDS V.1.2.3 version.

S. No.	Sample No.	Approx. Yield (ng/ µl)	Mean cat IPC	Mean Ct Sample
1	Exhibit marked-A (Source: Blood Positive Saya Cuttings- undergarment)	Undetected	33.58	Undetected
2	Exhibit marked-B1 (Source: Blood and semen Positive Janghiya Cuttings-undergarment)	Undetected	34.36	Undetected
3	Exhibit-C1(Source: Blood and semen Positive Janghiya Cuttings- undergarment)	Undetected	35	Undetected

Table 2: Autosomal STR DNA profile Comparative Chart of allele distribution (genotype) of 15 different loci of the DNA tested.

S. No.	Name of loci	Exhibit marked-A (Source: Blood Positive Saya Cuttings)	Exhibit marked-B1 (Source: Blood and semen Positive Janghiya Cuttings)		Exhibit-C1(Source: Blood and semen Positive Janghiya Cuttings)
			Male fraction	Female fraction	
1.	D8S1179	15, 16	10, **15, 16**	15, 16	13, 15
2.	D21S11	30, 30	29, **30, 30**. **2**	30, 30	28, 29
3.	D7S820	8, 11	8, 11	8, 11	8, 12
4.	CSF1PO	11, 13	10, **11**, 12, **13**	11, 13	10, 12
5.	D3S1358	14, 16	**14**, 15, **16**	14, 16	16, 18
6.	THO1	8, 9	6, 7, **8, 9**	8, 9	8, 9
7.	D13S317	8, 11	**8, 11**, 12, 13	8, 11	8, 12
8.	D16S539	11, 13	10, **11, 13**	11, 13	9, 11
9.	D2S1338	17, 21	**17, 21**, 22, 25	17, 21	23, 23
10.	D19S433	13, 13	**13, 13**.2, 14, 14.2	13, 13	13, 13
11.	vWA	16, 17	15, **16, 17**, 18	16, 17	15, 19
12.	TPOX	9, 9	8, **9**, 11	9, 9	8, 8
13.	D18S51	11, 15	**11**, 12, **15**, 17	11, 15	14, 19
14.	D5S818	11, 13	11, 13	11, 13	13, 13
15.	FGA	19, 22	**19, 22**, 26	19, 22	22, 23
16.	Amelogenin	X, X	X, >Y	X, X	X, Y

DNA Isolation or extraction

Organic extraction method: Exhibit or sample (Blood stains) was subjected to Organic Extraction Method for DNA isolation [6]. Blood stains were submerged in 400 µl of extraction buffer (10 mM Tris-Cl, pH 8.0, 0.1M EDTA, pH 8.0, 20 µg/ml RNase A, 0.5% SDS) in 1.5 ml eppendorf tubes then 10 µl of Proteinase K was added and incubated for 2 hours at 56°C on water bath with shaking speed of 150 rpm. After incubation an equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added to each tube then mixed by inverting the tube up and down for 2 minutes. The tubes were then centrifuged at 5000 X g for 10 min at room temperature. The upper aqueous layer was then transferred in separate eppendorf tube. Then purification of DNA was done by precipitation with absolute ethanol (2X) and kept at -20°C for 30 min to settle down. After incubation tubes were then centrifuged at 15, 000 X g for 15 min. The pellet of DNA was washed with 70% ethanol to remove remnant salts and dried at room temperature. The DNA was resuspended in desired volume of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0). The tubes were stored at 4°C until the use.

Differential organic extraction method: Exhibit or sample (Blood stains mixed with semen) was subjected to Differential Organic Extraction Method for DNA isolation (separation of female DNA and sperm cells). To separate the male and female fraction exhibits were submerged in 400 µl of Tris/EDTA/NaCl (TNE) solution (ice cold), 25 µl of 20% Sarcosyl, 75 µl MilliQ, 5 µl Proteinase K, vortexed and kept on 37°C incubator for 2 hrs. After incubation vortexed the tubes, centrifuged (short spin) and transferred the supernatant in new fresh tube and centrifuged at 10,000 rpm for 5 min. Removed the supernatant (contained the female fraction) in fresh tube and kept at 4°C. The tube contained the male fraction was processed for wash with 1 ml TNE solution, vortexed, centrifuged at 10,000 rpm for 5 min, discarded the supernatant and this step was repeated for 3 times. Then added 150 µl TNE solution, 50 µl 20% Sarcosyl, 40 µl DTT (0.39M), 150 µl MilliQ, 10 µl Proteinase K in the tube containing male fraction and kept at 37°C for 2 hrs. Then, both the male and female fractions mixed with the equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1), centrifuged at 10,000 rpm for 6 min. Further, processed as organic extraction method for DNA purification. After purification re-solubilized the DNA in 36 µl Tris EDTA (TE) at 56°C for 2 hrs and tubes were stored at 4°C until the use.

Microcone purification

DNA samples after Organic and Differential extractions were further purified with Microcone™ 100 Concentrater Unit. Assembled the Microcone™ 100 tubes added 100 µl TE and kept the tubes at room temperature for 5 min. Then added the DNA samples, centrifuged at 2500 ×g for 10 min. Discarded the filtrate, added 200 µl TE and centrifuged at 2500 ×g for 10 min. Reverted the microcone, assembled in collector tube and added 20 µl TE, centrifuged the tubes at 2500 ×g for 10 min. The tubes were stored at 4°C until the use.

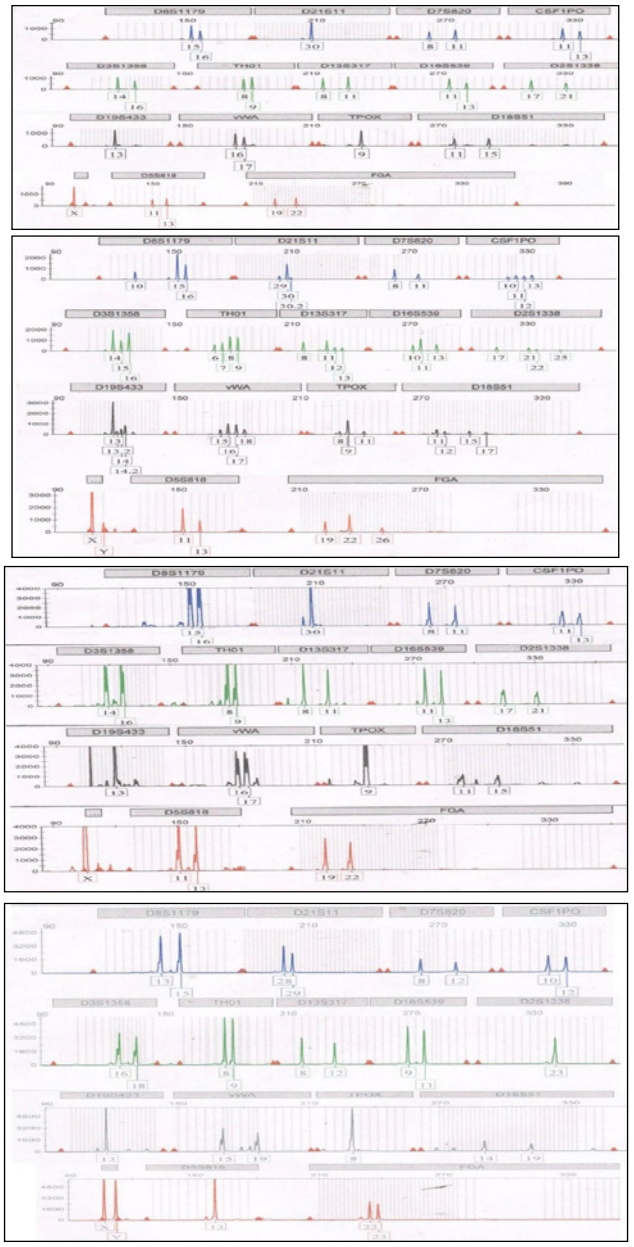

Figure 1: DNA profile of exhibit marked A, B1 m, B1 fm, and C1.

DNA quantification

The Real-Time polymerase chain reaction (RT-PCR) was performed for the DNA quantification of the samples (Table 1) by using the Quantifiler® Human DNA Quantification kit (Life Technologies Inc.) [7]. This kit provides the PCR reaction mix (Human Quantifier), primer sets (Human Quantifier) and standard DNA (200 ng/µl). RT-PCR reaction setup of 25 µl includes 10.5 µl of primer sets, 12.5 µl of reaction mix and 2 µl of DNA templates to each well and run the PCR using Real-Time PCR machine (Applied Biosystems) and Software: 7500 SDS V.1.2.3.

The eal-time RT-PCR did not yield the DNA concentration result (Table 1) of the samples used for quantification of DNA, perhaps due to the background noise present in the samples.

Identifier plus® and Y-filer™ PCR

AmpFISTR Identifiler Plus® kit [4] was used to multiplex PCR reaction for co-amplification of 15 autosomal STRs loci and a gender locus (Table 2). Using 25 µl PCR amplification mixture (10 µl of PCR reaction mix, 5 µl of Identifier Plus® Primer Sets and 10 µl of template DNA in TE buffer (1ng of DNA template), amplification was carried out under conditions of initial denaturation at 95°C for 11 min, followed by 28 cycles of denaturation at 94°C for 20 sec, annealing at 59°C for 3 min, extension at 72°C for 1 min and a final extension step at 60°C for 10 min. Similarly AmpFISTR® Y-filer™ Kit was used to multiplex PCR reaction for co-amplification of 17 Y-STRs loci (Table 3), (Figure 1). Using 25 µl PCR amplification mixture (9.2 µl of PCR reaction mix, 5 µl of Y-filer™ Primer Sets, 0.8 µl AmpliTaq Gold DNA polymerase and 10 µl of template DNA in TE buffer (1ng of DNA template), amplification was carried out under conditions of initial denaturation at 95°C for 11 min, followed by 29 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min, extension at 72°C for 1 min and a final extension step at 60°C for 60 min.

Electrophoresis

The PCR products were then examined using a 10-µl electrophoresis system containing 0.3 µl of Gene Scan™ 500 LIZ® Size Standard dye, 8.7 µl of Hi-Di™ formamide and 1.0 µl of PCR product or the AmpFlSTR® Identifiler Plus® allelic ladder. Capillary electrophoresis was performed on an ABI-3130 Genetic Analyzer using 36 cm 4-capillary array (Applied Bio-systems). Sizing of the DNA fragments was carried out using Gene Mapper ID software v3.2 with respect to Gene Scan™ 500 LIZ® Size Standard. The resultant allelic distribution (genotypes) obtained from the studied loci in the exhibits is shown in the Table 2, Table 3 and Figure 1.

Results

The DNA profile generated from the source of Exhibit marked-A (Source: Blood Positive Saya Cuttings) is from a human female. The DNA profile generated from the source of Exhibit marked-B1 (Source: Blood and semen Positive Janghiya cuttings) is mixed profile of a human male and also from a human female. Out of which the female profile matched with the DNA profile of source of exhibit marked-A (Source: Blood Positive Saya Cuttings). The DNA profile generated from the source of Exhibit marked-C1 (Source: Blood and semen Positive Janghiya cuttings) is from another human male source of origin. Which did not match with the male DNA profile of source of exhibit marked-B1 (Source: Blood and semen Positive Janghiya cuttings).

Discussion

In the present case study successful DNA profile were generated from the blood on female (victim) undergarment and blood mixed with semen on male (suspects) undergarments. In the challenged exhibits where soils rich in humic acid and co-purify during the DNA extraction process, hamper the PCR amplication by acting as inhibitor. Such a PCR inhibitors usually decrease the sensitivity during real-time PCR assays. To avoid the PCR inhibitors samples (Blood and Blood mixed with semen) were purified via microcone (Microcone™ 100 Concentrator Unit) which enable centrifugal concentration and greatly reduce the risk of contaminants of DNA. In the DNA profile generated it was observed that there is no drop out

Table 3: Y-filler DNA profile Comparative Chart of allele distribution (genotype) of 17 different loci of the DNA tested.

Sl. No.	Name of loci	Exhibit marked-B1(Source: **Blood and Semen positive Janghiya Cutting)**
1.	DYS456	15
2.	DYS3891	13
3.	DYS390	23
4.	DYS389II	28
5.	DYS458	16
6.	DYS19	14
7.	DYS385	12, 20
8.	DYS393	13
9.	DYS391	10
10.	DYS439	11
11.	DYS635	24
12.	DYS392	10
13.	GATAH4	11
14.	DYS437	15
15.	DYS438	9
16.	DYS448	19

of alleles or loci and complete DNA profiles were generated from the exhibits marked A, B1 and C1. Interpretation of STR data revealed that the DNA profile generated from exhibit marked A (evidence from victim) is from a human female and this female profile matched with the DNA profile generated from the source of exhibit marked B1 (evidence obtained from suspect).

Conclusion

The DNA test performed on the exhibits noted above is sufficient to conclude that: The DNA profile generated from the source of Exhibit marked-A (Source: Blood Positive Saya Cuttings) is from a human female. The DNA profile generated from the source of Exhibit marked-B1 (Source: Blood and semen Positive Janghiya cuttings) is mixed profile of a human male and also from a human female. Out of which the female profile matched with the DNA profile of source of exhibit marked-A (Source: Blood Positive Saya Cuttings). The DNA profile generated from the source of Exhibit marked-C1 (Source: Blood and semen Positive Janghiya cuttings) is from another human male source of origin. Which did not match with the male DNA profile of source of exhibit marked-B1 (Source: Blood and semen Positive Janghiya cuttings).

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