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Research Article

Challenging and Rare DNA Evidences Convicts the Accused in a High Profile Murder Case

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Abstract

Identifying the obscured exhibits is both a challenging and a commendable task in forensic science. Here we report on solving the case of a doctor's murder (victim) carried out for demand of gross ransom by five perpetrators. The crime scene happened in the Gumla District which lies in the Red Corridor of Jharkhand State (Eastern India). The incident took place at two spots (atrocities for demand at a house and final murder in a forest) leaving behind some challenging evidences such as few scalp hairs on the bed, cigarette stubs, chewed tobacco and few blood drops scattered in a forest soil. These rare evidences were collected by the forensic team of Jharkhand and proceeded for DNA extraction and forensic analysis. The DNA obtained from these evidences matched with the DNA profile of the three suspects out of five under police detention. This is rare and first case reported in Jharkhand where chewed tobacco and few fallen hairs on bed sheet have been used to solve a critical crime case. Gumla police and the state forensic team were honoured with first prize in whole Jharkhand for solving this case scientifically.

Keywords: Rare evidences; Forensic genetics; Jharkhand; India

Introduction

Forensic science often involves the study of challenging samples (legally known as exhibits) collected from the crime sites where indiscernible tissues or samples are carved out whose identity determination remains a big challenge in the current scenario. At the site of crime scene, the body fluids of human such as blood, semen, visceral fluid, vaginal fluid, saliva, and menstrual fluid [1] are washed out of its natural texture and are discoloured often due to meagre in quantity or drying/bleaching out in long exposure. Such exhibits present a huge challenge in front of DNA forensic scientists to determine the actual cause/severity of the crime from trace amount of samples collected. Here we report a high profile murder case of a Doctor from the naxalite-hit area of Jharkhand, India where rare forensic samples (chewed tobacco and fallen hairs on bed) along with other significant exhibits were collected from the crime scenes.

Crime and crime scene investigation

The incident occurred in Gumla district, which lies in the south-east part of Jharkhand State in eastern India, an area which falls in the Red Corridor region and fully prone to frequent Naxalite-Moaist insurgency. A government resident, Dr. ABC (identity has been concealed) was abducted from his clinic on 30.04.2015. While the nurse couldn't see the Doctor returning to his clinic till evening, she filed an FIR (First Information Report) to the nearest police station. The abductors had demanded a lump sum ransom of about Rs 50 lakhs from his wife through phone, although the complete information was not conveyed to the police due to fear. On the 6th day of his kidnap on 05.05.2015, the Doctor's body was found dead near to Chandaal Dam in Raidih forest of the Gumla district about 10 km away from Kashitoli village where they had initially kept him for ransom demands. The incident resulted in widespread protests in Jharkhand from the Doctors community as well as the civilians (External links – 1 ,2, 3, 4,). With the help of a telephone number (mobile number) which was used to call the wife of the Doctor by the perpetrators, the Gumla police traced and held five persons under their custody imposing the Indian Penal Code under sections: 364(A)/302/201/120(B)/34. The Director of the Forensic Laboratory was immediately informed by the Superintendent of Police, Gumla district and a team of four Scientific Assistants with an Assistant Director was constituted and deputed for investigation and collection of samples that could help nab the perpetrators. On reaching at the protected site of crime in Kashitoli village ushered by the police, a locked house (Figure 1) by

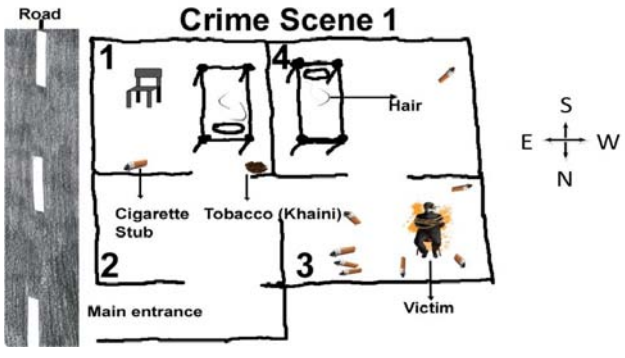

Figure 1: The crime scene 1 investigated by the forensic team.
In the west side of a road in Kashitoli village, the crime scene 1 was comprised of a house having two rooms (marked 1 and 4) and two verandas (marked 2 and 3) in all. The house was protected by seal and lock by the Gumla police immediately after the crime incident till the entering of the State forensic team into the house. Khaini (chewed tobacco) was collected from the room 1, a highly rare exhibit only can be observed in South-East Asia [8,9]. Cigarette stubs were collected from rooms 1and 4 and veranda 3. Fallen hairs on pillow and bed were collected from the rooms 1 and 4. Victim (Doctor) was kept tied in the day time in the veranda 3 as revealed from the perpetrators after solving the case with all these evidences.

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Table 1: Quantification of human DNA through RT-PCR using Quantifiler Human DNA kit.
*Chewed tobacco (Hindi)

S. No.	Sample / Exhibit	Yield of DNA (ng/µl) in 20 µl final volume
1	Exhibit marked – 1/A (source: hair strands)	1.710
2	Exhibit marked – 1/B (source: cigarette stub)	0.671
3	Exhibit marked – 1/C (source: Chewed khaini*)	12.79
4	Exhibit marked – 1/D1 (source: cigarette stub)	1.941
5	Exhibit marked – 1/D2 (source: cigarette stub)	0.086
6	Exhibit marked – 1/D3 (source: cigarette stub)	0.447
7	Exhibit marked – 1/D4 (source: cigarette stub)	0.110
8	Exhibit marked – 1/D5 (source: cigarette stub)	0.086
9	Exhibit marked – 1/D6 (source: cigarette stub)	0.030
10	Exhibit marked – 1/D7 (source: cigarette stub))	0.106
11	Exhibit marked – 1/D8 (source: cigarette stub)	0.342
12	Exhibit marked – 1/EA (source: hair strands from Room No-4)	0.119
13	Exhibit marked – 1/F (source: cigarette stub from Room No-4)	0.131
14	Exhibit marked – 1/G (source: blood stained earth from Sabi Toli)	-----
15	Exhibit marked – 1/I (source: a piece of cloth)	0.015
16	Exhibit marked – 3/A (source: blood of suspect 1 soaked on gauze)	31.33
17	Exhibit marked – 3/B (source: blood of suspect 2 soaked on gauze)	2.162
18	Exhibit marked – 3/C (source: blood of suspect 3 soaked on gauze)	22.86
19	Exhibit marked – 3/D (source: blood of suspect 4 soaked on gauze)	0.935
20	Exhibit marked – 3/E (source: blood of suspect 5 soaked on gauze)	2.62

Gumla police having two rooms (1 and 4) and two verandas (2 and 3) was the first site to be investigated. The police helped to unlock the door with the key with them and the forensic team headed for investigation and collection of samples. Photography of each room and each possible forensic sample was properly collected to recreate the crime scene. No forensic samples could be recovered from the first veranda where a broken couch was lying with some bed sheets and blankets which were wrapped round each other. Then after entering into the first room and after a long search with the help of halogen torch, two hairs about 3 cm and 5 cm length were picked with the help of sterile forceps from the bed lying over there, sealed in an envelope and labelled. Surprisingly some chewed tobacco (dried) was found glued in north of the wall that was collected by sterile forceps, wrapped in a paper, sealed in a paper envelope and labelled. Then the team entered into the second veranda where there was an iron made chair and there were 7 cigarette stubs lying on the ground, each of which were picked up and kept in separate envelopes. As later on revealed from one of the perpetrators, the chair was used to tie down the Doctor in the day time. The second room was facing south of the second veranda (Figure 1) and was previously closed with a big lock by the perpetrators. The lock was broken with the help of a hammer and a big rod of iron. The second room had too some hair falls on the bed sheet of a bed, lying in the east of the room that were collected with the help of sterile forceps and sealed in a separate envelopes and labelled. After having the comprehensive search-through of the site, the forensic team headed towards the Raidih forest, the second site of crime scene where the dead body of the Doctor was recovered and already sent to post mortem in the very morning. It was about 7:30 PM in the evening, the blood scattered in the forest soil near Chandaal Dam was confirmed by the Kastle-Meyer test [2,3] as well as Luminol chemiluminescence [4]. The blood positive soil as well as nearby soil with negative blood test for reference were also collected for comparative physical analysis. All the samples collected were sealed with molten lac-gum as well as with signature on the envelope closure and handed over to the investigating officer of police Mr. XYZ and was asked to channel these exhibits with full custody to the forensic laboratory along with blood samples of suspects under police detention in dry state on gauze pieces.

Materials and Methods

Descriptions of exhibits

Exhibits from Room marked-1: Exhibit marked – 1/A (source: hair strands), exhibit marked – 1/B (source: cigarette stub) and exhibit marked – 1/C (source: khaini). *Exhibits from Veranda marked-3:* Exhibit marked – 1/D1 (source: cigarette stub), exhibit marked – 1/D2 (source: cigarette stub), exhibit marked – 1/D3 (source: cigarette stub), exhibit marked – 1/D4 (source: cigarette stub), exhibit marked – 1/D5 (source: cigarette stub), exhibit marked – 1/D6 (source: cigarette stub), exhibit marked – 1/D7 (source: cigarette stub), exhibit marked – 1/D8 (source: cigarette stub). *Exhibits from Room marked-4:* Exhibit marked – 1/EA (source: hair strands), exhibit marked – 1/F (source: cigarette stub), exhibit marked – 1/I (source: a piece of cloth). *Exhibits as blood soaked on gauze piece from accused:* Exhibit marked – 3/A (source: suspect 1), exhibit marked – 3/B (source: suspect 2), exhibit marked – 3/C (source: suspect 3), exhibit marked – 3/D (source: suspect 4), exhibit marked – 3/E (source: suspect 5). *Other exhibits:* Exhibit marked – 1/G (source: blood stained earth from Raidih forest).

Table 2: Comparative chart of the allele distribution (genotype) of different loci of the DNA tested.

S. No.	Name of locus	Exhibit marked – 1/A	Exhibit marked – 1/B	Exhibit marked – 1/C	Exhibit marked – 1/D1	Exhibit marked – 1/D2	Exhibit marked – 1/D3
1	D8S1179	14, 15	13, 16	13, 16	10, 14	10, 12, 13, 14, 15	10, 12, 13, 14, 15
2	D21S11	29, 31.2	29, 30	29, 30	28, 28	28, 29	28, 29, 31.2, 32.2
3	D7S820	8, 11	11, 12	11, 12	9, 10	11, 11	8, 11, 12
4	CSF1PO	10, 12	10, 11	10, 11	10, 12	11, 12	9, 12
5	D3S1358	15, 17	17, 17	17, 17	16, 17	14, 16, 17	14, 15, 16, 17, 18
6	THO1	7, 9	9, 9	9, 9	8, 9	8, 9	7, 8, 9, 9.3
7	D13S317	8, 12	10, 12	10, 12	8, 11	9, 10, 12	8, 10, 11, 12
8	D16S539	10, 12	13, 13	13, 13	8, 11	10, 12, 13	10, 11, 12, 13
9	D2S1338	19, 21	21, 23	21, 23	21, 21	26, ….	17, 18, 19, 20
10	D19S433	13, 14	13, 13	13, 13	12, 14	13, 14, 15.2	13, 14, 15, 15.2, 16.2, 17.2
11	vWA	15, 15	14, 14	14, 14	17, 18	15, 16, 17, 18	15, 16, 17
12	TPOX	8, 11	9, 10	9, 10	8, 8	8, 8	8, 10, 11, 12
13	D18S51	13, 13	15, 21	15, 21	13, 13	14, 18	13, 14, 15
14	D5S818	12, 12	11, 12	11, 12	10, 12	10, 11, 13	10, 11, 12
15	FGA	20, 22	22, 25	22, 25	20, 20	22, 25	20, 21, 22, 25
16	Amelogenin	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y

DNA isolation

Twenty exhibits (Table 1 and listed above) collected from the crime scene 1, crime scene 2 and from the accused persons and were subjected to organic extraction method for DNA isolation [5]. Briefly the mouth-end filter of cigarette stubs, hair with roots, chewed tobacco and blood swabs on gauze piece 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 separate 1.5 ml capacity eppendorf tubes for 1 hours at 37 °C. Proteinase K was then added and incubated for further 3 hours at 56 °C with periodic swirling. The solution was brought at room temperature and an equal volume of Tris-equilibrated phenol (pH 8.0) was added and mixed by turning the tube end over end for 5 minutes. The tubes were then centrifuged at 5000 X g for 10 min at room temperature. The aqueous supernatant was then transferred in another fresh eppendorf tube. The DNA in the aqueous solution was precipitated by adding 2X vol of absolute ethanol and kept at -20 °C for 30 min. The tubes were then centrifuged at 15, 000 X g for 15 min. The pellet obtained was washed with 70% ethanol to remove remnant salts contaminated with DNA. The tubes were 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 its use. Amplifiable DNA could not be extracted from exhibits 1/D6 and 1/I.

DNA quantification

DNA quantification of the 20 samples (Table 1) was performed with real time polymerase chain reaction (RT-PCR) using the Quantifiler® Human DNA Quantification kit (Life Technologies Inc.) [6] containing DNA standard solution (200 ng/µl), Quantifiler Human Primer mix, and Quantifiler PCR Reaction Mix. Human Primer Mix (10.5 µl/sample) and PCR Reaction Mix (12.5 µl/sample) were mixed and then dispensed into reaction wells (23 µl each) followed by the addition of 2 µl of sample or standard DNA of known concentration to each well, to obtain a 25 µl PCR reaction system. The amount of DNA was calculated by the real time PCR machine (Applied Biosystems).

Identifiler plus® pcr and electrophoresis

AmpFISTR Identifiler Plus® kit [7] 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 (10.5 µl of PCR reaction mix, 5.5 µl of Identifiler Plus® Primer Set, 9.0 µl of nuclease-free water and 1 µl of DNA template), amplification was carried out under conditions of initial denaturation at 95°C for 10 min, followed by 29 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min and extension at 72°C for 1 min, and a final extension step at 60°C for 60 min. The PCR products were then examined using a 10-µl electrophoresis system containing 0.3 µl of GeneScan™ 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 Biosystems). Sizing of the DNA fragments were 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 Software: 7500 SDS V.1.2.3 (Applied Biosystems) of the studied loci in the exhibits is shown in the five Tables (2-6). Similarly multiplexing of the Y chromosome alleles for the DNA where mixed population was obtained above exhibits was carried out using AmpFISTR Y-filer® PCR amplification kit and protocol.

Results

The exhibit marked -1/A (hair strands from room No-1), exhibit marked -1/F (cigarette stub from room no-4), and exhibit marked 3/E (Suspect 5) were having DNA profile of one and the same individual. The exhibits marked-1/B (cigarette stub from room no-1), 1/C (khaini from room no-1) and 3/C (Suspect 3) were having DNA profile of one and the same individual. The exhibits marked- 1/

Table 3: Comparative chart of the allele distribution (genotype) of different loci of the DNA tested.

S. No.	Name of locus	Exhibit marked – 1/D4	Exhibit marked – 1/D5	Exhibit marked – 1/D7	Exhibit marked – 1/D8	Exhibit marked – 1/EA	Exhibit marked – 1/F
1	D8S1179	10, 12, 13, 14, 15	10, 12, 13, 14, 15	10, 11, 12, 13, 14, 15	10, 12, 13, 14, 15	13, 15	14, 15
2	D21S11	28, 29, 32.2	27, 28, 29, 32.2	28, 29, 31.2, 32.2	28, 29, 30	29, 30	29, 31.2
3	D7S820	8, 9, 10, 11, 12	8, …..	8, 11	8, 9, 10	8, 10	8, 11
4	CSF1PO	9, 10, 12	12, 12	9, 10, 12	10, 11, 12	11, 11	10, 12
5	D3S1358	14, 15, 16, 17, 18	14, 15, 16, 17, 18	14, 15, 16, 17, 18	16, 17	17, 17	15, 17
6	THO1	7, 8, 9, 9.3	7, 8, 9, 9.3	7, 8, 9	6, 7, 8, 9	6, 7	7, 9
7	D13S317	8, 11, 12	11, 12	8, 11, 12	8, 11, 12	8, 12	8, 12
8	D16S539	8, 10, 11, 12, 13	11, 12, 13	10, 11, 12	8, 11, 13	11, 13	10, 12
9	D2S1338	17, 18, 20, 21	NA	17, 20	21, 23	23, 23	19, 21
10	D19S433	12, 14, 15, 15.2, 16.2, 17.2	13, 14, 14.2, 15, 15.2, 17.2	13, 14, 15, 15.2, 16.2, 17.2	12, 13, 14	13, 13	13, 14
11	vWA	15, 16, 17, 18	14, 15, 16, 17	15, 16, 17	15, 16, 17, 18	16, 18	15, 15
12	TPOX	8, 11, 12	8, 10, 11	8, 10, 11, 12	8, 9	9, 9	8, 11
13	D18S51	13, 14, 15	14, 14	13, 14	12, 13, 14	12, 14	13, 13
14	D5S818	10, 11, 12	10, 11, 12	10, 11, 12	10, 12, 13	12, 13	12, 12
15	FGA	20, 21, 22, 25	21, 22, 25	20, 21, 22, 25	20, 23, 25	23, 25	20, 22
16	Amelogenin	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y

Table 4: Comparative chart of the allele distribution (genotype) of different loci of the DNA tested.

S. No.	Name of locus	Exhibit marked – 1/G	Exhibit marked – 3/A	Exhibit marked – 3/B	Exhibit marked – 3/C	Exhibit marked – 3/D	Exhibit marked – 3/E
1	D8S1179	13, 15	10, 14	14, 15	13, 16	9, 14	14, 15
2	D21S11	29, 30	28, 28	28, 29.2	29, 30	30, 32.2	29, 31.2
3	D7S820	8, 10	9, 10	8, 12	11, 12	9, 11	8, 11
4	CSF1PO	11, 11	10, 12	10, 11	10, 11	10, 10	10, 12
5	D3S1358	17, 17	16, 17	15, 15	17, 17	14, 16	15, 17
6	THO1	6, 7	8, 9	6, 9	9, 9	9, 9	7, 9
7	D13S317	8, 12	8, 11	8, 13	10, 12	8, 11	8, 12
8	D16S539	11, 13	8, 11	9, 10	13, 13	10, 12	10, 12
9	D2S1338	23, 23	21, 21	23, 24	21, 23	17, 18	19, 21
10	D19S433	13, 13	12, 14	13, 14	13, 13	13, 14.2	13, 14
11	vWA	16, 18	17, 18	15, 15	14, 14	16, 17	15, 15
12	TPOX	9, 9	8, 8	8, 11	9, 10	11, 11	8, 11
13	D18S51	12, 14	13, 13	17, 17	15, 21	12, 17	13, 13
14	D5S818	12, 13	10, 12	11, 11	11, 12	11, 11	12, 12
15	FGA	23, 25	20, 20	20, 20	22, 25	21, 23	20, 22
16	Amelogenin	X, Y	X, Y	X, Y	X, Y	X, Y	X, Y

D1 (cigarette stub from veranda marked-3) and 3/A (Suspect 1) were having DNA profile of one and the same individual. The exhibits marked-1EA (hair strands from room no-4) and 1/G (blood stained earth from Raidih forest) were from one and the same individual (Doctors profile), which connects the crime scene 1 with crime scene 2. The exhibit marked-3/D did not match with any of the exhibits. The profile generated from the exhibits viz. 1/D2, 1/D3, 1/D4, 1/D5, 1/D7 and 1/D8 were from more than one individual (Table 2-6). DNA profile could not be generated with the isolated DNA obtained from the exhibits 1/D6 and 1/I.

Discussion

Solving a forensic case dauntlessly and scientifically is a reward in itself for the scientific experts and the police involved in the case. Rare and challenging exhibits often obscure the crime scene and present a huge challenge in forensic study as long as the perpetrators are getting smarter to elude the crime scene. This was the high profile case where Forensic Laboratory had deployed its newly recruited forensic experts of 2015 batch to solve a forensic case in a naxalite-hit area of Jharkhand, a state which lies in eastern part of India. The incident occurred after kidnapping and murdering a doctor in a dense forest

Table 5: Comparative chart allelic distribution (genotypes) of different loci of DNA tested using Y filer kit.

S. No.	Y Filer locus	Exhibit marked-1/D2	Exhibit marked-1/D3	Exhibit marked-1/D4	Exhibit marked-1/D5	Exhibit marked-1/D7	Exhibit marked-1/D8
1	DYS456	15	15	16	14, 15	14, 15, 16	15, 16
2	DYS389I	15	12	13	NA	12	12, 13
3	DYS390	24	23	19	NA	23	22, 25
4	DYS389II	31	27	29	NA	27	27, 29, 30
5	DYS458	17	15	17	15, 17	15	15, 16, 17
6	DYS19	16	15	15	15	15	15
7	DYS385a/b	10, 14	12, 16	15, 17	NA	12, 16	11, 14, 15, 17
8	DYS393	13	13	12	13	13	12, 14
9	DYS391	10	10	10	NA	10	10, 11
10	DYS439	10	12	12	NA	NA	10, 12
11	DYS635	23	21	20	NA	21	20, 23
12	DYS392	12	11	11	NA	11	11
13	Y GATA H4	13	11	12	11	11	11, 12, 13
14	DYS437	14	14	14	NA	14	14
15	DYS438	11	11	9	NA	11	9, 11
16	DYS448	20	21	19	NA	NA	19, 20

Table 6: Comparative chart allelic distribution (genotypes) of different loci of DNA tested using Y filer kit.

S. No.	Y Filer locus	Exhibit marked-1/EA	Exhibit marked-1/G	Exhibit marked-3/A	Exhibit marked-3/B	Exhibit marked-3/C
1	DYS456	16	16	16	16	16
2	DYS389I	12	12	13	14	12
3	DYS390	25	25	22	22	25
4	DYS389II	30	30	29	30	29
5	DYS458	16	16	17	16	15
6	DYS19	15	15	15	15, 16	15
7	DYS385a/b	11, 14	11, 14	15, 17	14, 15	15, 18
8	DYS393	14	14	12	13	14
9	DYS391	11	11	10	10	11
10	DYS439	10	10	12	12	12
11	DYS635	23	23	20	21	22
12	DYS392	11	11	11	11	13
13	Y GATA H4	13	13	12	13	11
14	DYS437	14	14	14	16	14
15	DYS438	11	11	9	10	10
16	DYS448	20	20	19	21	18

of Gumla District leaving behind rare and challenging exhibits at two crime sites. The exhibits were collected and presented as evidences to reconstruct the crime scene with full effort from forensic team as well as Jharkhand police. This is highly rare case reported in Jharkhand where Khaini (chewed tobacco), cigarette stubs and few fallen hairs on beds (besides collecting other pertinent exhibits systematically as listed in Table 1) have been used to solve a critical crime case. All the five perpetrators were convicted of plotting and executing the murder crime of Dr. ABC from end to end thorough investigation by the Gumla police although the DNA profiles of the three accused (Suspect 1, Suspect 3 and Suspect 5) out of five were able to be obtained in the laboratory and matched with the DNA profiles obtained from the crime scene 1. Gumla police headed by Investigating Officer Mr. DEF and the forensic team were awarded first prize by the Chief Minister of Jharkhand in the concluding year of 2015 for solving this case dauntlessly and scientifically.

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