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RESEARCH ARTICLE

ESTABLISHMENT OF PATERNITY IN SEXUAL OFFENCES CRIME REGISTERED UNDER POCSO ACT, 2012 FROM THE SAMPLE OF CHORIONIC VILLI- A CASE STUDY

Himjay Kumar[1], Ashu Kumar Jha[2], Priti Bala[2] and Prakash Kumar[3]

[1,2]Assistant Director, Forensic Science Laboratory, Bihar, Patna
[2,3]Senior Scientific Assistant, Forensic Science Laboratory, Bihar, Patna

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**Corresponding Author:*
Debasmita Debbarma

ABSTRACT

DNA is present in most of the cells in our body, which is unique in each and everyindividual, and we leave a trail of it everywhere we go. This has become an advantagefor forensic investigators who use DNA to draw conclusion in identification of victimand accused in crime scenes. Chorionic villi are villi that sprout from the chorion to provide maximal contact area with maternal blood.They are an essential element in pregnancy from a histomorphology perspective, and are, by definition, a product of conception. Branches of the umbilical arteries carry embryonic blood to the villi. After circulating through the capillaries of the villi, blood returns to the embryo through the umbilical vein. Thus, villi are part of the border between maternal and foetal blood during pregnancy.Child sexual abuse is one of the major problems of India. In regard to this problem, a special law has been enacted named as Protection of Children from Sexual Offences Act (POCSO) with a view to protect the rights of children. Undoubtedly, passing of this Act has been a major step in securing children's right. In the present case a girl child of aged about 11 years was sexually abused by her school teacher. Upon arising of symptoms her mother doubted and asked the child about facts. Mother took her to the doctor where she tested positive for 9 weeks pregnancy, subsequently a FIR was lodged in a Mahila P.S of District. As it was not feasible to deliver in such a minor age, a legal abortion was carried out upon intervention of hon'ble trial court. The product of conceptus (Chorionic villi) along with reference blood samples of accused and victim were received in Forensic Science Laboratory, Bihar, Patna for establishing paternity. The DNA examiners team at FSL, Patna experienced first time such kind of sample and took it as a challenge. The sample of chorionic villi were subjected to DNA extraction using Automate DNA Extraction Prepfiler Express method and DNA from reference Blood Samples were extracted separately. The DNA were further subjected to Quantitation using 7500 RT PCR instrument and Quantifiler Duo amplification kit followed with 16 STR multiplexing using 9700 PCR machine and Identifiler plus amplification kit. The amplified DNA were subjected to 3130, 4-capillary Genetic Analyzer for fragment analysis.The conclusion revealed that the accused found to be biological father of the product of conceptus(chorionic villi) and upon submission of detailed report, the hon'ble trial court sentenced accused for Death Penalty.

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INTRODUCTION

Forensic DNA examination is basically utilized for identification of any individual using methodology of genetic principles. In modern era, the choice of genetic material are short tandem repeats. The variable (Polymorphic) nature of the STR regions that are analyzed for forensic testing intensifies the discrimination between one DNA profile and another. The system of DNA profiling used today is best on PCR (polymorphic chain reaction) and uses simple sequences. This method uses highly polymorphic regions that have short repeated sequences of DNA (the most common is 4 bases repeated, but there are other lengths in use, including 3 and 5 bases). These STR loci (locations on a chromosome) are targeted with sequence-specific primers and amplified using PCR.

The DNA fragments that result are then separated using electrophoresis, the commonly method of separation and detection today used is capillary electrophoresis. The Chorionic villi are villi that sprout from the chorion to provide maximal contact area with maternal blood. They are an essential element in pregnancy from a histomorphology perspective, and are, by definition, a product of conception. Branches of the umbilical arteries carry embryonic blood to the villi. After circulating through the capillaries of the villi, blood returns to the embryo through the umbilical vein. Thus, villi are part of the border between maternal and foetal blood during pregnancy. It is one of the challenging sample for establishing paternity as it has maximal contact with mother and to extract nuclear DNA of the offspring needs extra precaution and exclusion of contamination.

About 5 ml of liquid blood of accused and victim (in EDTA vial) were received separately in this laboratory along with Chorionic villi in separate vial. The nuclear DNA were extracted from each of the samples separately using DNA Automation method and subjected to human duo quantifiler method using real time PCR for estimation of quantity and quality of DNA. The amplification of extracted DNA were carried out using 16 STR (Autosomal) multiplex reaction. The DNA fragment analysis were done using capillary electrophoresis and the data were interpreted with gene mapper ID-X software.

MATERIAL AND METHOD

Material

Applied Biosystem Automate express – Prepfiler Express Forensic DNA Extraction kit. Chemical and Consumables – 1.5 ml Eppendorf tube, 10 to 1000 µl filter tips, 10 to 1000µl variable micro pipettes, 15 ml falcon tube, Prepfiler Express Cartridges, LySep Column, 0.2 ml PCR Tube, 96 well optical plate, Adhesive film, 96 well plate Septa, 1 M DTT Solution, Lysis buffer, Quantifiler Duo RT PCR Amplification kit, 16 STR (Autosomal) multiplexing Identifiler plus Amplification kit, Hidi Formamide, EDTA buffer, POP-4 (Performance Optimized Polymer), Gene Scan 500 LIZ size standard.

- Eppendorf Thermomixer
- Real Time PCR 7500 – HID Method
- Thermal Cycler – 9700 PCR Machine
- Eppendorf minispin and Cryo-centrifuge
- Plate Centrifuge
- 130 Capillary Electrophoresis Genetic Analyzer (5 Dye matrix Chemistry)

Method

The preserved Chorionic Villi contained in vial were transferred to sterilised petridish and macerated. 50 mg of macerated lump was transferred into 1.5 ml Eppendorf tube and subjected for lysis using 500 µl Prepfiler lysis buffer and 5 µl 1 M DTT solution and placed on thermomixer for incubation at 70^{O} C with 750 RPM shake and left for 40 minutes. The lysate was transferred into 1.5 ml sample tube fitted with lysep column and centrifuged at 10000 g for 2 minutes. The sample tube filled with lysate subjected in Automate Express workstation fitted with DNA Extraction Prepfiler Express Cartridge and run for 30 minutes for extraction of nuclear DNA. 25 µl of isolated DNA collected into separate 1.5 ml Eppendorf tube. Similarly, 40 µl of liquid blood sample of accused and victim were respectively transferred into 1.5 ml Eppendorf tubes and subjected for nuclear DNA extraction using Prepfiler Express Forensic DNA extraction method as described above. The extracted nuclear DNA were further subjected to quality assessment and quantitative analysis using real time PCR Quantifiler Duo Kit method.

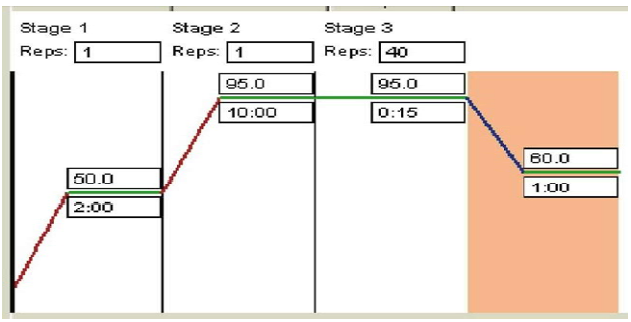

Fig 1 – Real time PCR temperature condition

Master mix including 10.5µl PCR mix and 12.5 µl primers were prepared for all the samples (2 µl each) and 8 standard (with dilution factors –50 ng/µl, 16.7 ng/µl, 5.56 ng/µl, 1.85 ng/µl, 0.62 ng/µl, 0.21 ng/µl, 0.68ng/µl, 0.023 ng/µl) respectivelysubjected for PCR at following conditions.

Table 1. PCR temperature variations with number of cycles

Initial incubation step	Cycle (29 cycles*)		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE	Anneal/Extend	HOLD	HOLD
95°C 11 min	94°C 20 sec	95°C 3 min	60°C 10 min	4°C ∞

The extracted DNA were further subjected to 16 STR (autosomal) multiplexing using applied biosystems Identifiler plus amplification kit method and 9700 Geneamp PCR machine.Master mix including 10µl and 5 µl primers were prepared for all the samples (10 µl each) respectively the PCR tubes loaded with prepared sample subjected to following PCR conditions. The amplified products were subjected for fragment analysis using 3130 model capillary electrophoresis (4-capillary sequencer) with 5-dye matrix chemistry, Genescan 500 LIZ size standard and POP-4(polymer)

- Mixture of 9.7 µl of amplified DNA along with 0.3 µl 500 liz size standard and 1 µl Hidi formamide prepared in respective well of 96 well plate.
- The loaded 96 well plate subjected to centrifuge for removal of any bubble.
- The prepared samples in 96 well plate were subjected to thermal cycler for denaturation at 95^{O}C for 3 minutes and subsequently cooled down.
- The prepared samples in 96 well plate subjected in 3130 4-capillary genetic analyser for the analysis of fragments.
- The resultant genotype were studied using I-DX software.

RESULT AND DISCUSSION

The quality and quantity of extracted DNA from Chorionic villi was analysed using real time PCR and following resultswere observed

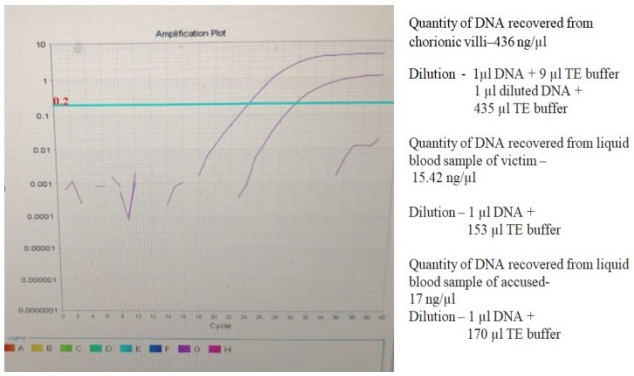

Fig. 2. Amplification plot of the sample Chorionic Villi (HID software)

The raw data confirms the quality of DNA fragments analyzed with I-DX software

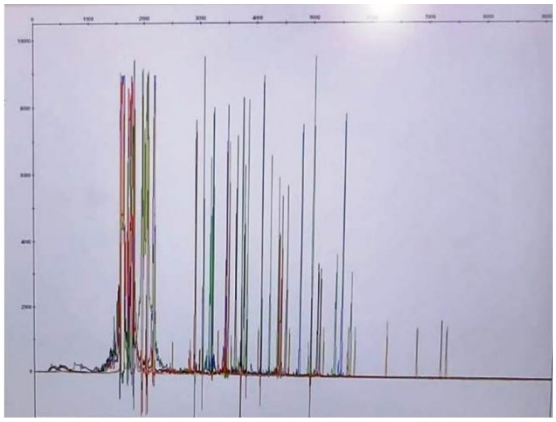

Fig. 3. The raw data confirms the quality of DNA fragments analyzed with Gene mapper ID-X software

Genotype of samples

Table 2. Allelic distribution of 15 STR markers along with Amelogenin marker analysed with ID-X software

Sl. No.	Name of STR Loci Marker	Accused	Chorionic villi	Victim
1	D8S1179	15, 16	10, 15	10, 14
2	D21S11	29, 32.2	29	29, 32.2
3	D7S820	8, 10	8	8
4	CSF1PO	9, 12	12	12, 13
5	D3S1358	17	16, 17	15, 16
6	TH01	7, 9	7, 9	6, 9
7	D13S317	11, 13	8, 11	8, 9
8	D16S539	11, 12	12	11, 12
9	D2S1338	24	18, 24	18, 23
10	D19S433	13	13	12, 13
11	VWA	14, 17	14, 17	16, 17
12	TPOX	9, 11	9, 11	9, 11
13	D18S51	14, 16	14, 15	15
14	D5S818	10, 11	11, 12	11, 12
15	FGA	21, 25	21, 22	22, 24
16	Amelogenin	XY	X	X

CONCLUSION

On the basis of conclusion the accused was found to be biological father of the embryo (extracted part of chorionic villi i.e., product of conceptus) the learned trial court convicted the accused to capital punishment.

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