

Use of DNA Technique to Search and Identify the Mother of Abandoned Foetus – A Case Study

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Abstract: *Feticide is increasing due to prejudiced thinking in the society. Reason for feticide is mainly due to discrimination against girl child. This crime mainly goes unsolved because no clue is found about the criminal. But in present case right investigation and use of advanced technique i.e. DNA fingerprinting made it possible to catch the criminal mother. Two fetuses (described as of male & female) were found in the garbage of a village of Kaithal District of Haryana. The crime was very heinous but no clue was available but police detective started investigation to search for any woman who was pregnant in the nearby and found one in a local village. The suspected woman was subjected to DNA profiling to compare with the DNA profile of two fetuses. Surprisingly one of the fetuses was found to be biological child of the woman. Further both fetuses who were thought to be twins and described as male & female were found to be both male and biologically different through DNA fingerprinting and gave new direction to police investigation and helped catch the mastermind of the racket.*

Keywords: Fetus, Femur, STR, feticide, DNA profiling, Haryana

1. Introduction

DNA profiling has now become an important tool in individualization and comparison in many criminal cases like paternity, rape, homicide, assault, missing identity and illegal immigration etc. Due to high discrimination power of DNA patterns, it is accepted in judiciary beyond doubt. The witness may turn hostile in the court but DNA profiling becomes strong evidence

It gained its popularity because of its use in many famous controversial and high profile cases such as ND Tiwari case, tandoor murder case of Delhi and various rape and disputed paternity cases. DNA fingerprinting refers to the characterization of one or more relatively rare features of an individual's genome or hereditary makeup (Kirby, 1990). It is like signatures that resemble the bar codes on market goods. Unique genetic structure of each individual can be detected by recombinant DNA techniques.

Short tandem repeats (STR) are one of the most commonly analyzed and informative PCR based genetic markers for individualizing the biological material (Butler and Becker, 2001). The STR loci are tandemly repeated sequences of 1-6 bp core units and range from 50-300bp. (Goodwin et al., 2007) Because of the relatively small size of STR alleles, amplification by PCR is relatively easy, where even low amount and degraded samples may be amplified, with high sensitivity (Tautz, 1989). In addition, STR loci can be amplified simultaneously in a multiplex PCR. Thus, substantial information can be obtained in a single analysis with the ancillary benefits of using less template DNA, reducing labour and chances of contamination (Murphy, 2017).

2. Case History

Feticide is a heinous crime. Reason for feticide is generally due to discrimination towards girl child or apprehension of

facing humiliation by becoming mother without marriage in our society. Such woman is seen with very much hatred and family has the fear of social isolation. Due to which generally the child is aborted by the mother or the family. In the present case two dead fetuses, described as one male and another female, were found in the garbage of a village of Kaithal District of Haryana. Police lodged a case and started investigating the case. There was no clue of the criminals. Although the investigation was started and the police officer heard through informers about an unmarried woman who was suspected to have been pregnant recently as she was looking very weak as if she has undergone abortion or delivery of a child. Police searched and detained the woman and got her medically examined. After medical examination it was established that she has undergone abortion recently. Suspicions were on that woman to be the mother of the abandoned twin fetus found in the garbage. But there was no evidence or witness to establish legality of the allegation. The concerned investigating officer approached the DNA branch of Forensic Science Laboratory, Haryana where he was advised to send the suitable sample of fetus and the blood sample of suspected woman.

3. Materials and Methods

Sample Pre-processing: Small femur bones of each the fetuses and blood sample of suspected woman were received in DNA Division of our laboratory. Femur bones were foul smelling and thus cleaned with sterile water and ethanol. The femur bone samples (approximately 1gm) were crushed to powder by mixing in liquid nitrogen with the help separate sterile pestle and mortar.

DNA Extraction from bone: DNA was extracted by organic extraction method. Bone powder was first decalcified by incubating with 0.5 M EDTA, pH 8.0. Decalcified material was then subjected to DNA extraction by adding lysis buffer (50 mM Tris-HCl, 100 mM NaCl, 50 mM ethylenediamine tetraacetic acid [EDTA]), proteinase K

and SDS for digestion of proteins and incubated at 37°C overnight with shaking. The extracted DNA from bone sample was purified in Nanosep-30K. (Jakubowska)

DNA Extraction from blood: DNA was extracted from blood sample by first adding lysis buffer-I (Tris-HCl – 30mM, EDTA – 5mM, NaCl – 50mM), freezing at -80°C and thawing at 65°C. It was then centrifuged, pellet was washed with normal saline. The pellet was incubated with lysis buffer-II (NaCl – 75mM, EDTA – 2mM), proteinase K and SDS at 37°C with mild shaking overnight. DNA was dissolved in sufficient amount of Tris-EDTA buffer (pH 8.0) after Phenol:Chloroform:Isoamyl-alcohol extraction (Sambrook and Russell, 2001).

Quantification: DNA samples were quantitated using Nano-Drop-1000 spectrophotometer and checked for quality by further performing electrophoresis in 0.8% Agarose gel.

Amplification of STRs: PCR was performed in thermal cycler, Gene Amp 9700 of Applied biosystems by using AmpF/STR identifier kit, which uses 5-dye technology, to amplify 15 Autosomal STR markers and amelogenin (Applied Biosystems, 2001). DNA was diluted before PCR and PCR reaction mix was prepared from the available Identifier kit for Autosomal STRs by pipetting PCR Master mix (9.5 µl), PCR Primer mix (5.0 µl), Taq Polymerase (0.5 µl), Template DNA (10 µl at concentration of 0.125ng/µl) in a single 0.5ml micro centrifuge tube. All the contents were mixed uniformly and PCR was run as per the following program:

Table 1: PCR thermal cycle set in ABI 9700 thermal cycler.

HOLD	30 CYCLES			HOLD	HOLD
95°C	94°C	59°C	72°C	60°C	4°C∞
11 minutes	1 minute	1 minute	1 minute	30 minutes	

These tubes were stored at -15°C to -25°C till further analysis.

Genotyping: The PCR amplicon was then processed for capillary electrophoresis in 36cm Capillary Array filled with POP4 polymer to separate different STRs in ABI3130 Genetic Analyzer of Applied Biosystems, USA. Initially HIDI Formamide (8.7 µl) and Liz (0.3) was taken into the well of reaction plate. Wells in reaction plates were loaded with positive control, negative control and allelic ladder alongside 1µl of Post PCR product of samples. 96 well Sample tray was capped with a septum. Septa was checked whether having a slit prior to capping tubes. Sample amplicons were denatured at 95°C in a thermal cycler or heat block for 3 minutes and snap-chilled at 4°C for 2 minutes. Sample tray was then loaded in auto sampler. Electrophoresis in the genetic analyser was then started in ABI3130 Genetic Analyzer. The results were then analyzed using Genemapper IDv3.0 software (Thangaraj *et al.*, 2004).

4. Results and Discussion

Blood sample of woman was marked as exhibit-A and femur bones of each fetus, described male and female, were marked as exhibit-B and C respectively. Both the femur bones marked exhibit-B and C showed amplification on all the 15 autosomal STR and amelogenin loci using AmpF/STR identifier kit as tabulated in table-1. The DNA profile of suspected woman was also generated. After comparison of DNA profile of femur samples of fetuses (exhibit-B and C) with that of woman it was observed that

here DNA profile was sharing the half of the alleles at each STR loci with the DNA profile of fetus marked as exhibit-B. DNA profile of fetus marked as exhibit-C was not sharing any allele at eight STR loci (D8S1179, D21S11, CSF1PO, TH01, D2S1338, D18S51, D5S818, FGA). Thus, it can be stated with absolute certainty that suspected woman was the biological mother of the fetus-B and was not biologically related to fetus-C.

With the help of DNA profiling, a new fact was revealed that fetuses were not biological twins and both were male fetus as both indicated XY genotype. Another fetus might have been abandoned by another person(s) and need to be investigated. With right approach of investigation and then use of advanced technique i.e. DNA profiling, the criminal mother could be found. Thus DNA analysis gave new direction to investigating agency. Later a local nursing home was found to be involved in illegal abortion banned by the law in India. DNA Profiling is now broadly accepted in medico-legal cases.

5. Conclusion

Through DNA profiling the criminal in this case was confirmed to have committed the crime which was otherwise no possible by any other method. Not only had this it also gave clue to investigating agency about a racket helping in feticide. Use of DNA profiling in criminal investigation has become deterrent for the criminals and it is also a best method to exonerate innocent falsely implicated in any case.

Table 2: The results of autosomal genetic markers contained in AmpF/ Identifier kit of the woman and both fetuses

Sr. No.	STR Locus	Exhibit-A (Suspected Woman) (corresponding allele)	Exhibit -B (corresponding allele)	Exhibit -C (corresponding allele)
1.	D8S1179	11, 14	10, 14	13, 16
2.	D21S11	31, 32.2	31.2, 32.2	29, 29
3.	D7S820	11, 11	7, 11	11, 11
4.	CSF1PO	11, 12	11, 12	10, 10
5.	D3S1358	15, 17	16, 17	16, 17
6.	TH01	6, 8	8, 9.3	7, 7

7.	D13S317	8, 12	8, 10	10, 12
8.	D16S539	11, 12	11, 12	8, 12
9.	D2S1338	18, 24	19, 24	17, 17
10.	D19S433	13, 15.2	13, 13	13, 14
11.	vWA	16, 17	16, 17	14, 17
12.	TPOX	11, 11	9, 11	11, 11
13.	D18S51	14, 15	14, 19	18, 18
14.	AMELOGENIN	XX	XY	XY
15.	D5S818	12, 12	10, 12	13, 13
16.	FGA	24, 25	23, 24	23, 23

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