

Molecular insights of saliva in solving paternity dispute

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Abstract

Everyone is born with a unique genetic blueprint i.e. its own genome. Special locations called loci on different chromosomes display predictable inheritance patterns that could be used to determine biological relationships. These locations contain specific DNA sequences, called markers, which forensic scientists use as identifying marks for individuals. Saliva is a potentially useful source of genomic DNA for genetic studies. Paternity testing is based on the premise that we inherit half our DNA from our father and half from our mother. Therefore, persons who are biologically related must share similar DNA profile. Conversely, the absence of similarities in the DNA profiles of the child and the alleged father is used as proof that no biological relationship exists. In this paper, a female complained for being raped a year back by Mr. X and accused him of being father of her 3-months-old baby girl. DNA testing was done using saliva for the child and blood sample from the mother and the suspected father. The finding presented here allows the use of saliva as an alternative source of blood.

Key words: Deoxyribonucleic acid, human leukocyte antigen, paternity test, restriction fragment length polymorphism, short tandem repeat

Introduction


Everyone is born with a unique genetic blueprint known as DNA. As half of the DNA of an individual is inherited from the biological mother and half from the biological father, the comparison of the DNA sequences of a person to that of another person can show if one of them is derived from the other or not. Specific sequences are usually looked at to see if they are copied verbatim from one of the individual's genome to the other.^[1] Saliva is a potentially useful source of genomic DNA for genetic studies since it can be collected in a painless and non-invasive manner.^[2]

Paternity testing simply means establishing fatherhood. DNA identification provides a conclusive and definitive way to establish biological relationships. Consequently, DNA testing has become the most accepted method to determine identity within the legal and scientific communities.^[3]

The DNA parentage testing is the most reliable and powerful method of proving or disproving parentage for legal, personal, or medical reasons. This test also resolves disputes, and helps streamline court proceedings to settle child support and custody cases. The standard methods for paternity testing are polymerase chain reaction (PCR), short tandem repeat (STR), restriction fragment length polymorphism (RFLP), and DNA sequencing. These tests are sometimes ordered by courts when proof of paternity is required.^[3,4]

Case Report

A female victim aged 24 years lodged a complaint in Barabanki police station for being raped a year back by Mr. X. Victim claims to be mother of his 3 months old baby (Girl)

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and wanted justice and compensation. The case was referred to the Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow for paternity testing. Saliva samples were collected from the oral cavity of the baby, and blood samples were collected from the mother and suspected father.

Methods

Extraction of genomic DNA in both blood and salivary samples was done using phenol-chloroform method published in our previous research article.^[5] It was followed by its qualitative and quantitative analysis using spectrophotometer and amplification of DNA by using PCR. Further panel of markers were selected for this purpose; few of them were used because of their positive response shown in our previous study like ApoB3'HVR, Hum-vWF-1, Hum-F13A.^[6] Other STRs markers used were Hum-vWA, Hum Tho1, Hum-TPO, Hum-FES, Hum-HPRT, and Hum-FGA. All the above markers were analyzed on poly-acrylamide gel electrophoresis (PAGE). We analyzed few of the autosomal STR markers on 310 fragment analyzer; these were D3S1342, D8S4321, and D11S432. Also, we analyzed Human Leukocyte Antigen (HLA) gene complex markers like Class I- A, B, C and Class II- DR β , DQ α on agarose gel.

Results

Analysis of Hum-vWF-1 marker [Figure 1] showed that bands in the suspected father (SF) did not match with the bands in the baby (B), whereas bands in the baby matched with the bands in mother (M). Analysis of Hum-FES marker showed that bands in the suspected father did not match with the bands in the baby, whereas one of the bands in the baby neither matched with the mother nor with the suspected father.

Analysis of HLA -A gene complex using agarose gel [Figure 2] showed that one out of 2 bands in the suspected father did not match with the bands of the baby. The analysis of HLA -C gene complex using agarose gel [Figure 3] showed that one out of 2 bands in the suspected father did not match with the bands in the baby.

Based on the DNA analysis, the pattern of bands revealed in the child's DNA was compared with that in the mother's DNA. All bands that matched in position and relative intensity are, or could be, maternal in origin. Thus, all of the remaining bands in the DNA fingerprint of the child must have been inherited from the biological father. In this present case, all of these remaining bands in the child were not present in the DNA fingerprint of the alleged father. This excludes Mr. X (suspected father) as the biological father of the baby because they do not share sufficient genetic markers as there was no

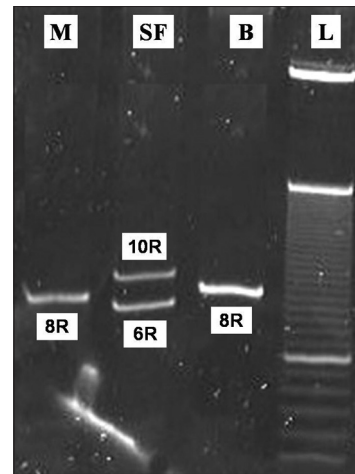


Figure 1: Analysis of Hum-vWF-1 marker using PAGE, L- DNA ladder

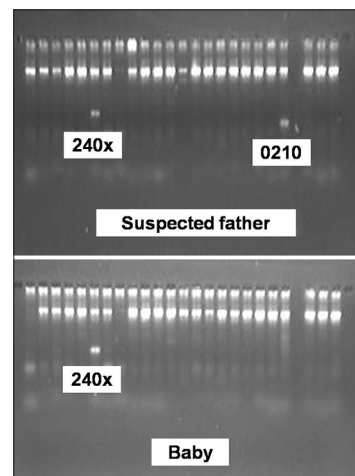


Figure 2: Analysis of HLA -A gene complex using agarose gel

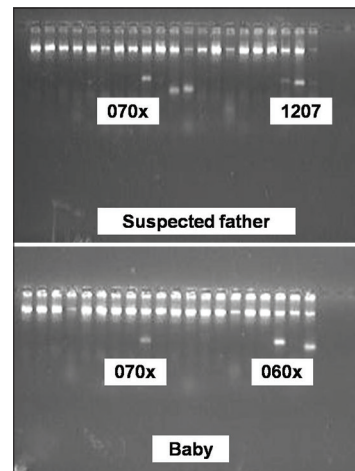


Figure 3: Analysis of HLA -C gene complex using agarose gel

common allele between the putative father and baby at 5 loci. Comparing the DNA sequence of suspected father, mother and the baby [Figure 4] showed that one of the sequences in the baby was solely derived from the mother. As the tested alleged father does not match the child

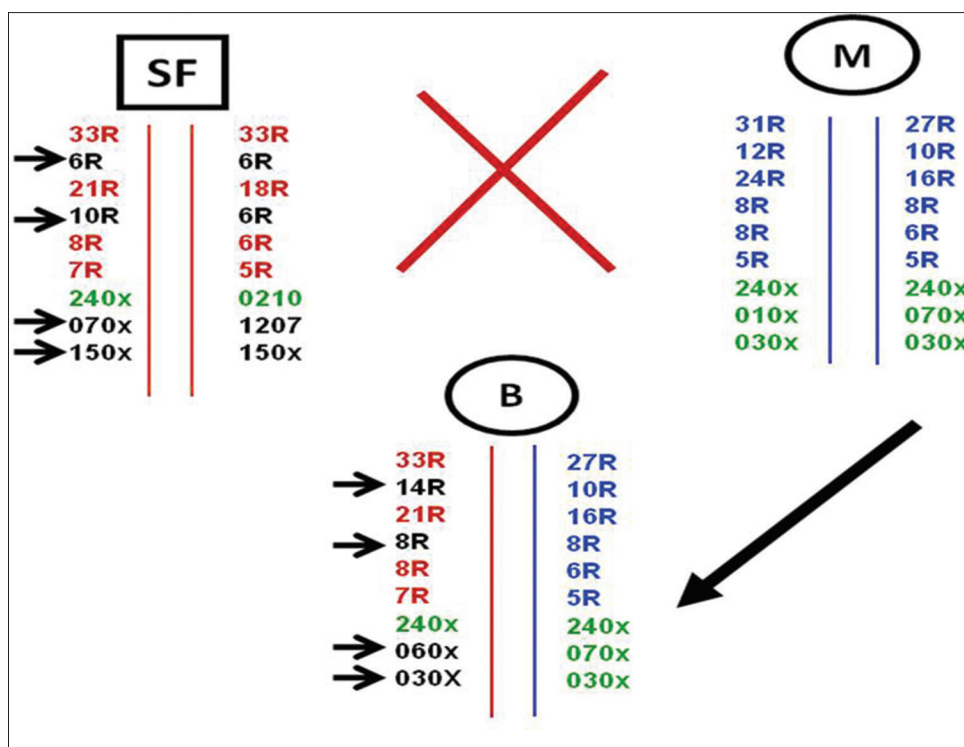


Figure 4: Comparison of the DNA sequence of suspected father, mother, and the baby

on 3 or more genetic sites, the alleged father is not the child's biological father.

Discussion

Forensic science (Legal medicine) is a specialism that aims to help judges and juries solve legal issues, not only in criminal law but also in civil cases.^[7] DNA fingerprinting is a technique used especially for identification (as for forensic purposes) by extracting and identifying the base-pair pattern of an individual's DNA.^[8] It is also known as DNA typing or genetic fingerprinting.^[8] The process of DNA fingerprinting was developed by Alec Jeffreys in 1984, and it first became available for paternity testing in 1988.^[8]

The basic function of paternity testing is to exclude the maximum number of individuals that could be biological fathers of the child in question. This is done by identifying the obligate allele in the child and determining if the alleged father also carries this allele.^[9]

For a paternity test to be considered to be of high quality, at least 16 loci must be analyzed during testing, which was done in the present case.^[10] If DNA of the child and alleged father show exclusions at 3 or more loci, then the alleged father is considered to be excluded as the biological father of the child.^[10] In the present case, the probability of paternity would be 0.00% as 5 of the bands present in baby did not match the bands of suspected father.

Generally, fresh whole blood or blood-stained material is the primary source of an individual's DNA used as a standard for comparison to evidentiary material in DNA typing.^[11] Various studies along with our previous study have demonstrated that saliva and saliva-stained materials are good source of DNA.^[5,6,12] Moreover, the DNA banding patterns obtained from saliva or saliva-stained material are indistinguishable from the patterns obtained from blood or hair from the same individual.^[11] In the present case, since the baby was 3-months-old, fairly large volumes of saliva was collected in a painless and non-invasive manner.

Conclusion

Most paternity testing is done for financial reasons, i.e. to establish legal responsibility and provide for child support. Such testing is clearly an important tool in civil courts when the paternity of a child is in question. As saliva is a rich source of genomic DNA, it can be used as an alternative source of DNA for known standards (Blood). As it is well said, it's important to give justice to the victim when the alleged father is proved to be the biological father, but it is also equally important to exclude the innocent.

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