

The Child's DNA: A Silent Witness in a Sexual Assault Case

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ABSTRACT

This paper delves into a high-profile Sexual assault case in Rajasthan, India, where the birth of a child became a pivotal element in the forensic investigation, ultimately providing irrefutable evidence for legal proceedings. The victim, a young girl, reported repeated assaults over a year, culminating in the birth of a daughter. Due to the lapse of time between the assaults and the investigation, traditional forensic evidence, such as semen or blood which are usual evidences in sexual assault cases, was no longer readily available. This presented a significant challenge in connecting the accused to the crime. However, the victim's pregnancy and the subsequent birth of a child opened a new avenue for investigation, utilizing the power of DNA analysis.

Blood samples from the victim, the accused, and the child were meticulously collected and analysed using advanced forensic DNA profiling techniques. These techniques, which involve the examination of specific genetic markers, are highly reliable in establishing biological relationships. The results conclusively confirmed the accused as the biological father of the child, providing unquestionable scientific evidence for lawful accounts.

The case represents the critical role of forensic DNA examination in establishing parentage, especially in criminal cases where traditional physical evidence-like blood and semen is not available. The use of DNA testing of the newly born in this case not only provided a strong basis for conviction but also highlighted the importance of scientific advancements in seeking justice for victims of sexual assault. This case study emphasizes the transformative potential of forensic DNA analysis in achieving justice in cases where evidence may be limited or compromised.

Keywords: DNA Profiling, Physical evidences, Paternity testing, Sexual assault case.

INTRODUCTION

Cases of sexual assault often present significant challenges in finding corroborative evidence, particularly when the assaults occurred months or years prior to the investigation. The passage of time can make forensic evidence like semen or blood, difficult or impossible to retrieve. The absence of this immediate biological evidence can make it extremely difficult to connect the accused to the crime, often leading to insufficient evidence for conviction and a sense of injustice for the victim. This can leave victims feeling unheard and further traumatized by the legal process.

However, in cases where assault results in pregnancy and the birth of a child, DNA testing of the child, victim, and accused can be a decisive factor in establishing a biological link and ensuring justice. This type of evidence can provide a powerful tool for investigators and prosecutors, offering a way to connect the perpetrator to the crime even when evidences like semen and/or blood is unavailable.

The victim, subjected to repeated assaults over an extended period, eventually gave birth to a daughter. While the alleged perpetrator was arrested, the significant time gap between the assaults and the investigation made retrieval of evidences like semen more difficult. This presented a significant hurdle for the investigators and the legal system in establishing a clear link between the accused and the crime, potentially jeopardizing the chances of securing a conviction. However, DNA testing of the newly born emerged as the vital piece of evidence in this case, offering a new path towards justice.

Paternity determination through DNA analysis offered irrefutable scientific proof of the accused's involvement. Establishing the biological relationship between the child and the accused not only substantiates the victim's claim but also enables the legal system to take appropriate action against the offender. This case exemplifies the transformative power of DNA technology in achieving justice for victims of sexual assault, especially in cases where forensic evidence is scarce or unavailable. DNA evidence can provide a crucial voice for victims, ensuring their experiences are heard and acknowledged, and that perpetrators are held accountable for their actions.

Materials and Methods

Sample collection

For DNA profiling, blood samples were collected from the victim, the accused, and the child by medical jurist and forwarded to FSL in sealed EDTA K2 blood collection vial with 0.25M EDTA (Nasmed Diagnostic Pvt. Ltd. India) maintaining cold chain. All the blood samples were meticulously labelled and documented to establish a clear chain of custody.

DNA Extraction and Quantitation

To extract DNA, a magnetic bead-based methodology was, selected for its ability to yield high-purity DNA even from complex biological evidences. This method was executed using the AutoMate Express™ Instrument, an automated extraction platform developed by Thermo Fisher Scientific specifically for forensic applications. The instrument utilizes the PrepFiler™ Express DNA Extraction Kit; a robust system certified for forensic DNA analysis [13]. This kit includes reagents optimized to maximize the efficiency and yield of DNA extraction, essential for obtaining reliable results in downstream applications. The magnetic bead-based technology ensures that impurities and PCR inhibitors are minimized, enhancing the quality of extracted DNA. Following extraction, DNA quantification was conducted using a 7500 Real-Time PCR instrument [8,15], which provides accurate concentration measurements critical for ensuring consistent DNA input in subsequent steps. All the samples provided good quantity of DNA which is diluted to 1ng with Tris-EDTA buffer for PCR.

DNA Amplification

PCR is used for amplify short tandem repeat (STR) loci, commonly used in forensic and paternity testing. The amplification process was performed using the AmpFISTR Identifiler® Plus PCR Amplification Kit, (Thermo Fisher Scientific, Waltham, MA, USA) known for its sensitivity and reliability in forensic applications [3]. This kit targets 15 autosomal STR loci, along with the Amelogenin locus, which provides sex determination. The highly polymorphic nature of these loci allows for detailed individual identification and genetic relationship analysis [5]. DNA amplification was carried out under controlled environmental conditions, with strict adherence to contamination control measures, such as using separate pre- and post-PCR work areas and wearing personal protective equipment (PPE), to ensure the integrity of the results.

DNA Fragment analysis

After amplification, the STR amplicons were analysed using capillary electrophoresis, a technique that separates DNA fragments by size and enables precise differentiation of STR alleles. The ABI 3130XL Genetic Analyzer, a capillary electrophoresis instrument validated for forensic use, was employed for this purpose [4]. This instrument facilitates high-resolution separation of STR alleles, generating data essential for accurate profiling. The data from the electrophoresis were analysed using GeneMapper ID-X® software, designed specifically for forensic DNA interpretation. The analytical threshold adopted for allele calling was 50 RFU. The software enables detailed analysis of allelic patterns, automates the assignment of alleles to specific loci, and provides a reliable mechanism for comparison between sample profiles. Through allelic matching across loci, the software generates a comprehensive genetic profile for each sample, allowing for the clear identification of familial relationships or individual identity.

Statistical Calculations

To determine the Probability of Paternity [10,14], the obligate allele present in the DNA profile of the alleged father is first identified. The allele frequency of this obligate allele in the relevant population is then obtained [9]. The Paternity Index (PI) is calculated by dividing the prior probability of transmission by the allele frequency. The prior probability refers to the likelihood that the alleged father has transmitted the obligate allele to the child—this value is 1.0 if the father is homozygous for the allele and 0.5 if he is heterozygous.

$PI = 0.5 \text{ or } 1 \div \text{Frequency of obligate allele} \quad (1)$

The Combined Paternity Index (CPI) is derived by multiplying the individual PI values across all tested loci.

$CPI = \text{Multiplication of all individual PI} \quad (2)$

The Probability of Paternity (POP) is then calculated using the formula:

$POP = (CPI \div CPI + 1) \times 100 \quad (3)$

In addition, the Random Man Not Excluded (RMNE) is calculated using the formula:

$RMNE = 1 - (1 - \text{allele frequency at a given locus}) \quad (4)$

The Combined Random Man Not Excluded (CRMNE) is obtained by multiplying the RMNE values across all loci. The Probability of Exclusion (PE) is finally calculated using the formula:

$PE = 1 - CRMNE \quad (5)$

Results

The DNA profiling analysis yielded a comprehensive genetic profile for each individual involved in the case: the victim (mother), the accused (alleged father), and the child. The child's DNA profile indicated a female genetic profile, confirmed by the presence of two X chromosomes at the Amelogenin locus, which is a standard marker for sex determination in forensic DNA analysis and presently being used to determine sex of DNA donor [2]. Upon comparing the STR profiles of the child with those of the victim and the accused, it was observed that each allele presents in the child's profile matched alleles found in either the mother's or the accused's DNA profile. Specifically, at each STR locus analysed, the child possessed one allele inherited from the victim and one from the accused (Table 1). This allele-sharing pattern across all 15 autosomal STR loci signifies a complete match, providing a robust basis for parentage determination. Table 1 presents the allelic data for each individual at each locus, highlighting the inheritance pattern of each allele from the victim and the accused. For example, at locus D8S1179, the child's alleles (14,14)

matched one allele from the victim (14) and the other from the accused (14), demonstrating biparental inheritance. This pattern was consistent across all loci analysed, with no discrepancies or mismatches, underscoring the genetic relationship between the individuals.

Discussion

Before the development of DNA fingerprinting by Alec Jeffreys in 1985 [6], the blood group was the primary method for determining biological relationships in paternity cases. DNA fingerprinting plays an important role in the establishment of the paternity of an individual [11]. Paternity disputes can be solved in certain cases by blood groups matching but with the advent of DNA fingerprinting technology the blood group methods became less important. In most of the cases ABO blood group information can be used to exclude potential fathers, rather than confirm the presence of a parental relationship [1]. In forensic science, analysing materials from cases involving abortion has become crucial for resolving paternity disputes and prosecuting sexual offenses [7,12]. DNA typing enables the exclusion of an alleged father with absolute certainty hence, DNA technology serves not only to establish guilt but also to affirm innocence when applicable. The basic function of all forensic testing is to exclude the maximum number of individuals possible. This is done in the present case by identifying the obligate allele and determining if the alleged father has this allele. To further support the findings, a statistical analysis was conducted to calculate the probability of a coincidental match between the child's and the accused's DNA profiles. Given the high polymorphism and discriminative power of the selected 15 STR loci, the probability that another random individual, unrelated to the child, would show the same allelic match across all loci is exceedingly low. This probability can be expressed as a Paternity Index (PI) and Combined Paternity Index (CPI). The CPI value obtained in this analysis is significantly high, reinforcing the conclusion of biological Probability of Paternity (POP) with a confidence level exceeding 99.999%. The Probability of Exclusion (PE) is 0.99999 which is significantly low. Hence, DNA analysis in this case provides conclusive indication that the accused is the biological father of the child. This finding is aligned with the victim's account, and the high statistical confidence afforded by the DNA profiling strengthens the scientific validity of the conclusion, effectively ruling out any alternative biological parentage. The DNA evidence, therefore, substantiates the allegations, adding a critical layer of scientific certainty to the case that supports the victim's claims and aids in the judicial determination. In this way DNA-based evidence has transformed the forensic landscape, enabling investigators to solve cases that lack traditional evidence. In paternity cases related to sexual assault, DNA testing allows for the indirect corroboration of the crime, providing justice for victims in cases that might otherwise lack adequate evidence. DNA profiling, as demonstrated in this case, exemplifies the capability of modern forensic techniques to deliver definitive answers, reinforcing the reliability of DNA testing as a crucial tool in criminal justice.

The results of DNA analysis in the present case provide certain evidence that the child is the biological offspring of both the victim and the accused, thereby supporting the victim's account and enabling the legal system to pursue justice. This case underscores the essential role of DNA profiling in sexual assault cases where traditional evidence may not be available. As demonstrated here, forensic DNA analysis not only strengthens the evidentiary basis of the case but also serves as a powerful instrument in criminal justice, ensuring accountability and supporting the rights of victims. In the legal context, DNA profiling serves as highly credible evidence. The child's genetic profile acts as a silent witness, establishing a biological connection that directly implicates the accused. This evidence is especially compelling in court as it offers a scientifically robust means of confirming paternity, which is difficult to refute.

Although the determination of paternity in the present case was used to solve alleged Sexual assault case. Still, the use of a child's DNA to establish paternity in criminal cases raises ethical considerations, particularly surrounding privacy and the child's future autonomy. There is an inherent ethical tension in balancing the child's right to privacy with the need for justice in cases of serious criminal allegations, such as sexual assault. DNA testing in such cases must be handled with sensitivity, ensuring that protocols are in place to protect the privacy and rights of all individuals involved, especially minors. To address these concerns, forensic DNA testing is conducted under judicial oversight, and privacy protocols are rigorously followed to respect ethical guidelines while obtaining crucial evidence. However, in situations where the evidence is crucial for justice, such as in cases of sexual assault, the benefits of such testing outweigh the ethical concerns. Protocols are followed to ensure that DNA testing is conducted with full respect for privacy and under judicial oversight.

The intersection of science and the courtroom is far from new. For centuries, forensic evidence has played a pivotal role in the judicial process, aiding in the administration of justice through the application of scientific principles. However, the advent of advanced forensic technologies, especially DNA analysis, has fundamentally transformed the landscape, requiring careful examination and regulation to ensure accurate, reliable, and ethical applications

In the courtroom, DNA profiling stands as a compelling form of evidence due to its scientifically robust foundation. The child's genetic profile effectively acts as a "silent witness," establishing a biological link between the accused and the child in a manner that is difficult to dispute. Given the statistical probability of a coincidental match, the evidence presented is especially persuasive in court, underscoring the role of DNA as a credible and near-irrefutable means of confirming paternity. Through advanced forensic techniques and careful analysis, the legal system can rely on DNA evidence to bolster the evidentiary basis of a case, supporting victims' rights while holding perpetrators accountable.

Conclusion

The results of the DNA examination provided undeniable evidence that the child is biological daughter of the accused. This alignment of scientific and judicial goals highlights DNA profiling's role as an essential instrument in the criminal justice system, delivering precise and objective findings that support fair adjudication. While ethical considerations remain an important part of forensic DNA applications, the decisive role of DNA profiling in securing justice, particularly in sensitive cases involving sexual assault, is invaluable. This case serves as a reminder of the ethical responsibility and scientific rigor required to responsibly apply forensic DNA analysis in the pursuit of truth and justice.

References

1. Adams J.: Paternity testing "Blood types and DNA" Nature Education 2008, 1(1):146
2. Ahmad A. and Khan S. "Amelogenin (genetic marker) role in sex determination of human" International Journal of Biochemistry and Molecular Biology 2022, 1(1):01-03
3. AmpFSTR® Identifiler® Plus PCR Amplification Kit, user guide 2015
4. Applied Biosystems 3500/3500xL Genetic Analyzer, user Guide" 2010.
5. Butler J.M., Shen Y., Mccard B.R. "Forensic DNA typing: biology and technology behind STR markers" 2001, London: Academic Press.
6. Jeffreys A. J., Wilson V., Thein S.L. "Individual-specific fingerprints of human DNA" Nature, 1985, 316, 76–79

7. Karger B., Rand S.P., Duchesne A. "DNA analysis of abortion material assisted by histology screening" American Journal of Forensic Medicine and Pathology.2001; 22:397-9.
8. Knight K.L. Mauriello A., Williams G.: "Quantitation of DNA using the Applied Biosystems Quantifiler® Trio DNA Quantification Kit" Methods in Molecular Biology.; 2023,2685:175-88.
9. Kumawat R. K., Shrivastava P. , Shrivastava D., Mathur G. K., Dixit S. "Genomic blueprint of population of Rajasthan based on autosomal STR markers" Annals of Human Biology 2020, Volume 47, Issue 1, 70-75.
- 10.10.Macan M., Uvodic P., Botica V. "Paternity testing in case of brother and sister incest" Croatian Medical Journal, 2003, 44 (3),347-349
11. Mishra A, Sathyan S. "Role of DNA fingerprinting in disputed paternity". Med Phoenix; 2016, 1(1):44-46
12. Nair N.P, Boban A., Krishna D.A., Rana A.K.: "Comparative DNA Profiling of conceptus parts In Paternity disputes and Rape investigations: A case study" Sri Lanka Journal of Forensic Medicine, Science & Law, 2024,15(1):11-17
13. PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits User guide 2017 for use with AutoMate Express™ Forensic DNA Extraction System
14. Primorac D., Schanfield M.S. "Application of DNA testing in the legal system" Croatian Medical Journal, 2000, 41(1), 32-46
15. Quantifiler™ HP and Trio DNA Quantification Kits User Guide, 2017.

TABLE 1: Allelic data analysis in the DNA profiles of the exhibits

Locus	Victim	Child	Accused	Obligate allele	allele Frequency	Paternity index	RMNE=1-(1-AF) ²
D8S1179	14,14	14,14	14,15	14	0.155	$0.5 \div 0.155 = 3.22$	0.2859
D21S11	28,32.2	28,32.2	28,30	28	0.159	$0.5 \div 0.159 = 3.14$	0.2927
D7S820	8,8	8,8	8,12	8	0.251	$0.5 \div 0.251 = 1.99$	0.4389
CSF1PO	10,12	12,13	10,13	13	0.073	$0.5 \div 0.073 = 6.84$	0.1406
D3S1358	15,18	16,18	15,16	16	0.327	$0.5 \div 0.327 = 1.52$	0.5470
TH01	7,9.3	9.3,9.3	8,9.3	9.3	0.170	$1 \div 0.170 = 5.88$	0.3111
D13S317	10,11	10,11	11,11	11	0.263	$1 \div 0.263 = 1.90$	0.4568
D16S539	11,11	9,11	8,9	9	0.180	$0.5 \div 0.180 = 2.77$	0.3276
D2S1338	19,25	18,25	18,23	18	0.158	$0.5 \div 0.158 = 3.16$	0.2910
D19S433	12,14	12,14	14,15	14	0.230	$0.5 \div 0.230 = 2.17$	0.4071
vWA	14,15	14,20	18,20	20	0.014	$0.5 \div 0.014 = 35.71$	0.0278

TPOX	10,11	10,12	12,12	12	0.046	$0.5 \div 0.046 = 10.86$	0.7084
D18S51	13,15	15,16	16,16	16	0.118	$0.5 \div 0.118 = 4.23$	0.2212
D5S818	9,12	10,12	10,12	10	0.120	$0.5 \div 0.120 = 4.16$	0,2256
FGA	19,20	20,23	23,28	23	0.181	$0.5 \div 0.181 = 2.76$	0.3292
Amelogenin	X, X	X, X	X, Y				
						CPI =85628	CMNE =5.04X10 ⁻⁹
						POP= 99.99%	PE =1- 5.04X10 ⁻⁹ =0.999999

Abbreviations used:

CPI = Combined Paternity Index

CRMNE =Combined Random Man Not Excluded

PE =Probability of Exclusion

PI = Probability Index

POP =Probability of Paternity

RMNE= Random Man Not Excluded

PE =Probability of Exclusion