DOI: 10.21088/ijfmp.0974.3383.19326.4

Indian Journal of Forensic Medicine and Pathology
Volume 19, Number 3, July-September 2026

ORIGINAL ARTICLE

When DNA Lies: A Forensic Dilemma Caused by Twin Absorption and Chimerism

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HOW TO CITE THIS ARTICLE:

Bhawna Poonia, Sangita Gupta. When DNA Lies: A Forensic Dilemma Caused by Twin Absorption and Chimerism. Indian J Forensic Med Pathol. 2026; 19(3): 309-316.

ABSTRACT

Background: Nuclear genomic markers, like short tandem repeats (STR), are frequently employed for paternity verification and investigating crimes. As per Mendel's law of inheritance, every living cell of an individual are expected to inherit identical DNA profile. However, extremely rare conditions such as chimerism or genetic mosaicism, arising from the fusion of two fraternal embryos in early development (Tetra gametic origin), make possible the shared existence of two genetically distinct cell populations within a single individual.

Method: This paper explores a case study in which a foetus displayed both male and female genetic markers—one from the femur and another from blood—regardless of the same origin. Blood and femur bone exhibited chimerism at all twenty three informative autosomal loci tested, provided in Powerplex®-Fusion 6C PCR amplification kit (Promega). The existence of genetically male cell population in the chimeric foetus was confirmed by typing a Y-chromosome haplotype.

Result: It would have been reasonable to assume that these samples came from two distinct individuals. However, after putting together data from different genetic researches and contextual information, the most plausible explanation suggested that the foetus was a chimera or a genetic mosaic due to the shared existence of two genetically distinct cell populations.

Conclusion: This case underscores that chimerism may pose challenges for forensic analyses, as it can result in false paternity exclusions or render it futile to correlate the offender to a stain detected at a crime scene. Cautious data interpretation, an amended sampling plan, as well as efficient recognition of mosaics or chimeras, should enable avoiding such errors.

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➢ **Received:** 11-04-2026 ➢ **Accepted:** 14-06-2026

KEYWORDS

• DNA evidence • Tetra-gametic chimerism • Short Tandem Repeat (STR) analysis
• Forensic investigation

INTRODUCTION

Deoxyribonucleic acid or DNA is the hereditary material in humans.[1] It serves as a fundamental tool in forensics for identifying individuals and establishing biological relationships. Regardless of the bodily part being tested, DNA will remain the same. However, chimerism challenges this dogma. Chimerism, a condition where a person has more than one DNA profile, can create difficulties in interpreting forensic results and determining sex accurately.[2] Conventional forensic methods rely on the assumption that each individual possesses a single, consistent, unique genetic profile, referred as genetic fingerprint or code,[3] but when the individual from whom the biological sample originates is genetically mosaic or a chimera it leads to misinterpretation of evidence, furthermore it can pose significant challenges for the legal system.

According to Greek mythology, Chimera was a creature which is a messup of a lion in front, a goat in between and a reptile behind.[4] In 1989, Churchill defined a chimera as "an organism composed of two or more genetically distinct cell types" in his Medical Dictionary.[5] Later, scientific community borrows Greek terms to characterize a peculiar combination of certain species and organism types.[6]

In the year 2015 in United States, a typical case of a man who 'failed' a test for paternity simply due to genetic profile in his saliva differing from that in his sperm, published by the Independent newspaper. Apparently, this was the first time that a 'human chimera' had been known to "fool" a paternity test.[7] In this case, extra genes present in chimera, had taken from a twin perished in the initial stages of pregnancy, making the son's 'true' genetic father was the man's deceased twin, who had never been born.

Although mosaicism and chimerism are often considered synonyms, yet individual with genetically distinct cell lines emerging from a single zygote are said to be mosaic, whereas those that ultimately originating from more than one zygote are considered to be chimeric.[8] However, it was extremely challenging to ascertain, whether the cohabitation of numerous STR identities within a person is a result of mosaicism or chimerism. Chimerism is generally classified as either acquired or congenital. The acquired form is seen through transplants or transfusions. It is also observed in women during pregnancy, when fetal cells infiltrate the mother's bodily tissues.[9,10]

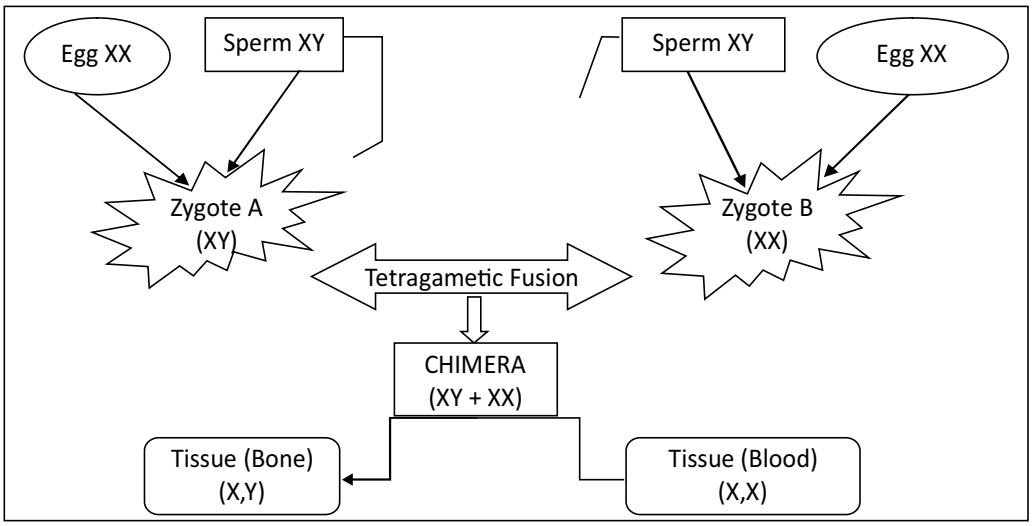

Figure 1: depicts the process of tetragametic chimerism. (Chimerisation begins when two separate eggs are fertilized by two separate sperm, resulting in two fraternal zygotes. Instead of developing into two separate embryos, these two zygotes fuse during the blastocyst stage of embryonic development - one twin gets absorbed into the other and form an entire being. Instead of dying off completely, cells from the absorbed twin become integrated into the surviving twin's body - two different sets of DNA – Tetragametic Chimera — one from each original zygote. These two cell lines (genetically distinct) are distributed in various tissues and organs throughout the body of an individual).[11,13]

Congenital chimerism can arise due to several reasons. Chimerism can occur in fraternal twins that share a placenta through blood vessel anastomoses that allow stem cell transfusions from either the vanishing twin or the other born twin.[11] Alternatively, congenital chimerism may arise from inter-embryonic cell circulation,[12] or early fusion of two embryos.

Although most chimeric persons are healthy and do not show obvious traits, congenital chimerism is rare and often remains undiagnosed throughout life. However, abnormalities of sexual development and reproduction are prominent exception,[12,14,15] which could occur if the fraternal twins are of the opposite gender.[16] Additional physical manifestations of chimerism include asymmetrical body parts, skin patches, Blaschko lines and different eye colours.[12] However, scientific data indicated that chimerism is not an uncommon event. According to a clinical review, some singleton pregnancies start out as twin, with 15-36% of twin pregnancies losing one fetus early.[17] However, there is currently no population-wide screening for chimerism in place.[7] This event may be underestimated due to the recent increase in the use of assisted reproductive technologies (ART). Some ART techniques increase the likelihood of multiple pregnancies, including mono and dizygotic twins as well as triplets. The most common cause of tetragametic chimerism is thought to be dizygotic multiple pregnancies.[7]

When seeking a patient primed for a tissue or organ transplant, histocompatibility testing may reveal congenital chimeras. Additionally, they can also be distinguished by DNA profiling,[16] kinship testing,[7] and certain medical diagnostic tests.[14]

In this work, we describe a case study in which STR-based DNA profiling of the two independent separate samples taken from the single foetus consistently displayed two distinct profiles. The objective of this study was to determine the underlying cause of this unexpected discrepancy by considering probable biological factors and ruling out technical or procedural sources of error, since it is assumed that a single individual will yield similar STR patterns. Unbeknownst, the best suited reason for the outcome was that the foetus inherited more than one genome, a state known as chimerism. Specifically, the case was identified as tetragametic chimerism, a form of congenital chimerism arising from the fusion of two zygotes.

CASE STUDY OVERVIEW

A fetal autopsy case presented an unprecedented genetic discrepancy. Samples from deceased foetus were submitted for preparing DNA profile in order to biologically identify the deceased foetus. The bone sample collected from the foetus showed a male (XY) STR profile while the blood sample revealed a female (XX) STR profile, even when taken from the same foetus. Both profiles showed shared allelic pattern indicating a common clonal origin from the same parent which were consistent with a sibling profile. This discrepancy aroused higher concerns about chimerism rather than maternal blood contamination.

MATERIAL AND METHODS

The Maxwell FSC Extraction System (Promega) was used to extract DNA using the Casework Extraction Kit (Promega). The Powerplex®-Fusion 6C (Promega) multiplex PCR system kit was then used to amplify the isolated DNA, enabling the amplification of a total of 27 STR markers (D3S1358, D19S433, Penta D, D22S1045, D1S1656, D2S441, SE33, D21S11, D13S317, Penta E, D8S1179, D16S539, D18S51, CSF1PO, FGA, D7S820, D5S818, D10S1248, TPOX, D12S391, D2S1338,vWA and TH01) and Amelogenin, along with three additional markers indicating male origin (DYS391, DYS576 and DYS570). PCR amplification was performed using a half reaction volume on Eppendorf Mastercycler Nexus X2. The PCR amplicons of the DNA fragment were analyzed using the 3500 Genetic Analyzer (Applied Biosystems). The data was subjected to fragment analysis by GeneMapper™ ID-X software v1.6 (Thermo Fisher Scientific). The allelic ladder supplied with the kit was used to designate the allele number in the DNA profile. All procedures were run under the protocol recommended by the manufacturer.

As the samples demonstrated that deceased foetus was male, Y-STR profiling was performed using the Powerplex® Y-23 (Promega) system kit with standard laboratory practices. A total of 23 polymorphic Y-STR loci were genotyped.

Quality assurance: Appropriate controls and decontamination precautions were taken to avoid any potential cause of contamination during extraction. A positive control DNA template (2800 M) and negative control (nuclease-free water) provided along with the kit, were included in all experimental runs to ensure that the results were reproducible and accurate. The peak height thresholds were 50 and 200 RFU for the heterozygous and homozygous alleles respectively.

RESULTS

The first hands-on DNA examination of both samples from the deceased foetus, was carried out using Powerplex®-Fusion 6C (Promega) system kit. The outcomes of amplifying the 23 autosomal STRs plus gender defining Amelogenin marker displayed two distinct profiles – the femur bone exhibited a male profile while the blood exhibited a female profile. Unexpectedly, comparison revealed that half of the alleles in the autosomal STR profile of the femur bone sample matched half of the alleles in the STR profile of the blood sample (Table 1). Preliminary results indicated a potential familial relationship between the two profiles, with approximately 50% allele sharing consistent with a mother-son relationship (Figure 2). Using more than one kit invariably increases the discrimination power. Nonetheless, we performed supplementary testing using Investigator Argus X-12 kit (Table 2), and Figure 3 displays all the investigated loci across various X-STR markers. The Investigator Argus X-12 kit is a commercially available forensic DNA typing kit developed by QIAGEN for analyzing 12 highly polymorphic X-chromosomal STR markers. In this case, Investigator Argus X-12 kit was employed to rule out maternal contamination and to provide genetic evidence of tetragametic chimerism with high precision. Based on the samples' STR data analysis, genotyped through Powerplex®-Fusion 6C (Promega)system kit and Investigator Argus X-12 kit, the most logical explanation for two DNA profiles from the same individual showing a parent-offspring like relationship is that they arose from two separate zygotes-fraternal twins - that fused into a single body early in development, resulting in chimerism.

The fetus was male and his identity was established using a Y- chromosome STR marker kit. The femur bone showed a strong male DNA profile, while a partial male profile was procured from the provided fetal blood sample, as evident by Figure 4, using the Powerplex® Y23 (Promega) system kit (Table 3).

Table 1: Allelic data analysis of Powerplex®-Fusion 6C (Promega) system Kit

Locus	Femur bone sample of foetus	Blood sample of foetus
Amelogenin	**XY**	**XX**
D3S1358	15,17	15,17
D1S1656	11,14	11,15
D2S441	11,14	10,11
D10S1248	14,16	14,15
D13S317	8,12	12,12
Penta E	16,17	13,16
D16S539	11,12	8,12
D18S51	13,14	14,21
D2S1338	18,21	18,23
CSF1PO	10,12	12,12
Penta D	11,11	11,11
TH01	8,8	7,8
vWA	16,18	16,17
D21S11	29,29	28,29
D7S820	9,11	9,10
D5S818	11,13	11,12
TPOX	8,10	8,11
D8S1179	14,14	14,15
D12S391	17,24	18.3,24
D19S433	14.2,15.2	12,14.2
SE33	12,18	18,18
D22S1045	15,17	15,15
DYS391	10	-
FGA	19,25	21,25
DYS576	17	-
DYS570	20	-

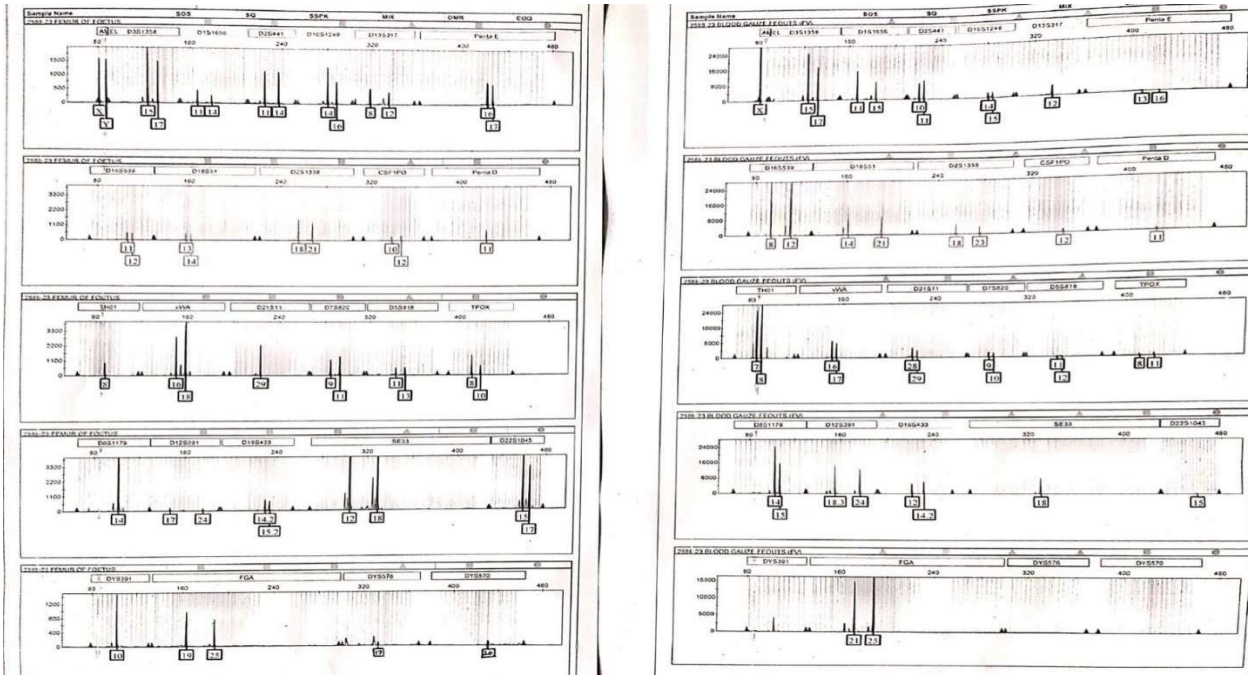

Figure 2: Electropherogram of STR genotypes of (a) Male DNA profile shown by femur bone of foetus, and (b) Female DNA profile shown by blood sample of foetus, at 27 different loci of the Powerplex®-Fusion 6C (Promega) system Kit.

Table 2: Allelic data analysis of Investigator Argus X-12 kit

Locus	Femur bone sample of foetus	Blood sample of foetus
QS1	Q	Q
Amelogenin	**XY**	**XX**
DXS10103	19	19,21
DXS8378	11	10,11
DXS10101	29.2	29.2, 32.2
DXS10134	36	36, 37
DXS10074	17	17, 17
DXS7132	14	14, 15
DXS10135	-	20, 22
DXS7423	15	14, 15
DXS10146	29	29, 29
DXS10079	23	20, 23
HPRTB	13	13, 15
DXS10148	23	23, 24
D21S11	29	28, -

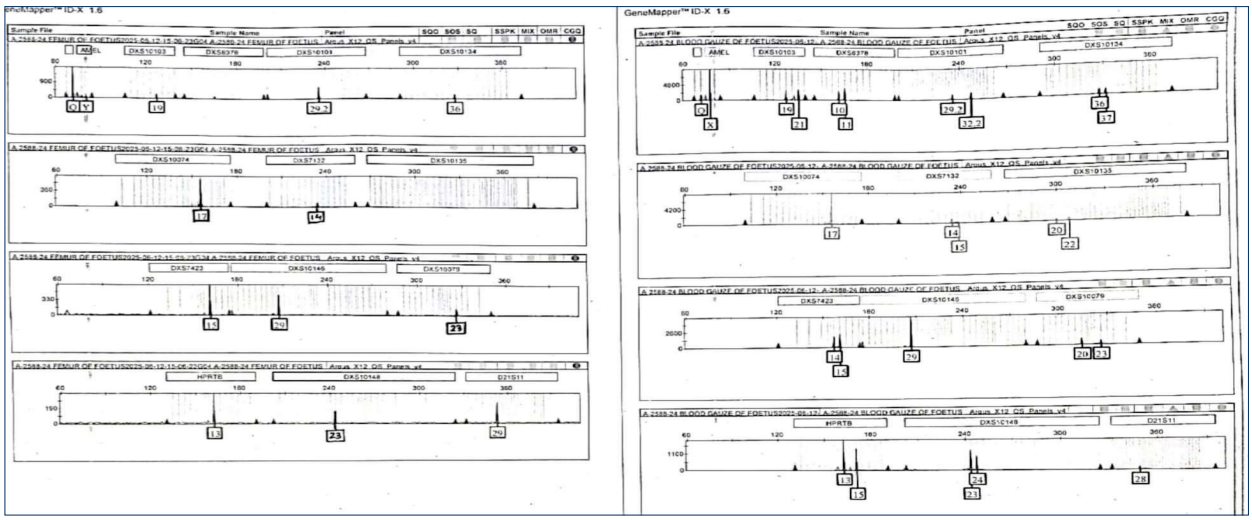

Figure 3: Electropherogram of STR genotypes of (a) Male DNA profile shown by femur bone of foetus, and (b) Female DNA profile shown by blood sample of foetus, at 15 different loci of the Investigator Argus X-12 kit

Table 3: Allelic data Analysis of Powerplex Y23 (Promega) system kit

Locus	Femur bone sample of foetus	Blood sample of foetus
DYS576	17	17
DYS389I	14	14
DYS448	20	-
DYS389II	31	31
DYS19	15	-
DYS391	10	10
DYS481	23	23
DYS549	13	13
DYS533	12	12
DYS438	11	-
DYS437	14	-
DYS570	20	20
DYS635	24	-
DYS390	24	-
DYS439	10	10
DYS392	11	11
DYS643	10	-
DYS393	13	13
DYS458	16	16
DYS385a/b	11,14	11,-
DYS456	15	15
Y-GATA-H4	12	-

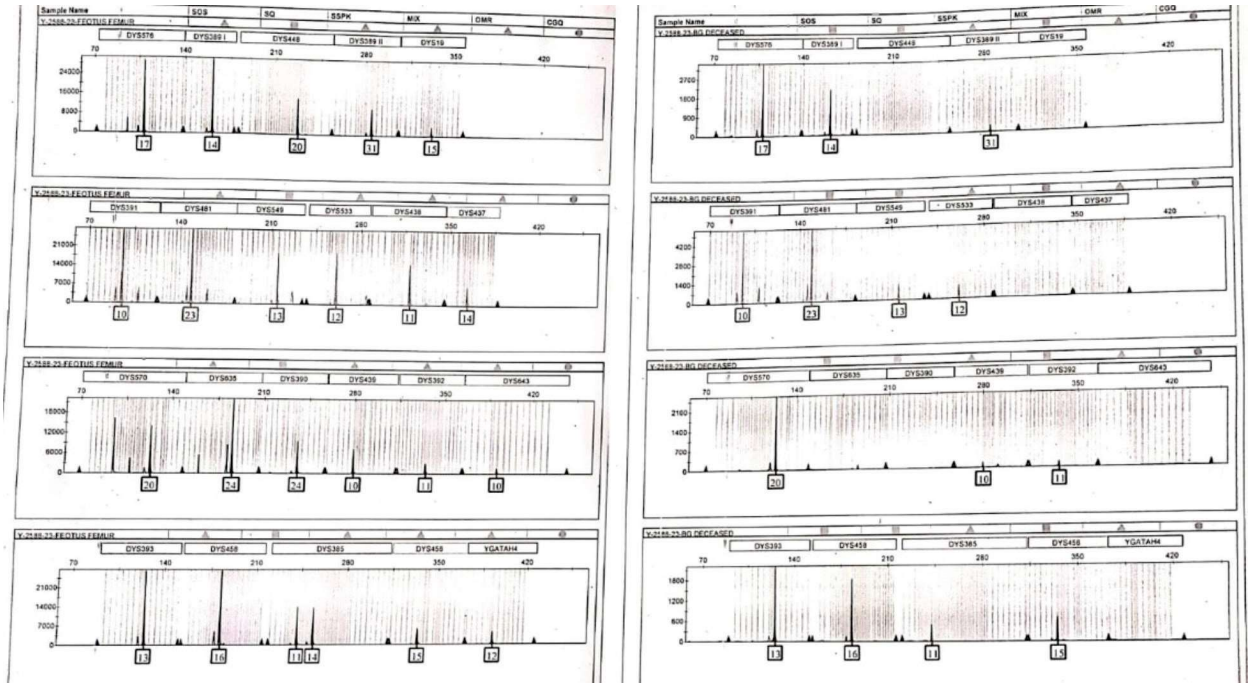

Figure 4: Electropherogram of STR genotypes of (a) Male DNA profile shown by femur bone of foetus, and (b) Partial male DNA profile shown by blood sample of foetus, at 23 different loci of the Powerplex®-Y23 (Promega) system kit

DISCUSSION

Individualization is a fundamental aspect of forensic science, achieved through accurate sex determination using DNA analysis.[18] However, chimerism presents unique challenges that can compromise the identification techniques by standard genetic analysis. If DNA had been extracted from either tissues of chimeric person, a forensic or kinship conclusion would have misrepresented the individual's full genetic identity.

In this case, analysis of STR profiles from two tissue sources—blood and bone—revealed striking differences. The blood sample exhibited a consistent female profile (XX), while the bone sample showed a distinct male profile (XY), across autosomal STR loci. Such sex-discordant results from the same individual cannot be explained by normal genetic variation, laboratory error, or contamination alone. The possibility of maternal cell admixture was also ruled out due to partial male alleles in the blood and the consistency of the autosomal profile, which would be highly unlike in a simple maternal-fetal mixture.[19,20] To resolve the discrepancy, we employed the Argus X-12 kit, a specialized X-STR panel designed to detect variations on the X chromosome. The analysis confirmed:

- The blood sample had two alleles at all loci, indicating a typical female (XX) profile.

- The bone sample had single allele at each locus, consistent with a male (XY) who possesses only one X chromosome.

The use of the Argus X-12 kit was critical in demonstrating the presence of two distinct X-chromosomal haplotypes, thereby strengthening the evidence for sex-based tetragametic chimerism.[21] Without this additional analysis, the discordance between autosomal STRs alone might have been misinterpreted as technical error or mixed sample contamination.

The forensic implications of tetragametic chimerism are significant. If unrecognized, it can lead to false exclusions in paternity testing,[19] misidentification in criminal investigations,[22] and complications in organ transplantation compatibility. The well-known case of Lydia Fairchild (2002, USA) illustrates that how chimerism can lead to serious misinterpretation in forensic and legal investigations. DNA testing shows no biological relation between her and her children.[23] Van Dijk, *et al.*, (2016) reported a similar problem in which a person's blood and buccal swabs had distinct DNA profiles because of tetragametic chimerism.[24] Another case comes from Yu *et al.*'s research from (2022), where a biologically female person appeared to have male DNA markers in her blood as a result of receiving blood transfusions.[25] There were lots of such cases of chimerism in the human population which go undiagnosed.

In summary, the current study also highlights the diagnostic value of combining autosomal STR typing with X-STR analysis in resolving complex chimeric profiles. Such cases, though rare, underline the importance of considering chimerism when encountering intra-individual genetic discrepancies, particularly those involving sex chromosome discordance.

CONCLUSION

In forensic casework, biological anomalies like chimerism can lead to significant misinterpretation—ranging from wrongful exclusions in kinship analysis to challenges in establishing the identity of a suspect or victim. This case highlights the necessity of conducting comprehensive multi-tissue analysis in cases where discordant STR profiles are incongruent with phenotypic sex or known familial relationships.[26] Chimerism, while rare, presents real and profound challenges to forensic reliability. The notion that "DNA doesn't lie" must be nuanced by biological complexity, particularly in conditions like tetragametic chimerism.

Forensic methodologies must be evolved to address the rare—but significant—possibility that an individual may not be genetically uniform. It is necessary to learn more about the detection limits of SNP microarrays, STR and other DNA analyses used to diagnose and identify chimerism. Forensic and parental DNA testing labs may re-examine such cases that were sent to them but were deemed inconclusive because of contamination. In order to detect chimerism in tissues other than blood, autopsy specimens may be useful.[19]

These findings emphasize the need for increased awareness of chimerism among forensic examiners and the importance of including it in differential diagnoses of genetic inconsistency. Future guidelines should incorporate chimerism screening protocols in complex or contradictory cases to avoid misconduct of justice. As the field of forensic genetics evolves, rare but impactful conditions like tetragametic chimerism must be recognized and systematically investigated to ensure scientific and legal accuracy.[27]

Acknowledgements

We express our gratitude to the Director of Forensic Science Laboratory, Jaipur, Rajasthan, India, for doing all the tests and related interpretations for this case.

Conflict of Interest

The authors declare no competing interests.

Ethical Approval

All procedures were conducted in accordance with ethical standards.

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