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Microdeletion Screening of Azoospermia Factor d (AZFd) at Y Chromosome

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Abstract: ***Aim: To determine the frequency of Yq chromosome microdeletions in infertile men from Gujarat. Introduction: Male infertility, contributing to 9% of global infertility cases, is often linked to Y-chromosome microdeletions, especially in the AZF region. Methods: Fifty-eight male infertility samples (40 normozoospermic, 13 oligozoospermic, 5 azoospermic) were analyzed. Sperm morphology was assessed via Papanicolaou staining. DNA was extracted using the Phenol-Chloroform method, and PCR detected Yq microdeletions using sY152 and SRY sY14 markers. Results: Among the samples, 67.24% had reduced motility, 79.31% exhibited >14% morphological defects, and 10.34% showed AZFd microdeletions. Conclusion: Sperm morphological defects and Yq microdeletions contribute to infertility. Y-chromosome deletion testing has diagnostic and prognostic significance, aiding screening for IVF clinics.***

Keywords: Microdeletions, Azoospermia factor (AZF), Y chromosome, Deletion frequency, PCR analysis, IVF clinic

1. Introduction

Infertility, defined as the inability to conceive after one year of unprotected intercourse, affects 15% of couples worldwide, with male infertility contributing to 9% of cases. Genetic factors account for 30% of male infertility, with Y chromosome microdeletions in the AZF region being the second most common cause after Klinefelter syndrome. These deletions can impair spermatogenesis, leading to conditions like azoospermia and oligozoospermia.

Over 90% of male infertility cases involve poor sperm quality, yet the exact cause remains unknown in more than half of them. The Yq11 region regulates spermatogenesis, and its microdeletions can disrupt sperm development. Despite increasing research, Y chromosome microdeletion studies remain limited in Gujarat, India. PCR-based genetic testing using sequence-tagged site (STS) markers helps detect these deletions, particularly in the AZFd region, which is linked to abnormal sperm morphology.

This study aims to determine the prevalence of Y chromosome microdeletions in azoospermic and oligozoospermic men, assess their correlation with sperm defects, and enhance the genetic diagnosis of male infertility.

2. Review Literature

Multiple studies highlight the role of Y chromosome microdeletions in male infertility, particularly in the AZF regions. Kent-First et al. (1999) reported a 3.5% AZFd microdeletion frequency, while Hassan Osman et al. (2021) found deletion rates of 18.8% in azoospermic and 7.1% in oligospermic men. Barbhuiya et al. (2013) observed a 25.3% deletion frequency in infertile men in Northeast India, with the highest rate (28%) in azoospermic cases.

Studies in Malaysia, Iran, and Turkey confirm similar trends. Akbarzadeh Khiavi et al. (2020) reported a 32% deletion rate in azoospermic men, while Hussein et al. (2015) found 7.4% in oligospermic men. Al-achkar et al. (2013) identified a high prevalence of AZFc deletions, while Liu et al. (2016) and Zhang et al. (2013) validated the link between microdeletions and infertility.

Chen et al. (2023) found a 3.31% deletion frequency in a Chinese cohort, with the AZFd region, particularly the sY152 marker, being most affected. These studies emphasize the importance of genetic screening for Y chromosome microdeletions in diagnosing and managing male infertility.

3. Materials and Methods

Fifty-eight semen samples (40 normozoospermic, 13 oligozoospermic, 5 azoospermic) were collected from Gujarat. The study was conducted at ARIBAS and approved by the G.J. Patel Institute of Ayurvedic Hospital. Participants aged 22–45 with primary infertility were included, while those with immunological or hormonal infertility and men over 45 were excluded.

Morphological Analysis & DNA Isolation: Sperm morphology was assessed using the Rapid Papanicolaou Staining Kit and observed under a microscope. DNA was extracted using the phenol-chloroform method, with quality and quantity assessed via agarose gel electrophoresis and NanoDrop analysis.

AZF Microdeletion Analysis: PCR amplification was performed using STS markers sY152 (AZFd) and sY14 (SRY control). PCR conditions included 34 cycles with denaturation (94°C), annealing (57°C), and extension (72°C). The amplified products were analyzed using 2% agarose gel electrophoresis with a DNA ladder for comparison. The AZFd sY152 gene produced a 125 bp band, and the SRY sY14

control gene produced a 472 bp band, visualized under a UV transilluminator.

4. Results

Sperm Morphology Analysis: Papanicolaou staining revealed abnormalities in sperm structure (head, midpiece, and tail). Among 58 samples, 67.24% (39) had motility below 40%, and 79.31% (46) exhibited morphological defects exceeding 14%.

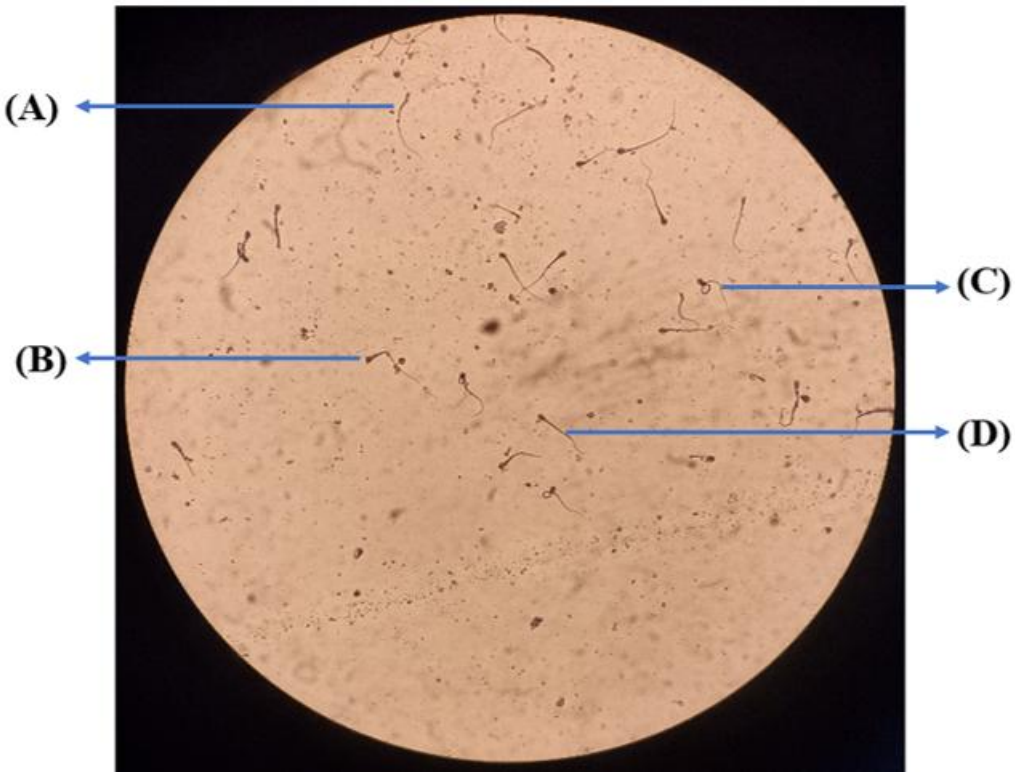

Figure 1: Morphology of sperm from sample No. 18 showing neck defect (A), Head defect(B), Tail defect (C) and Normal sperm (D).

DNA Analysis: Qualitative analysis using 1% agarose gel electrophoresis showed sharp DNA bands under UV transillumination, while quantitative assessment was performed using a NanoDrop.

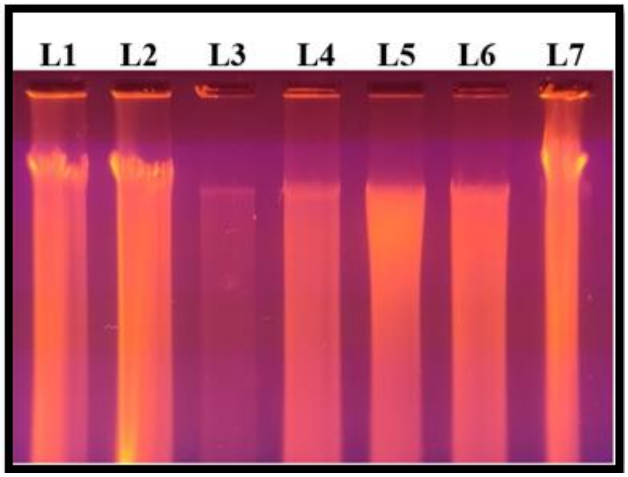

Figure 2: Electrophoretic pattern of isolated DNA. (L1 to L7= Sample No. 31 to 37)

PCR Amplification Analysis: PCR products were analyzed on 2% agarose gel with a 100 bp DNA ladder. The presence of a 472 bp SRY gene confirmed Y chromosome DNA. A 125 bp sY152 band indicated a normal AZFd gene, while its absence suggested AZFd microdeletion.

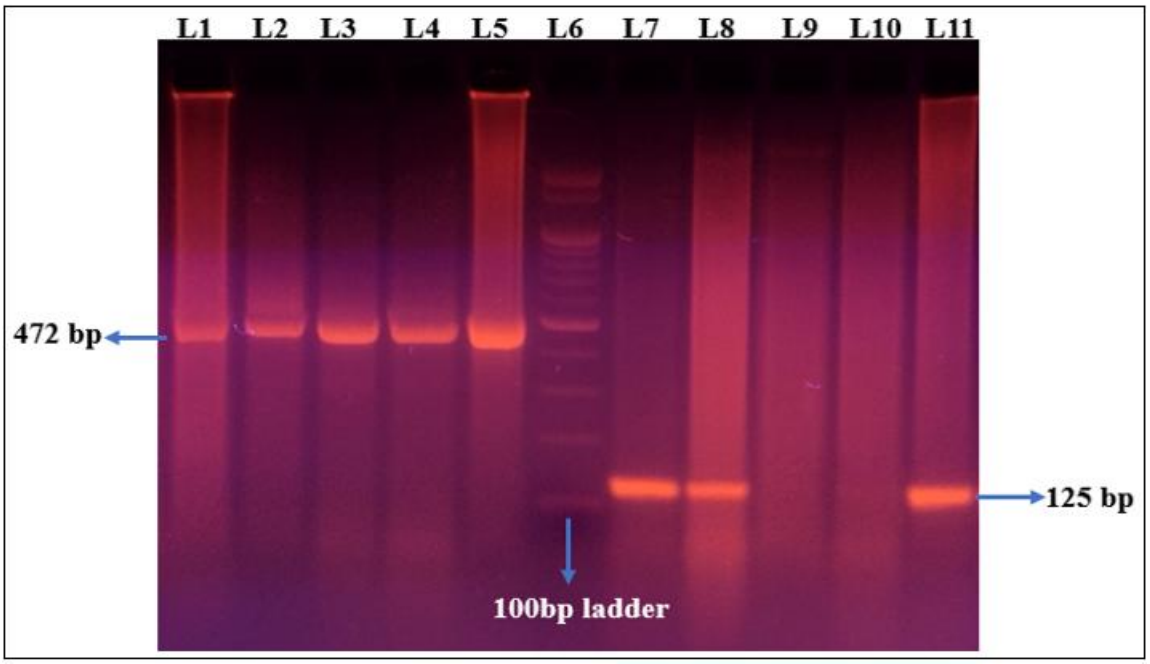

Figure 3: PCR products of SRY & sY152 gene L1 to L4 = Sample No. 35 to 39, L6=100bp DNA ladder L7 to L11 = Sample No. 31 to 35.

5. Discussion

Genetic defects, particularly microdeletions, are a major cause of male infertility after Klinefelter syndrome. This study identified AZFd subregion microdeletions in 6 out of 58 samples: 2 azoospermic, 1 oligozoospermic, and 3 normozoospermic. Normozoospermic samples with deletions exhibited normal sperm count and motility but had morphological defects exceeding 14%. The oligozoospermic sample showed low sperm count but normal motility and abnormal morphology. The positive fructose test confirmed the absence of obstructive oligospermia.

Findings suggest that AZFd deletions impact sperm morphology and count, implicating its role in spermatogenesis. Though less frequent, AZFd deletions contribute to male infertility, aligning with global studies linking AZF microdeletions to sperm quality and concentration. Male infertility is multifactorial, but AZFd deletions may play a role in sperm morphogenesis.

6. Conclusion

The analysis of 58 male samples (normospermic, oligozoospermic, and azoospermic) revealed significant sperm morphological defects, even in normospermic individuals. Among these, 67.24% exhibited reduced motility (<40%), and 79.31% had high morphological abnormalities (>14%), based on WHO (2021) criteria. The fructose test confirmed the absence of obstructive oligoospermia.

AZFd subregion microdeletions were detected in 6 samples (10.34% prevalence), affecting sperm morphology and count. The findings suggest that infertility results from a combination of microdeletions and sperm abnormalities.

7. Future Scope

Y chromosome deletion screening has diagnostic, prognostic, and preventive value, aiding in infertility management by reducing unnecessary treatments and guiding sperm retrieval strategies. Advancing molecular techniques and standardized protocols could enhance the accuracy, efficiency, and accessibility of Y chromosome microdeletion testing in fertility clinics.

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