Role of Y Chromosome Microdeletions in the Clinical Evaluation of Infertile Males

Stacy Colaco, Aiman Lakdawala, Deepak Modi

ABSTRACT

Infertility is a multifaceted condition, which is on the rise in the last few decades. A stage of great importance in the development of human male gametes is spermatogenesis, which is governed by a set of genes located on the q arm of the Y chromosome. Loss of these genes can cause disruptions in spermatogenesis and, thus, lead to male infertility. Studies have identified several deletions on the long arm of the Y chromosome, called Yq microdeletions, which occur in three distinct loci termed AZFa, AZFb, and AZFc. In addition to these, there exist small subdeletions in the AZFa locus, called gr/gr, b1/b2, or b2/b3 subdeletions. Such deletions can lead to azoospermia or oligozoospermia by causing Sertoli cell-only syndrome, impairment in spermatogenesis, or maturation arrest. Testing for Y chromosome microdeletions is clinically significant for several reasons, since these deletions are exclusively associated with male infertility and their detection can help identify the cause of infertility. Knowing the presence or absence of Y chromosome microdeletion also aids in predicting the prognosis of oligozoospermic males, who are usually known to progress to azoospermia over time. The occurrence and type of Yq microdeletions are correlated with testicular phenotype in infertile males and, thus, serve as a good predictor of sperm retrieval. Vertical transmission of Y chromosome microdeletions from father to the male offspring is common in pregnancies achieved via assisted reproductive technologies; hence, the diagnosis of these deletions becomes imperative in such couples to prevent perpetuation of infertility in the next generation. Screening for Yq microdeletions is, thus, clinically significant and must be offered to all infertile males.

Keywords: Azoospermia, Male infertility, Microdeletions, Sperm count, Y chromosome.


Source of support: Indian Council of Medical Research

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INTRODUCTION

The term infertility can be defined as the inability to bear offspring which brings a sense of failure to many couples. The World Health Organization defines infertility as “the inability of a couple to conceive within two years of exposure to the risk of pregnancy.” About 48 million couples worldwide suffer from infertility, while reports from India suggest that approximately 33 million couples of reproductive age face infertility. Clinical analysis of infertile couples show that in nearly 40% of cases, the defects occur in the male partner, in another 40%, the defects occur in the female partner, while in the remaining 20% cases, the cause of infertility is unknown. These observations have given rise to a clinical condition known as male infertility.

Male Infertility

Male infertility refers to the inability of the male partner to cause pregnancy in a clinically normal female. However, the incidence of male infertility is not well reported, especially in countries where cultural differences and patriarchal societies preclude accurate statistics from being assembled. A recent literature survey suggests that almost 30 million males worldwide are infertile, with the largest niches of male infertility occurring in Central and Eastern Europe (8-12%) and Australia (8-9%). Male infertility can be clinically addressed as the following conditions:

- Azoospermia – Absence of sperm in the ejaculate
- Oligozoospermia – Less than 15 to 20 × 10⁶ spermatozoa in the ejaculate
- Severe oligozoospermia – Less than 5 × 10⁶ spermatozoa in the ejaculate
- Normozoospermia – Normal values of sperms in the ejaculate
- Asthenozoospermia – Low levels of motility observed in less than 50% of sperms
- Teratozoospermia – Less than 30% of sperms have normal morphology
- Aspermia – Failure in ejaculating semen

Male infertility can be classified as non-idiopathic (cause of infertility is known) or idiopathic (cause of infertility is unknown) in nature. Some known causes of male infertility include cryptorchidism (absence of one or both testes in the scrotum), varicocele (abnormal enlargement of the pampiniform venous plexus in the scrotum), hormonal imbalances, alcohol consumption, and chemotherapy. However, in most cases, the cause of male infertility remains unknown and, in such cases,
genetic causes are believed to be potential candidates responsible for the infertility. Some of the genetic defects observed in infertile males are karyotypic abnormalities, gene copy number variations, single gene mutations, and polymorphisms or deletions on the long arm of the Y chromosome (Y chromosome microdeletions or Yq microdeletions). All these genetic defects interfere with the development of the male gonads, the urogenital tract, arrest of germ cell production and maturation, or lead to the production of nonfunctional spermatozoa. Among the various genetic factors, karyotypic abnormalities and Yq microdeletions are the leading causes of male infertility. The Yq microdeletions are submicroscopic deletions of the Y chromosome that are undetectable on routine karyotype analysis. These microdeletions have been recognized as a cause of male infertility, and their presence has been noted in azoospermic males, while males with a normal spermiogram have shown absence of these microdeletions. In this review, we shall focus on the Yq microdeletions and how they cause male infertility.

In order to comprehend how Y chromosome microdeletions cause male infertility, it is essential to understand the structure of the human Y chromosome and the different genes that are found on it.

**Y Chromosome Structure**

The human Y chromosome is found only in males, haploid in nature, and does not undergo meiotic recombination. As compared with the other chromosomes, the Y chromosome is one of the smallest chromosomes (~60 million base pairs (Mb)), representing around 2 to 3% of a haploid genome. This chromosome is gene sparse with more than 50% of its sequence comprising of repeat elements. However, the human Y chromosome plays a focal role in testicular development and male infertility. Cytogenetically, there are three distinct regions of the Y chromosome (Fig. 1), which include:

1. Two pseudoautosomal regions (PAR1 and PAR2),
2. Heterochromatic region, and
3. Euchromatic region.

The PAR1 is located at the terminal region of the short arm (Yp) and the PAR2 at the tip of the long arm (Yq). These regions are called PARs because they behave like autosomes during meiosis. The PARs are regions where the Y chromosome pairs and exchanges genetic material with the PAR of the X chromosome. The genes present within the PAR are inherited in the same manner as autosomal genes. The PAR1 and PAR2 represent only 5% of the entire Y chromosome. The remaining 95% of the Y chromosome comprises the so-called “non-recombining Y” (NRY), which includes the euchromatic and heterochromatic regions of the chromosome. The heterochromatic region comprises the distal Yq, a region assumed to be genetically inactive and polymorphic in length in different male populations. It is composed primarily of two highly repetitive sequences families, DYZ1 and DYZ2, containing about 5,000 and 2,000 copies of each respectively.

The euchromatic region lies distal to the PAR1, and consists of the short-arm paracentromeric region, the centromere, and the long arm paracentromeric region (Fig. 1). The euchromatic NRY region does not recombine with the X chromosome, and, hence, it is called the non-recombining region. The NRY sequences contain 8 Mb of Yp and 14.5 Mb of Yq. These sequences are subdivided into three discrete classes: X-transposed, X-degenerate, and ampiclonic. The ampiclonic sequences are characterized by eight massive palindromes, six of which contain testis-specific protein-coding genes. Compared with the autosomes, the NRY has a limited number of genes. Approximately 115 genes have been mapped to the Y chromosome, of which 43 genes have been identified on the short p arm of the Y chromosome. Some of the genes on this arm include SRY (sex-determining region Y), ZFY (zinc-finger Y), and AMELY (amelogenin Y).

**Fig. 1: Gene regions on the Y chromosome**

<table>
<thead>
<tr>
<th>Pseudoautosomal region (PAR1)</th>
<th>Centromere</th>
<th>AZFa</th>
<th>AZFb</th>
<th>AZFc</th>
<th>Heterochromatin</th>
<th>Pseudoautosomal region (par2)</th>
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<td>Euchromatin</td>
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**Genes on the Short Arm of the Y Chromosome (Yp)**

The SRY, the mammalian Y chromosomal testis-determining gene, is located adjacent to PAR1, and is composed of a single exon, which encodes a protein of 204 amino acids. This gene is essential for initiating testis development and differentiation of the bipotential gonad into the testicular pathway. The SRY has been proposed to be the master gene regulating the cascade of testis determination. The ZFY encodes a zinc finger-containing protein that functions as a transcription factor. The binding of ZFY to deoxyribonucleic acid (DNA) is mediated by the interaction of the GGCC core base pairs with zinc fingers 12 and 13. A significant paralog of this gene is ZFX and
amplification of this gene is often used to determine the sex of DNA samples. The AMELY encodes a member of the amelogenin family of extracellular matrix proteins. Amelogenins are involved in biomineralization during tooth enamel development. This gene also has a paralog on the X chromosome, AMELX.

**Genes on the Long Arm of the Y Chromosome (Yq)**

The long arm of the Y chromosome (q arm) contains several genes implicated in spermatogenesis. Some of these genes include USP9Y (ubiquitin-specific protease, Y), DBY (DEAD box, Y), UTY (ubiquitously transcribed tetratricopeptide repeat gene, Y-linked), CDY (chromodomain, Y), RBMY (ribonucleic acid [RNA]-binding motif, Y), EIF1AY (translation initiation factor 1A, Y), and DAZ (deleted in azoospermia). Many studies have demonstrated that deletions in these genes often lead to male infertility.

The first correlation between spermatogenic failure and an underlying genetic cause was demonstrated by Tietpol and Zuffardi. These Italian researchers studied the Y chromosome of several azoospermic males and observed microscopically detectable deletions at the distal section of band q11 of the Y chromosome in six males. Based on this observation, the authors for the very first time suggested the role of the Y chromosome in spermatogenesis. The authors also proposed the existence of a spermatogenesis factor, called the “azoospermia factor” (AZF) encoded by a gene on distal Yq. Following this report, a map of the Y chromosome was created by Vergnaud et al. in 1986, using 23 Y-specific restriction fragments. Later studies confirmed the location of AZF in Yq11.

With the advent of molecular biology tools, Vollrath et al. created a deletion map of the human Y chromosome by testing 96 individuals with partial Y chromosomes for the presence or absence of many DNA loci. Their studies resolved the euchromatic region (short arm, centromere, and proximal long arm) of the Y chromosome into 43 ordered intervals, defined by naturally occurring chromosomal breakpoints and averaging less than 800 kilo base pairs (kb) in length. A later study by Vogt et al. proposed the presence of not one, but three spermatogenesis loci in Yq11, with each locus being active during a different phase of male germ cell development. This group observed that Y chromosome microdeletions occurring in infertile males followed a certain deletion pattern, with three recurrently deleted nonoverlapping subregions in proximal, middle, and distal Yq11. The three loci were designated “AZFa,” “AZFb,” and “AZFc” respectively.

**AZFa Locus and Its Genes**

The AZFa subregion spans about 800 kb and encodes single-copy genes, which are essential for normal spermatogenesis. Candidate genes of spermatogenesis in the AZFa locus include USP9Y (ubiquitin-specific peptidase 9, Y-linked), DBY, and UTY. The USP9Y was the first gene to be identified in the AZFa region. This gene was previously known as DFFRY (Drosophila fat facets-related Y). It consists of 46 exons and spans 159 kb of genomic DNA. It encodes an ubiquitin-specific protease and belongs to the peptidase C19 family. The USP9Y is not a testis-specific gene, but is expressed in multiple tissues because its homologous gene on the X-chromosome can escape X-inactivation.

The UTY encodes a protein-containing tetra-tricopeptide repeats, which are thought to be involved in protein–protein interactions. The encoded protein is also a minor histocompatibility antigen, which may prompt graft rejection of male stem cell grafts. This gene encodes a large number of alternatively spliced transcripts; however, the full-length nature of some of these variants has not been determined. The DBY is made up of 17 exons, which encode a putative ATP-dependent RNA helicase belonging to the DEAD box proteins. Deletion of AZFa is known to be a major cause for the occurrence of a severe testicular pathology, the Sertoli cell-only syndrome (SCOS).

**AZFb Locus and Its Genes**

The AZFb locus is located in the central region of Yq11 and overlaps with the AZFc region by 1.5 Mb. The AZFb region contains several single-copy genes as well as multicopy gene families. Single-copy protein-coding genes found within the AZFb include KDM5D (lysine-specific demethylase 5D), EIF1AY, and CYORF15 (chromosome Y open reading frame 15A and 15B). The AZFb region also contains a set of seven multicopy gene families: XKRY (XK, Kell blood group complex subunit-related, Y-linked), HSFY (heat shock transcription factor, Y-linked), RBMY, PRY (PTPN13-like, Y-linked), CDY, BYP2 (basic protein Y2, Y-linked), and DAZ. Twenty members from these gene families are located in AZFb, but several genes also occur on the AZFc region.

The RBMY proteins are characterized as having an auxiliary C-terminal domain containing four 37 amino acid repeats and a single RNA-binding domain. The RBMY is a multicopy gene, but its role in human spermatogenesis is challenging to prove because no damaging mutations have been identified in it. In the testis, RBMY is expressed in spermatogonia, spermatocytes, pachytene cells, and spermatids. Beyond the germ cells, RBMY has also been detected in the tail of human spermatozoa. Furthermore, an antibody against human RBMY has been found to block sperm motility, implying that this protein may be involved in motility. The EIF1AY encodes an abundantly expressed translation initiation factor, Y isoform of eIF-1A. The EIF1AY is involved in translation initiation.
AZFc Locus and its Genes

The AZFc locus codes for 21 candidate genes and 11 families of transcription units that are exclusively expressed in the testis. This locus is palindromic and repetitive in nature, and, hence, highly susceptible to intrachromosomal rearrangements during meiotic recombination. This is why the AZFc locus is prone to deletions, duplications, and copy number variations of the eight gene families that are harbored within it. Some of the genes belonging to the AZFc locus include DAZ (deleted in Azoospermia), GOLGA2LY (Golgi autoantigen, golgin subfamily A, 2-like, Y-linked), TTY4 (testis-specific transcript, Y-linked 4), CSPG4LY (chondroitin sulfate proteoglycan 4 pseudogene, Y-linked) CDY1 (chromodomain protein, Y-linked, 1), BPY2 (basic charge, Y-linked, 2), and TTY3 (testis-specific transcript, Y-linked 3)

Among these genes, the DAZ gene is thought to be decisive in determining male infertility by playing an important role in spermatogenesis. The DAZ gene is vital in stimulating germ cell progression to meiosis and has four functional copies in the AZFc locus (DAZ1, DAZ2, DAZ3, and DAZ4) that encode for a RNA-binding protein. Each of the four copies is highly polymorphic and expressed in the testis. The human DAZ proteins carry out transportation, translational activation of developmentally regulated transcripts and their storage. Infertile males showing a loss of individual copies of DAZ genes are highly predisposed to azoospermia or severe oligozoospermia.

The GOLGA2LY gene is present in two copies (GOLGA2P2Y and GOLGA2P3Y) on the AZFc locus. This gene is believed to encode a protein consisting of 108 amino acids, which is transcribed in the testis. No function has been attributed to GOLGA2LY; however, males harboring GOLGA2P3Y deletion display decreased sperm concentration and motility compared with males without deletion or with deletion of GOLGA2P2Y, thus suggesting the role of this gene in spermatogenesis. The BPY2 gene, which lies in the AZFc region, encodes for a highly charged protein, which is testis specific and is involved in cytoskeletal regulation in spermatogenesis. The BPY2A, BPY2B, and BPY2C are the three copies of BPY2. The BPY2 is present in the nuclei of spermatocytes, round spermatids, and spermatogonia. The function of BPY2 is yet to be established, but reports indicate it interacts with UBE3A, a ubiquitin protein ligase E3A. As UBE3A is expressed in the testis, it can be said that BPY2 is involved in modulating target specificity of UBE3A. The TTY4 gene has three copies, TTY4A, TTY4B, and TTY4C. This gene has not been studied in detail, and is considered to be RNA that does not encode any protein. The TTY3 is also a nonprotein coding RNA and has two copies. The CSPG4LY gene also has two copies and is regarded as a pseudogene.

The CDY1 gene exists in two copies (CDY1a and CDY1b) in the AZFc locus and encodes for chromo domain protein 1. The CDY proteins have two functional motifs namely a C-terminal domain that has CoA-dependent acetyl transferase activity and N terminal chromatin-binding domain (chromo domain), which aid in regulation of gene expression and chromatin remodeling. Deletions of CDY1 gene copies are also linked to infertility.

Yq Microdeletions

Studies involving molecular analysis and sequencing of the Y chromosome long arm have revealed eight large palindromic regions containing an array of different ampliconic sequences. Homologous recombination between any of these eight palindromic sequences leads to occurrence of Y chromosome microdeletions. The Yq microdeletions are defined as chromosomal deletions that span several genes, but are not large enough to be detected using conventional cytogenetic methods. They can be visualized only by sequence-tagged site (STS)-polymerase chain reaction (PCR) or Southern hybridization. Each of the three AZF regions located within the long arm of the Y chromosome contains several genes that play a role in different stages of spermatogenesis. It is known that only the AZFa and AZFb regions are needed to initiate spermatogenesis, but that without the AZFc region, spermatogenesis will not be completely normal. Deletions of AZFa lead to the complete depletion of germ cells and that of AZFb result in spermatogenic arrest, but both these deletions are less frequent. Deletions in the AZFc region are more frequent accounting for up to 90% of all Yq deletions with phenotypes varying from azoospermia to severe oligozoospermia. Complete deletions of the AZFc region may occur in two different ways: either as a result of a previous deletion within the AZFc or spontaneously from a normal AZFc region. Thus, microdeletions in the AZF regions of the Y chromosome are recognized to play an important role in determining male fertility, although the exact genotype–phenotype relationship of microdeletions and infertility in the AZF locus have not been fully explored.

gr/gr Subdeletions

The AZFc region is particularly susceptible to deletions because its structure is majorly composed of amplicons. Studies using DNA sequence alignments within the AZFc region of the Y chromosome have revealed the presence of smaller deletions called subdeletions in this region brought about by intrachromosomal recombination in
this AZF region. Detailed analysis of the AZFc region using molecular markers has confirmed the existence of three such subdeletions termed as gr/gr, b1/b3, and b2/b3.21,34 These subdeletions lead to loss of one or more copies of genes within the AZFc and produce a plethora of phenotypes, ranging from mild oligozoospermia to azoospermia.

The most prevalent subdeletion, gr/gr, is caused by recombination, resulting in the loss of two of the four copies of the DAZ gene and one of three copies of the BPY2 gene.21 The outcome of b2/b3 and b1/b3 deletions is similar, leading to the retention of two DAZ gene copies and one or two BPY2 gene copies. Several studies have associated this deletion as a risk factor for the loss of spermatogenesis,30,35,36 while others have failed to find a correlation.37 A study in the Han population in China reported that duplication of the gr/gr region is detrimental to fertility, further contributing to uncertainty about the role that this region plays in determining a male's fertility status.38

Prevalence of Y Chromosome Microdeletions

According to the present knowledge, the following recurrent microdeletions of the Y chromosome are clinically relevant, and are found in men with severe oligo or azoospermia: AZFa, AZFb (P5/proximal P1), AZFbc (P5/distal P1 or P4/distal P1), and AZFc (b2/b4). The most-frequent deletion type is the AZFc region deletion (~80%) followed by AZFa (0.5–4%), AZFb (1–5%), and AZFbc (1–3%) deletion. Deletions, which are detected as AZFabc are most likely related to abnormal karyotype, such as 46,XX male or iso(Y).11

Approximately 25 to 55% of males with severe testicular pathologies, such as hypospermatogenesis, sperm maturation arrest, and SCOS and 5 to 25% males with severe oligozoospermia or azoospermia harbor Y chromosome microdeletions, making them the most common known genetic cause of spermatogenic failure.36

There is wide variation in the prevalence of Y chromosome microdeletions world over. A report from the Middle East indicates a high prevalence of Y chromosome microdeletions (7.5%),39 and a study from Iran in azoospermic infertile males has reported a prevalence of 12%.40 A South American study has identified classical AZF microdeletions in 5.75% of Chilean patients with spermatogenic failure.41 Similarly, Pina-Neto et al42 have reported a prevalence rate of 7.5% in Brazilian infertile males, who seek assisted reproduction aid. A recent meta-analysis by Filho et al43 from Brazil revealed that 10.8% of infertile males showed presence of Y chromosome microdeletions. The overall prevalence of Y chromosome microdeletions in Korean infertile males was found to be 7.7%.44 While the frequency of AZF microdeletions was found to be significantly higher (11.75%) in a group of Chinese azoospermic males compared with severe oligozoospermic males (8.51%) in a study by Fu et al.45 Y chromosome microdeletions have also been identified in almost 15.6% infertile Serbian males,46 while a group from Italy found that the prevalence of microdeletions was 3.2% in unselected infertile males, 8.3% in males with nonobstructive azoospermia, and 5.5% in males with severe oligozoospermia.47

Prevalence of Yq Microdeletions in India

Studies from India have also reported a wide variation in the prevalence of Yq microdeletions in infertile males. A study by Abid et al48 in 200 males found a low prevalence of Y chromosome microdeletions (3%), while a study by Suganthi et al49 reported a higher microdeletion frequency of 36% when they examined South Indian infertile males. A more recent study by Mascarenhas et al1 noted that 8.3% of azoospermic males and 2.3% oligozoospermic males showed the presence of Y chromosome microdeletions. An extensive meta-analysis of the data available on the prevalence of Yq microdeletions in the Indian population by Sen et al46 revealed a prevalence rate of 5.8%). On segregating these data based on the type of AZF deletion and sperm count, it was observed that the AZFc microdeletion frequency is about 35% in azoospermic males (Graph 1A) and 70% in oligozoospermic males (Graph 1B).

It has been noted that the prevalence of Y chromosome microdeletions varies with geographical locations, the highest occurring among South Indian males and the lowest among Northern and Western parts of India. This suggests that ethnicity might also influence the prevalence of Y chromosome microdeletions.6 Sen et al6 have reported that the occurrence of Y chromosome microdeletions in Indian males with Klinefelter’s syndrome, varicocele, and cryptorchidism is not a rare phenomenon. These findings suggest that in the Indian population, clinically, it is necessary to offer Y chromosome microdeletion testing to all the classes of infertile males. As such, no clinical prediction regarding microdeletions is obtained from phenotypical parameters, such as testicular volume, mal-descended testis, hormone levels, infections, and varicocele, hence, making screening for Y chromosome microdeletions imperative for diagnosis of the right cause of male infertility in India.

Prevalence of gr/gr Subdeletions

Studies by Repping et al21 have shown that the gr/gr deletions do not completely abolish any testis-specific gene family, but decrease the copy number of gene
families on the Y-chromosome. They also noted that the dosage of one or more of these families affected the quality of sperm produced. Hence, the semen picture in gr/gr deletions may vary from azoospermia to normozoospermia with differences depending on ethnicity and geography. Several studies have reported different frequencies of gr/gr deletions. A number of studies have reported a significant association between the gr/gr deletions and spermatogenic failure; however, other studies suggest the absence of such an association.

The Y haplotype is one of the factors contributing to such discrepancies. Other partial deletions (b2/b3 and b1/b3) in the AZFc region are rare and, hence, have been studied less often. A study by Rozen et al has established that the b1/b3 deletion increased the risk of severe spermatogenic failure, while the b2/b3 deletions did not appear to be a risk factor for severe spermatogenic failure. They also found that the b2/b4 deletions increased the risk of severe spermatogenic failure 145 times. The gr/gr deletions have been found to be extremely common in Japanese males.

Although the gr/gr subdeletions are more common in infertile males, they have also been detected in fertile individuals. Extensive molecular analysis of males with AZFc subdeletions suggests that copy number variations or loss of copies of the DAZ gene, CDY1 gene, or the GOLGA genes increases the susceptibility for gr/gr deletions toward infertility.

Prevalence of gr/gr Subdeletions in the Indian Population

Till date, three studies have been reported regarding the presence of gr/gr deletions in the Indian population. In a case–control study, Shahid et al identified the presence of gr/gr, b1/b3, and b2/b3 subdeletions in azoospermic males as well as in oligospermic males (7.17%). Interestingly, they also identified presence of gr/gr deletions in normozoospermic males (2.9%). This group noted that deletions of DAZ genes could contribute differently toward the impairment of the spermatogenic process resulting in spermatogenic arrest, oligospermia, or SCOS. A study from our laboratory has found that the frequency of gr/gr is higher in oligozoospermic (10.5%) and azoospermic (11.6%) males as compared with controls (5.1%). Another Indian study found the frequency of gr/gr deletions was the highest (5.84%) and were significantly associated with male infertility (p = 0.0004).

Diagnosis of Y Chromosome Microdeletions

Initially, deletions on the Y chromosomes of infertile men were detected using karyotype analysis. However, Y chromosome microdeletions were found to be strenuous to be detected by standard karyotype evaluation and hence the PCR using STS markers technique was found to be a more feasible approach. The Y chromosome has been reported to contain around 300 STS that occur in the different AZF regions. These STS can be exploited for easier characterization of microdeletions. Many studies have demonstrated the utility of adopting this strategy by developing a targeted multiplex PCR using STS specific to their populations. Multiplex PCR offers the advantage of detecting numerous STS sites in one reaction. Presently, the STS PCR-based technique is widely accepted for detection of Y chromosome microdeletions. The European Molecular Genetics Quality Network recommends the use of six STS markers (sY84 and sY86 for AZFa, sY127 and sY134 for AZFb, and sY254 and sY255 for AZFc microdeletions) that are considered most robust, nonpolymorphic, and specific, that correctly identify the infertility causing microdeletions in more than 99% of cases with no false negativity. These six STS markers have been extensively utilized internationally for Yq microdeletion testing in various clinical settings. However, there
have been growing concerns regarding the clinical use of only these six recommended markers considering the heterogeneity in the Yq sequences in different populations. Sen et al. have shown that in the Indian setting, the six European Molecular Genetics Quality Network markers are not adequate to detect Y chromosome microdeletions, and have proposed a 13-marker panel for the same. This panel may be used for detection of the Y chromosome microdeletions in Indian males.

Clinical Implications of Yq Microdeletions

Although there is universal agreement on the need for chromosome analysis by karyotyping in the workup of male infertility, there is still a lack of consensus regarding the clinical utility of testing for Y chromosome microdeletions in azoospermic and oligozoospermic males. While the American Society of Reproductive Medicine recommends the use of both, karyotyping and Y chromosome microdeletion studies, in males preparing to undergo intracytoplasmic sperm injection (ICSI), the National Institute for Health and Care Excellence recommends only karyotyping for this group of patients. There is no such consensus in India for the same.

Screening for Y chromosome microdeletions has several clinical implications:

- Identifying the main cause of infertility: The Y chromosome contains several genes required for spermatogenesis and the loss of one or more of such genes can cause impairment of spermatogenesis. By investigating Y chromosome microdeletions in the male partner, we can determine the underlying genetic etiology of male factor infertility. It will also help clinicians to provide more effective solutions for problems faced by infertile couples. For example, low sperm count and motility can be treated with hormones, antioxidants, and lifestyle changes to improve the semenogram. However, these strategies of treatment will fail if the cause of infertility is genetic. Therefore, if the male partner is detected with a deletion, the couple can directly be offered assisted reproductive techniques (ARTs) and not be subjected to medical treatments to improve sperm count and motility.

- Predicting the prognosis of infertile males: It is known that oligozoospermic individuals harboring Y chromosome microdeletions progress to azoospermia over time. These males require being clinically followed up for their possible progression to azoospermia, genetically counseled, and updated regarding sperm cryopreservation for fertility options using ART in the future.

- Predicting outcome of testicular sperm aspiration (TESA): Most males with Y chromosome microdeletions would be infertile and have absence of or very few sperms in ejaculate. To achieve pregnancy, sperm can be retrieved directly from the testes using techniques like testicular sperm extraction (TESE) or TESA. These sperms can be used for ICSI, thus circumventing underlying spermatogenetic defects. The occurrence and type of Yq microdeletions have been found to correlate with testicular phenotype. Studies by Dada et al. have identified infertile males showing AZFa and AZFb deletions with a corresponding testicular cytopathology of absence of Sertoli cells and the complete absence of germ cells. Other infertile males investigated in this study showed the presence of AZFc microdeletions with a phenotype of hypospermogenetics and secondary spermatocyte maturation arrest, indicating that sperm may still be successfully retrieved using such procedures. Hence, screening for Y chromosome microdeletions before undertaking invasive procedures, such as TESE/TESA can be used as a good predictor of sperm retrieval using such techniques.

- Predicting the success of ART: Since most males harboring Y chromosome microdeletions would be infertile, they would be offered ART for achieving biological parenthood. Technologies, such as ART–ICSI allow males with suboptimal sperm quality to overcome natural selection mechanisms and produce a viable zygote. However, it is clinically relevant to know what possible outcomes the couples might express post-ART. Several studies reporting about ICSI performed in couples with male partners carrying AZFc deletions describe lower fertilization rate, poor embryo quality, a significantly impaired blastocyst rate, and lower overall success of the procedure. Hence, Yq microdeletion screening would aid in counseling couples regarding the probability of success rates after taking up ART.

- Prevention of vertical transmission of the genetic defects: The ART, like in vitro fertilization and ICSI, bypasses all the natural mechanisms and checkpoints related to normal fertilization. Thus, males carrying Y chromosome microdeletions perpetuate infertility in the next generation owing to 100% transmission of the genetic defect to the male offspring from the fathers. A study by Kleiman et al. reported how an AZFc microdeletion was transmitted in three generations of males, some of whom were born after ICSI. Although other reports indicate that most children conceived through ART seem normal, a slight increase in the prevalence of aneuploidy in the sex chromosomes of children born via ICSI (from 0.2 to 0.6%) and an increase in autosomal chromosome abnormalities (from 0.07 to 0.4%) have been reported. However,
these data are difficult to interpret because patients who use ICSI or other ARTs have a higher incidence of abnormalities due to their infertile status. Due to these reasons, Y chromosome microdeletion testing is highly recommended for all infertile males who opt for ICSI for biological parenthood. The patients can then be appropriately counseled and well informed before offering ART.

CONCLUSION

Infertility attributable to male factors is on the rise and constitutes 30 to 40% of cases of infertility. Studies have shown that AZF microdeletions occurring on the Y chromosome are a common cause of male infertility and occur in males with azoospermia and severe oligozoospermia. Screening for these microdeletions has several advantages, such as identifying the root cause of infertility, managing its treatment, predicting the outcomes of ARTs and invasive techniques like TESE and TESA.

The AZF microdeletions are vertically transmitted to offspring born using ART–ICSI, thus perpetuating infertility among generations. This information emphasizes the need to offer elaborate genetic counseling to infertile couples who wish to undertake the procedure. In the Indian scenario, screening of Yq microdeletions should not be merely an academic exercise, but should be offered to all cases of infertile males in a clinical setup in order to prevent the transmission of these small interstitial deletions to the male progeny and predict the embryo quality. Further research should be carried out to investigate the long-term impact on the children born to fathers who have adopted ART. Using this knowledge, clinicians will be able to treat infertile patients optimally and make knowledgeable decisions about the use of ART.

ACKNOWLEDGMENT

We wish to thank the Indian Council of Medical Research for funding.

REFERENCES


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