Male infertility: Y-chromosome deletion and testicular aetiology in cases of azoo-oligospermia

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The spermatogenesis locus azoo spermi a factor (AZF) in Yq11 has been delineated into three microdeletion intervals designated as AZFa, AZFb and AZFc. AZFc is the most frequently deleted region. We have studied 270 male infertile patients for various genetic disorders associated with infertile phenotype. In this study, we have presented results of our studies on Y-chromosome deletions, chromosomal abnormalities (Klinefelter syndrome) and histology of testis with the objective of seeing whether there were cases of gonosomic mosaicism and a causal correlation between the genetic disorder; and testicular aetiology could be drawn. In all the 13 cases of Y-chromosome microdeletion, AZFc region and DAZ gene were deleted, while no case of AZFa deletion was detected. This result was at variance with other reports from India, where a considerable fraction of cases showed deletion in AZFa region of the Y-chromosome. Both Y-deleted and non-Y-deleted cases revealed heterogeneous testicular phenotype with comparable severity. This disparity among testicular phenotype in cases with known genetic aetiology and even in cases of unknown aetiology can be attributed to different genetic backgrounds and effect of modifiers. Since male infertility is a multifactorial disorder, the contributions of environmental and occupational insults may not be underestimated.

Keywords: Azoospermia factor (AZF), Infertility, Male infertility, Y-microdeletion

Infertility is a global health problem and it affects 10-15% of married couples worldwide, nearly half of them being contributed by the defect in the male. In most cases of male infertility the common underlying identifiable causes are anatomic defects, gametogenesis dysfunction, endocrinopathies, immunologic problems, ejaculatory failure and environmental exposures. However, in approximately 10% infertile men the causal factor is genetic. X-chromosome aneuploidy, X or Y-autosome translocation and deletion of a part of the euchromatic region of the long arm of the Y-chromosome (the AZF region) are well known genetic contributors of male infertility. Molecular studies on the Y-chromosome microdeletion have identified AZF as a pure ‘male sterile locus’, subdivided into three non-overlapping regions, AZFa, AZFb and AZFc. Candidate genes for the three regions, respectively are DFFRY/USP9Y, RBMY, and DAZ. Nine distinct protein coding families serving as a candidate spermatogenesis gene encompass AZF with copy numbers ranging between 2 (VCY, XKRY, HSFY, PRY), 3 (BPY2), 4 (CDY, DAZ), 6 (RBM) and 35 (TSPY) throughout the ampliconic region. DAZ, a candidate infertility gene in AZFc that is most frequently deleted region has 4 copies present in two clusters. Deletion of the DAZ gene leads to spermatogenic failure. High richness of AZF in palindromic sequences, made up of amplicons present in multiple copies, would act as a natural substrate for intrachromosomal recombination resulting in recurrent deletions and infertility.

We have performed analysis of karyotypes, Y-microdeletion and testis histology in 270 azoospermic/oligospermic infertile patients and 204 fertile controls. This number includes those 180 patients whose analysis for Y-microdeletion has already been reported. The present paper is an extension of that study. Beside adding more data on the karyotypic and Y-microdeletion, the possibility of gonosomic mosaicism and correlation of chromosomal anomaly with testis histology have been attempted.

Materials and Methods

Patients—Otherwise healthy, but azoo-oligospermic men (270) suffering from primary idiopathic infertility were recruited from the outpatient clinic at
the University hospital and IVF and Urology clinics in the city. Patients were examined by a team of andrologist and gynaecologist to rule out the possibility of varicocele, hydrocele, physical injury, testis size and consistency, and absence of secondary sexual characters. Testis biopsy or FNAC reports were available for nearly one third of the patients and histological analysis of testis material revealed spermatogenic arrest at different stages. Consent for the molecular analysis of their blood and tissue material was obtained from all the donors. The study was approved by the ethical committee of the University’s Institute of Medical Sciences.

Chromosomal analysis—PHA-induced whole blood cultures were done to obtain good chromosome preparations. The slides were treated for G-banding and observed under microscope. Selected metaphase plates were karyotyped using cytovision software for karyotyping. Both numerical and visible structural anomalies were taken into consideration for the analysis.

Polymerase chain reaction (PCR) and Southern hybridization—DNA was extracted from the blood and PCR-based multiplex screening of microdeletions was done using more than 40 sets of STS markers covering all the three regions, (AZFa,b,c). Individuals showing deletions in any of these STSs were further screened by using flanking STSs’ to get a rough estimate of the location and the extent of the deletion. AZF deletion results were confirmed by Southern analysis using candidate gene specific probes.

Testis biopsy—Whenever testis biopsy was available it was fixed in Bouin’s fluid and subjected to histological sections from the fixed tissue. Paraffin sections (6μ) were cut and stained with haematoxylin and eosin. DPX mounted slides were observed under microscope to assess the testicular phenotype.

**Results**

Cytological examination—Patients were diagnosed for chromosomal aberrations both for numerical as well as structural anomalies. G-banded karyotype of 11 patients revealed chromosome complement of Klinefelter’s syndrome (47, XXY); three patients harboured a mixture of 46XY/45XO/47XXY cells. In the present study no microscopically detectable chromosomal rearrangements or deletion was observed.

Microdeletion analysis—Different STS primer sets harbouring AZFa, AZFb and AZFc region were analysed through multiplex PCR reaction for each patient. Employing multiplex PCR strategy microdeletion was observed in 13 patients (Fig. 1). All thirteen patients showed interstitial deletions in AZFc. However, in two cases deletion extended in AZFb region. No deletion was observed in AZFa region.

Analysis of testicular phenotype—Histological preparations of testis biopsy from the patients revealed a broad spectrum of testicular phenotype from Sertoli Cell Only Syndrome (SCOS) to meiotic arrest. The Y-chromosome deletion cases having deletion in the AZFc region showed heterogenous phenotype ranging from maturation arrest to SCOS I and SCOS II (Fig. 2). Generally, SCOS phenotype is associated with AZFa deleted cases. However, in the present study SCOS testicular phenotype was also observed in non-Y deleted cases (Fig. 3) and AZFc deleted cases.

![Fig. 1](image_url)—Multiplex PCR analysis for (a) AZFa, b, c regions, sY84 is used from AZFa, sY117 from AZFb and sY152 from AZFc. pt #117 shows deletion of sY152 of AZFc region; and (b) AZFc, pt #161 showing deletion of sY148.
Discussion

Microdeletions in the AZF region is one of the major genetic cause of idiopathic male infertility in azoospermic (10%) and oligospermic (6%) patients\(^7\). Though AZFc deletion is the most common, AZFa and AZFb deletions are also reported. The global frequency of AZFc deletion revolves around 8%, but there is considerable heterogeneity in different populations\(^13\). More particularly, in contrast to very low frequency in cases from countries of northern Europe (1-4%) those from Italy have greater than 15% incidence of Y-chromosome micro-deletion, suggesting a geographical region based heterogeneity in the molecular aetiology of male infertility. In India, several reports on Y-micro-deletion in male infertility have appeared from different regions\(^13,16,18\). As expected, Y-chromosome micro-deletion has been recorded in samples from all the regions, but there are differences in frequencies. In general, the frequency revolves around 8% in all the reports except the present report and the earlier one of Ambasudhan et al.\(^13\). This difference is largely attributable to the high frequency of AZFa deletions in Dada et al.\(^16\) (nearly 50% of the deletion cases involve AZFa) and Thangaraj et al.\(^17\). Thangaraj et al.\(^17\), who have the largest number of cases studied in India, discovered novel AZFa deletions using STSs different from those generally employed in other reports. We have examined both sets of primers of AZFa and yet got no deletions. The frequency of AZFc deletion in all the reports is rather on the lower side (5 to 7%) compared to the global incidence. AZFc deletion is homogenously low, however incidence of AZFa deletion seems to vary among Indian populations. It has been stated that the nature of deletion may determine the testis phenotype. AZFa deletion, for instance, is associated with more severe Sertoli Cell Only Syndrome (SCOS-I) where the germ cells are absent in the seminiferous tubules\(^5\). We have recorded a number of SCOS cases in our samples but either

Fig. 2—Haematoxylin-eosin stained sections (6 μ) of testes from AZFc,Y-deleted cases (a) Sertoli-Cell Only Syndrome (SCOS1). (b) Hyalinized seminiferous tubules with fibrous tissue and none/a few germ cells. (c) Germ cells arrested at primary spermatocyte stage and (d) Maturation arrest showing lack of spermatids.
they occur independent of the deletion or within AZFc. The complete lack of AZFa deletions in the cases hitherto examined by us does not appear to be due to paucity of selection parameters. They may, on the other hand, reflect a geographical selective preference due to some factors not immediately obvious to us.

Spermatogenesis is marked by an orderly progression of distinct cell types. We were able to analyse histology and molecular analysis of over 30 testis biopsies from the subjects of the present investigation. DNA results from testis were identical to those obtained from lymphocytes, suggesting absence of any gonosomal mosaicism. On the other hand, the phenotypic diversity of testis is of the same order both in the Y-deleted and non-Y-deleted cases. The possible explanation of SCOS1 like patients in AZFc deletion could be explained as a consequence of progressive regression of the germinal cells over time, a condition already reported in patients with AZFc deletions. However, the occurrence of similar phenotypes in non-Y-deleted cases affirms the multifactorial nature of this disorder. It is therefore quite likely that the phenotypic diversity in cases reflects a combination of genetic backgrounds and microenvironmental influences.

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