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Clinical and Laboratory Evaluation of Idiopathic Male Infertility in a Secondary Referral Center in India

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The genetic basis of infertility has received increasing recognition in recent years, particularly with the advent of assisted reproductive technology. It is now becoming obvious that genetic etiology for infertility is an important cause of disrupted spermatogenesis. Y-chromosome microdeletions and abnormal karyotype are the two major causes of altered spermatogenesis. To achieve biological fatherhood, intracytoplasmic sperm injection (ICSI) is performed in cases of severe infertility with or without genetic abnormalities. There is a concern that these genetic abnormalities can be transmitted to the male progeny, who may subsequently have a more severe phenotype of infertility. A total of 200 men were recruited for clinical examinations, spermiograms, hormonal profiles, and cytogenetic and Yq microdeletion profiles. Testicular biopsy was also performed whenever possible and histologically evaluated. Genetic abnormalities were seen in 7.1% of cases, of which 4.1% had chromosomal aberrations, namely Klinefelter's mosaic (47XXY) and Robertsonian translocation, and 3.0% had Yq microdeletions, which is very low as compared to other populations. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) were significantly increased in men with nonobstructive azoospermia (NOA) as compared to severe oligoasthenozoospermia ($P<0.0001$), whereas testosterone levels were significantly decreased in men with microdeletions as compared to men with no microdeletions ($P<0.0083$). Low levels of androgen in men with microdeletions indicate a need to follow-up for early andropause. Patients with microdeletions had more severe testicular histology as compared to subjects without deletions. Our studies showed a significant decrease ($P<0.002$) in the serum inhibin B values in men with NOA, whereas FSH was seen to be significantly higher as compared to men with severe oligoasthenozoospermia (SOAS), indicating that both the Sertoli cells as well the germ cells were significantly compromised in cases of NOA and partially affected in SOAS. Overall inhibin B in combination with serum FSH would thus be a better marker than serum FSH alone for impaired spermatogenesis. In view of the genetic and hormonal abnormalities in the group of infertile men with idiopathic severe oligozoospermia and NOA cases, who are potential candidates for ICSI, genetic testing for Y-chromosome microdeletions, karyotype, and biochemical parameters is advocated. J. Clin. Lab. Anal. 22:29–38, 2008. © 2008 Wiley-Liss, Inc.

Key words: Indian men; idiopathic infertility; Yq microdeletions; chromosomal aberrations; inhibin B; FSH

INTRODUCTION

Infertility affects nearly 15% of couples in the reproductive age (1). Although not life threatening, it is socially a traumatic condition associated with social stigma in certain societies. In many societies, including India, biological parenthood is considered one of the dominant social attributes, resulting in intense societal and parental pressure on infertile couples to attain

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biological parenthood. About 50% of the cases of infertility are attributed to the male factor, which clinically presents as either absent or low sperm counts resulting from altered spermatogenesis.

The etiology of male factor infertility is multifactorial, and little is known about the causative factors leading to decrease in spermatogenesis, which is a complex cascade of events. In addition to the identifiable causes, viz. varicocele, hydrocele, and chronic epididymo-orchitis, there is another subset in which the exact cause of infertility and decrease in spermatogenesis is not clear. These are identified as cases of idiopathic infertility, the pathophysiology of which is still an enigma. In the past few years, modern diagnostics to investigate male infertility have progressed, making it possible to understand the cause of decrease in spermatogenesis and thereby understanding the events of spermatogenesis in such cases. Improved molecular diagnostics such as polymerase chain reaction (PCR), chromosomal analysis, etc., have opened new vistas and given clinicians options for better management and improvement of pregnancy rates. In-depth studies of the causes of idiopathic infertility has thrown new light on the association of a particular clinical phenotype with genetic factors such as microdeletions of the Y-chromosome (2), abnormal karyotype (3), and biochemical markers such as serum inhibin B, follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone.

Leads have been obtained on the prognostic value of testicular histology (4), serum inhibin B, and FSH, with the success of sperm retrieval rates (5). New understanding has emerged on the risk of early andropause and osteoporosis (6) and other long-term health implications in association with severe infertility and low testosterone levels.

Keeping these in view, a comprehensive investigation in a secondary referral center with a research setup would be ideal in evaluating idiopathic infertile males. The present study has therefore been undertaken to comprehensively evaluate the complicated clinical entity of idiopathic male infertility in an urban secondary referral center. Specific objectives were to assess the clinical, biochemical, and genetic factors (Y-chromosome microdeletions and karyotype) among males with severe oligoasthenozoospermia (SOAS) or with non-obstructive azoospermia (NOA).

MATERIALS AND METHODS

Patients

We prospectively screened 660 infertile men between 29 and 35 years of age. They were referred to our clinic from December 2002 to December 2005 for the diagnosis and treatment of male factor infertility. The study was approved by the Institutional Ethics Committee and participants provided informed consent.

A total 200 severely infertile men were recruited for the study. A total of 100 of them had severe oligoasthenozoospermia (SOAS) and 100 had NOA. A group of 50 age-matched healthy men with proven fertility constituted the control group.

The clinical evaluation of the male partner included a detailed history, assessment of secondary sexual characters, local examination, and measurement of testicular volume. The laboratory analysis included assessment of semen samples after an abstinence of 3–5 days to assess sperm number, motility, and morphology according to World Health Organization (WHO) guidelines (7). Two samples were examined, at an interval.

Serum hormonal estimations included FSH, LH, total testosterone, and inhibin B using commercial kits from DSL (Webster, TX).

Genetic testing included karyotyping and detection for Yq microdeletions by PCR. Karyotyping was done using a standard G banding technique. At least 25 metaphases were counted and two were karyotyped to determine numerical or gross structural abnormalities. The number of metaphases counted was increased to at least 50 in cases of mosaicism.

Hormonal Evaluation

Serum FSH, LH, and testosterone (T) were measured by radioimmunoassay and inhibin B was measured by enzyme-linked immunosorbent assay (ELISA) (DSL). Reference ranges were as follows: FSH = 1.7–12.0 mIU/mL,

TABLE 1. STSs used for PCR amplification

STS	Primers	Size (bp)
AZFa		
sY84	5′AGAAGGGTCTGAAAGCAGGT3′ 5′GCCTACTACCTGGAGGCTTC3′	326
sY86	5′GTGACACACAGACTATGCTTC3′ 5′ACACACAGAGGGACAACCCT3′	320
AZFb		
sY127	5′GGCTCACAAACGAAAAGAAA3′ 5′CTGCAGGCAGTAATAAGGGA3′	274
sY134	5′ GTCTGCCTCACCATAAAACG3′ 5′ ACCACTGCCAAAACTTTCAA 3′	301
AZFc		
sY254	5′GGGTGTTACCAGAAGGCAAA3′ 5′GAACCGTATCTACCAAAGCAGC3′	400
sY255	5′GTTACAGGATTCGGCGTGAT3′ 5′CTCGTCATGTGCAGCCAC3′	126
SRY	5′GAATATTCCCGCTCTCCGGA 3′ 5′ GCTGGTGCTCCATTCTTGAG 3′	429
ZFY	5′ ACCRCTGTACTGACTGTGATTACAC3′ 5′ GCACYTCTTTGGTATCYGAGAAAGT 3′	300

LH = 0.5–10 mIU/mL, testosterone = 3–12 ng/mL, and inhibin B = 10–531 pg/mL.

PCR for Yq Microdeletions

Genomic deoxyribonucleic acid (DNA) was isolated from leukocytes of whole blood using a commercial kit. For Yq microdeletions, six pairs of sequence-tagged site (STS) primers spanning the azoospermia factor (AZF) a, b, and c regions were used for PCR. Sequences of the primers used are shown in Table 1. Primers were chosen on the basis of previous observations demonstrating high sensitivity and specificity in detecting Yq microdeletions and also as recommended by the European Academy of Andrology (EAA) guidelines for laboratory practices (8). PCR were performed in 25-mL reaction volumes, with 2.5 mL of 10 × buffer (ammonium sulfate buffer; MBI Fermentas, USA), 1.5–3 mL $MgCl_2$ (25 mmol/μl; MBI Fermentas), 1 mL of deoxyribonucleotide triphosphate (dNTP) mix (12.5 pmol/μL each dNTP; MBI Fermentas), 0.5 μL of each primer pair (12.5 pmol/μL), and 0.2 mL Taq recombinant DNA polymerase (5 U/μL; MBI Fermentas). Specific PCR conditions were as follows: 35 cycles with a presoak for 5 min at 94°C, denaturation for 1 min at 94°C, annealing for 1 min 55°C, polymerization for 2 min at 65°C, and extension for 5 min at 72°C. PCR products (10-μL aliquots) were analyzed on 2.0% agarose gels stained with ethidium bromide (Fig. 1a). Duplex PCR was performed on patient DNA using the STS primers and Sen determining region Y (SRY) and Zinc Finger on Y (ZFY) as PCR positive controls.

All reactions were performed with a male control sample, a female sample, and a negative control.

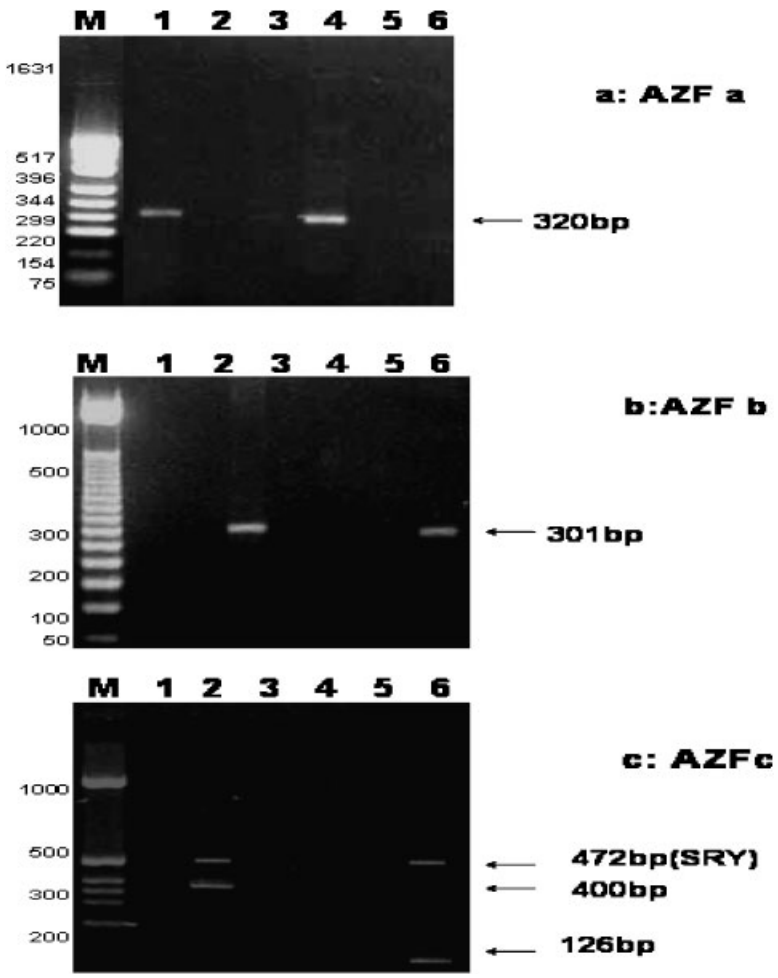

Fig. 1. PCR amplification for AZF deletions was carried out using six STS sets. (AZFa = sY84 and sY86; AZFb = sY127 and sY134; and AZFc = sY254 and sY255). Microdeletion of AZFa was observed in a single patient, AZFb was observed in two patients, and AZFc was observed in two patients. **a:** M, molecular weight marker. Lane 1, control male DNA. Lane 2, subject with deletion. Lane 3, PCR negative control. **b:** M, molecular weight marker. Lane 1, subject with deletion. Lane 2, control male DNA. Lane 3, PCR negative control. **c:** M, molecular weight marker. Lane 1, subject with deletion. Lane 2, control male DNA. Lane 3, PCR negative control.

Testicular Histology

Testicular histology was carried out in some of the subjects, who underwent testicular biopsy, to classify the stage of spermatogenic arrest (9).

The 5-μm-thick paraffin-embedded testicular sections (n = 26) were fixed on grease-free slides. The sections were deparaffinized in xylene for 15 min twice, followed by grades of alcohol, and stained with hematoxylin and eosin and at least 40 seminiferous tubules were evaluated. Classification was as follows:

- Sertoli cell only (SCO) no germ cells are seen, but presence of occasional tubules in which sperm production is normal is noted.
- Maturation arrest (MA) spermatogenesis is halted at the spermatocyte/spermatid level in the seminiferous tubules.
- Hypospermatogenesis (Hypo) all the stages of spermatogenesis including mature sperm are seen but with decreased number of cells (Fig. 2).

Statistical Analysis

The analysis was carried out using the statistical package Graph Pad Prism version 4 (Graph Pad Software, San Diego, CA). The frequency distribution of the genotype and phenotype in study group were done by chi-squared test and unpaired *t*-test with Welch's correction, respectively. The values were considered as statistically significant when the *P* value was less than 0.05.

RESULTS

Table 2 presents the clinical characteristics of subjects recruited. Y-chromosome microdeletions were observed in six of 200 (3%) subjects. As shown in (Table 3),

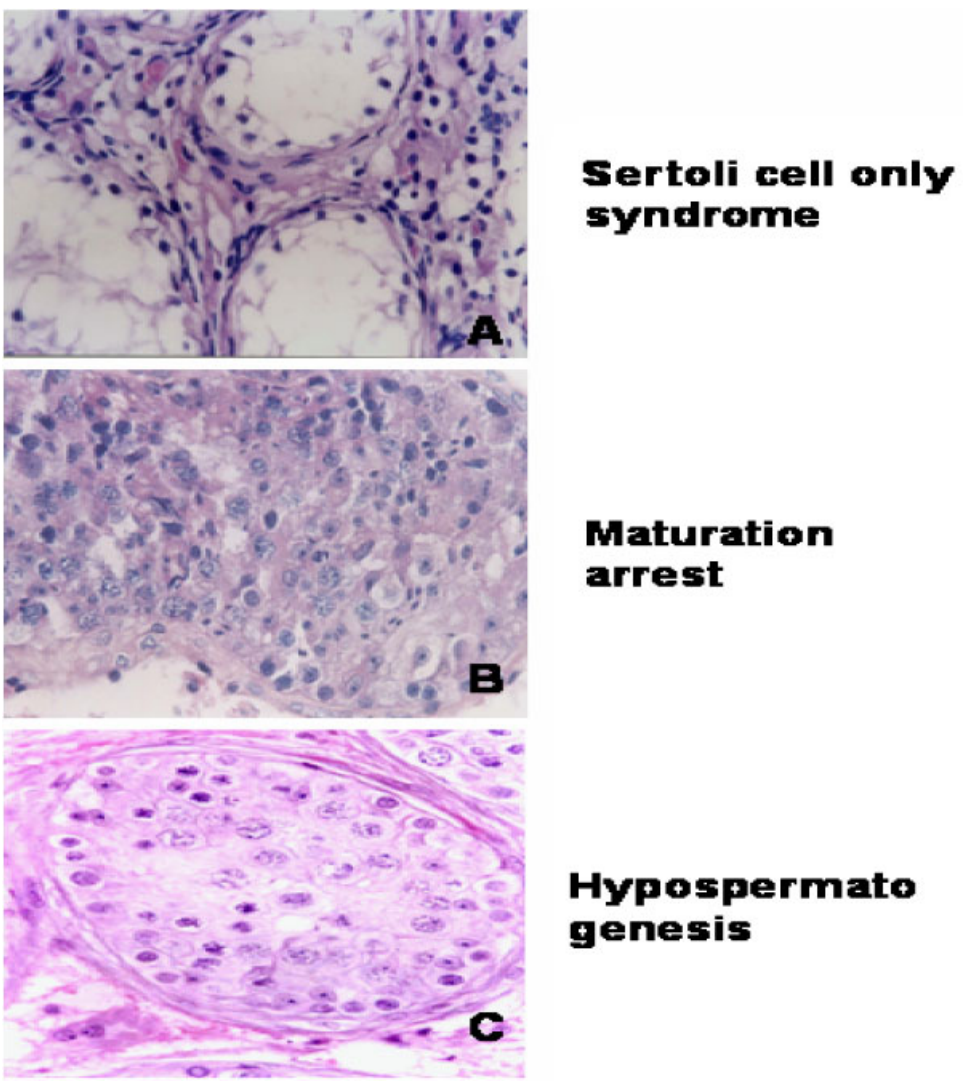

Fig. 2. Testicular morphology. **A**: SCOS. **B**: MA. **C**: Hypo.

subjects 11, 15, and 44 were azoospermic. Subject 11 had a large deletion of AZFb and AZFc and testicular histology showed SCO. No sperm were retrieved by testicular sperm aspiration for intracytoplasmic sperm injection (ICSI) in this case and the couple went for donor insemination. Subjects 15 and 44 had AZFb deletions. Subject 44 had four ICSI cycle failures. Subjects 17, 34, and 59 were phenotypically severely oligoasthenozoospermic. Testicular histology of subject 17 showed MA while SCO with pockets of spermatogenesis in few tubules was seen in subject 34. Subject 59 showed SCO. Microdeletions in the regions AZFc, AZFa, and AZFc were seen. The subjects with microdeletions had normal karyotypes. Table 4 shows hormonal and genetic profiles of the study group.

Abnormal karyotype (Klinefelter mosaic) was seen in eight in 194 (4.1%) of the subjects in which

TABLE 2. Clinical characteristics of the subjects

	NOA (n = 100)	Severe oligozoospermia (n = 100)
Age (years) (mean ± SD)	32 ± 3	32 ± 3
Duration of infertility(years) (mean ± SD)	6 ± 4	6 ± 4
Type of infertility: primary infertility (%)	96	96

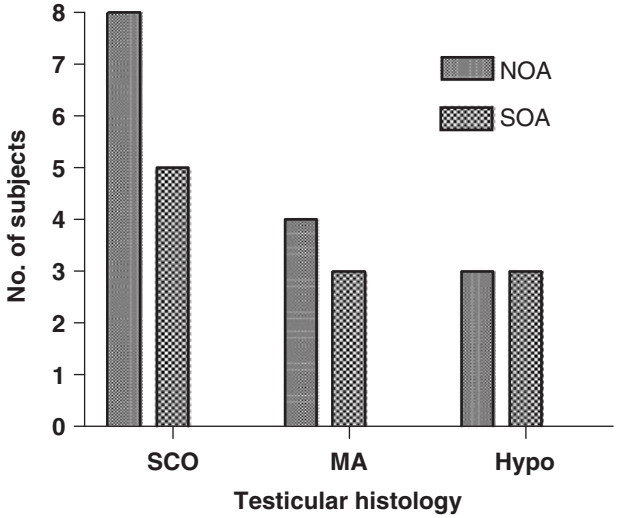

Fig. 3. Testicular histology pattern in men with NOA or with severe oligozoospermia, with and without microdeletions (n = 26).

TABLE 3. Genetic, hormonal, and testicular histology profile in subjects with Y-chromosome microdeletions

Patient no.	Phenotype	Age (years)	Sperm count	FSH (mIU/mL)	LH (mIU/mL)	Testosterone (ng/mL)	Karyotype	Histology	Yq deletion	Testicular volume
11	Azoospermia	31	0	21.2	11	0.35	46XY	SCOS	AZFb+c	L
15	Azoospermia	30	0	4.2	0.3	3.1	46XY	Maturation arrest	AZFb	N
44	Azoospermia	33	0	4.8	2.5	6.1	46XY	SCOS	AZFb (partial)	N
17	SOAS	34	1.0	10.4	1.0	5.9	46XY	Maturation arrest	AZFc	N
34	SOAS	28	1.2	14.0	6.2	3.8	46XY	SCOS II	AZFa	L
59	SOAS	30	0.1	10	0.8	2.4	46XY	SCOS	AZFc	N

L, low volume < 15 mL; N, normal > 15 mL; SCOSll, Sertoli cell only syndrome with few pockets of spermatogenesis.

TABLE 4. Hormonal and genetic profiles in the study group

	NOA (n = 100)	Severe oligozoospermia (n = 100)	Controls (n = 50)
FSH	20.1 ± 3.3 SE*	8.5 ± 0.9 SE	6.5 ± 0.67
LH	7.21 ± 1.1 SE*	4.26 ± 0.54 SE	5.5 ± 0.56
Testosterone	4.79 ± 0.45 SE	5.69 ± 0.44 SE	7.5 ± 1.24
Inhibin B	83.85 ± 15.7**	220 ± 27.3***	422.85 ± 31.4
Abnormal karyotype	6[a]	2[b]	None
Yq microdeletions	2 AZFb; 1 AZFb, c	2 AZFc; 1 AZFa	None

[a]Klinefelter's mosaic.
[b]Robertsonian translocation.
*$P = < 0.0001$.
**$P < 0.002$.
***$P < 0.024$.
SE, standard error.

no microdeletions were recorded. Testicular biopsy in NOA showed a more adverse histology in which eight of 15 had SCO syndrome and four of 15 had MA compared to SOAS (Fig. 3). Testicular histology of subjects with microdeletions was seen to have a prognostically adverse phenotype compared to those with no deletions (Fig. 4).

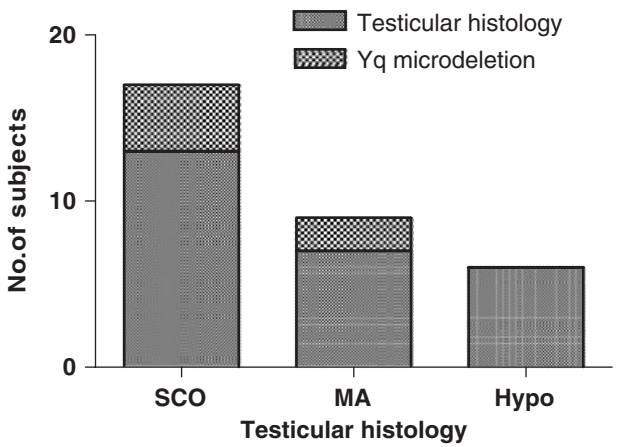

Fig. 4. Association of Yq microdeletions with type of testicular phenotype.

Hormonal Evaluation in Nonobstructive and Severely Oligozoospermic Men

The mean FSH concentration in patients with NOA was significantly higher as compared to severe oligozoospermia ($P<0.0001$) (Fig. 5a), the mean LH concentration in patients with NOA was significantly higher as compared to SOAS ($P<0.0001$) (Fig. 5b).

The mean testosterone concentration in patients with NOA showed no significant difference compared to SOAS (Fig. 5c).

Serum inhibin B values were significantly lower in men with NOA and in SOAS males ($P<0.002$ and $P<0.02$) (Fig. 5d), indicating that both the Sertoli cells and the germ cells were significantly compromised in NOA and partially compromised in the case of severe oligoasthenozoospermia.

Hormonal Evaluation in Microdeleted and Nonmicrodeleted Men

The mean FSH concentration in patients with microdeletions was significantly lower compared to nonmicrodeleted patients ($P<0.017$) (Fig. 6a).

The mean LH concentration was significantly higher in patients with microdeletions compared to nonmicrodeleted patients ($P<0.019$) (Fig. 6b).

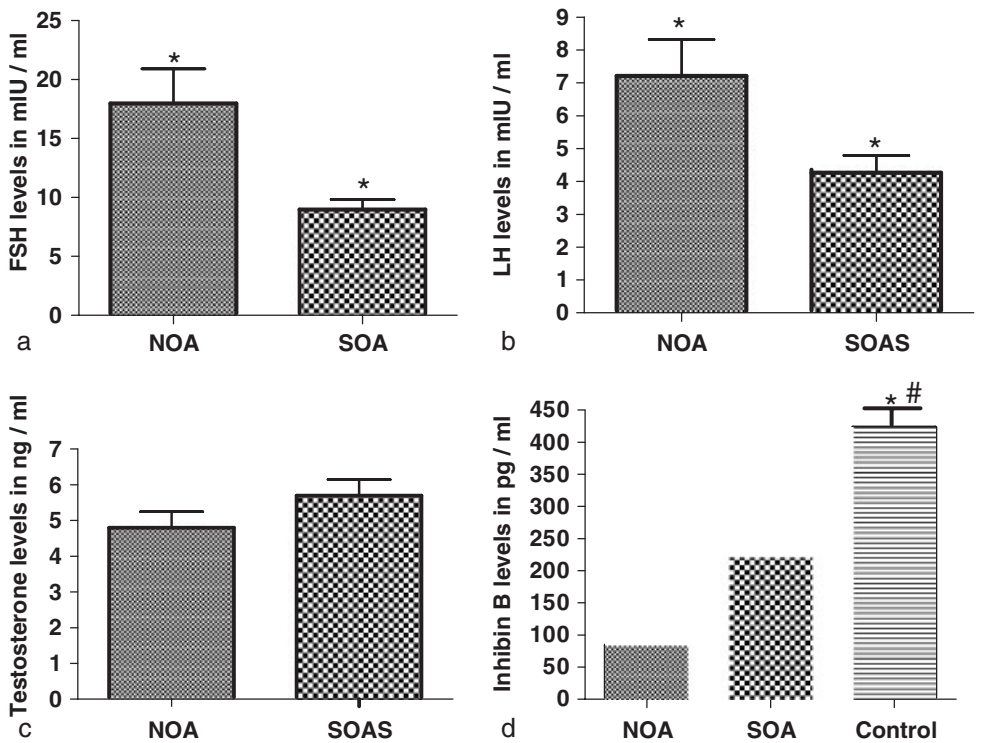

Fig. 5. **a:** Mean FSH levels in NOA compared to severe oligoasthenozoospermia; $P<0.0001$. **b:** Mean LH levels in NOA compared to severe oligoasthenozoospermia; $P<0.0001$. **c:** Mean testosterone levels in NOA showed no significant difference as compared to severe oligoasthenozoospermia. **d:** Mean inhibin B levels in NOA compared to severe oligoasthenozoospermia; $P<0.002$, $P<0.024$.

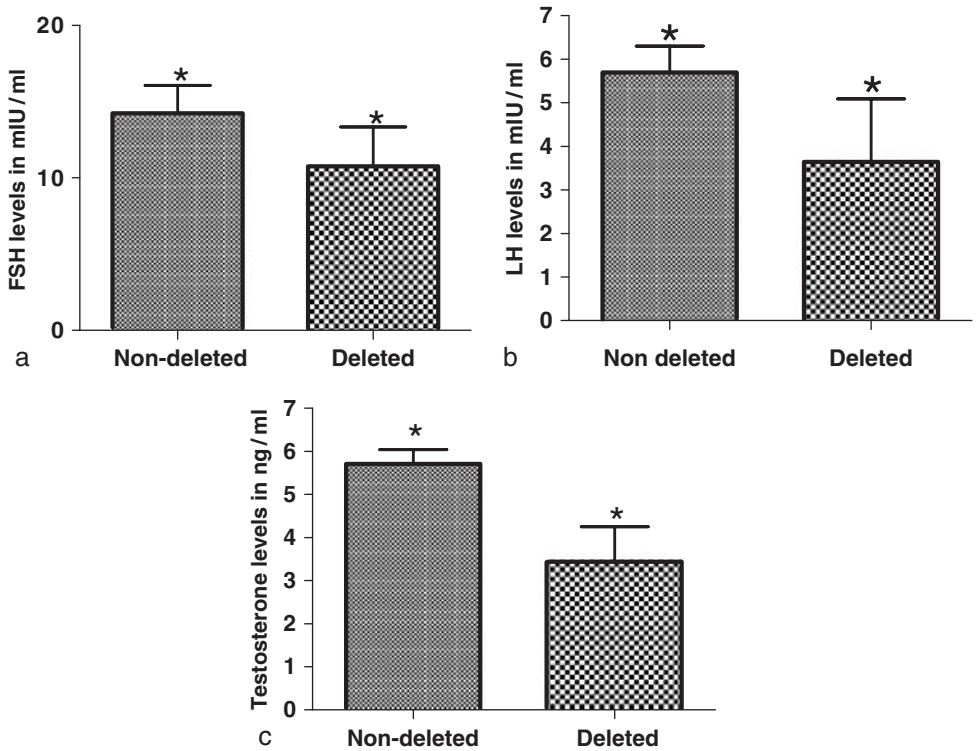

Fig. 6. **a:** Mean FSH levels in nondeleted compared to deleted; $P<0.017$. **b:** Mean LH levels in nondeleted compared to deleted; $P<0.019$. **c:** Mean testosterone levels in nondeleted compared to deleted; $P<0.0083$.

The mean testosterone concentration in patients with microdeletions was significantly decreased compared to nonmicrodeleted patients ($P<0.0083$) (Fig. 6c).

DISCUSSION

Infertility is a great social stigma and a major reproductive health problem. Worldwide reports suggest that infertility affects 10–15% of couples. Approximately 50% of these are accountable to the male partner. In more than 60% of these cases the origin of reduced testicular function is unknown (10). In recent years, genetic factors have been found to be involved in about 10% of the cases with male infertility (11). It is thus the most common molecularly diagnosable cause of spermatogenic failure in men (12). In about 15% cases of male infertility, no organic cause has been identified (idiopathic infertility). This entity of idiopathic male infertility is a multifactorial disorder, where environment, hormones, and genetic components interact variously. Data from regions with ethnic diversities, therefore, need to be generated to obtain a collective picture. In our study, we comprehensively evaluated the clinical, hormonal, and genetic abnormalities and testicular phenotype in a group of 200 idiopathic infertile males, who attended the institute's infertility clinic between 2002 and 2005. FSH is an important hormone to monitor in infertile men from the point of therapeutics and clinical intervention. Even though it is required for the initiation of spermatogenesis, the hormone by itself cannot be used as a marker for spermatogenesis. FSH twice the normal is indicative of germinal epithelial destruction, but normal or elevated FSH is associated with both normal as well as aberrant spermatogenesis, where testicular histology could show SCO syndrome, Hypo is seen where the quantity of spermatogenesis is decreased and is known to be associated with either normal or elevated FSH. (13) In our study, out of the three azoospermic men with microdeletions, two had SCO histology and one had spermatogenic arrest. The cases with SCO showed elevated FSH while it was normal in the other azoospermic male with deletion. The three patients with severe oligoasthenozoospermia had levels relatively elevated. Testicular histology in two of the cases showed Sertoli cell only syndrome (SCOS), one of them showing few tubules with spermatogenesis whereas the third patient had maturational arrest of spermatogenesis. Thus FSH values in the six cases with microdeletions did not indicate presence or absence of spermatogenesis. FSH by itself cannot be used as an indicator for sperm retrievals during ICSI due to the different histology

picture seen with normal/elevated FSH values. However, on the whole, our patients with NOA with/without microdeletions showed significantly higher values of both FSH ($P<0.0001$) and LH ($P<0.0001$) as compared to patients with severe oligozoospermia with/without microdeletions.

Inhibin B is an endocrine regulator of FSH secretion and has an inverse relationship with FSH. The Sertoli cells are generally considered as a major site of inhibin B production. In a recent study, however, the inhibin B subunit was also localized in certain stages of germ cell development; i.e, the pachytene spermatocytes and early round spermatids (14). Based on this observation, inhibin B in adult men appears to be a joint product of Sertoli cells and the germ cells. Studies on serum inhibin B levels related to the histological pattern of testicular biopsies have confirmed that: 1) inhibin B levels are reduced in men with severely affected spermatogenesis, particularly in men with SCO and spermatogenic arrest at an early stage (e.g., spermatogonia or spermatocyte arrest); 2) normal or near normal inhibin B levels are observed in azoospermic men with spermatogenic arrest at a later stage or with normal germ line, as in men with obstructive azoospermia (15). Precisely which germ cell types are involved in inhibin B regulation still remains to be established. Some studies point to pachytene spermatocytes where as others suggest that spermatids may be the important regulators for inhibin B secretion. Our studies showed a significant decrease ($P<0.002$) in the serum inhibin B values in men with NOA, where FSH was seen to be significantly higher as compared to severe oligoasthenozoospermic men, indicating that both the Sertoli cells as well the germ cells were significantly compromised in cases of NOA and partially affected in severe oligoasthenozoospermia. Overall, inhibin B in combination with serum FSH would thus be a better marker than serum FSH alone for impaired spermatogenesis (16). Taking into consideration the parameters; i.e., FSH, inhibin B values, and elongated spermatids in the histology, the combination augurs a higher sperm retrieval rate during ICSI.

Testosterone is an important hormone for the regulation of spermatogenesis; normal to lower normal values of testosterone is observed in men with moderate or severe infertility. Our study showed no statistically significant difference between men with severe oligoasthenozoospermia and with NOA, although testosterone values in men with NOA showed a trend toward low normal values. The hormone levels in the six cases that had Yq microdeletions in our study were significantly low compared to those without microdeletions ($P<0.0083$). Clinically, the six cases showed a trend toward hypoandrogenism. However, a large number of cases need to be studied to document the association of genetically-related infertility and low testosterone values. There is a growing concern regarding low testosterone values that are undetected clinically and its association with osteoporosis in infertile males (17) as well as the possibility of early andropause in this vulnerable group. The impact of lower normal range of serum androgens in terms of the long-term effect on general health and quality of life needs to be studied in this group of men.

With the advent of improved molecular tools in the diagnosis of genetic aspects associated with male infertility, there is an increasing observation of microdeletions of Y chromosome and abnormal karyotypes associated with severe forms of male infertility and decreased spermatogenesis. The reported frequency of deletions varies from 1% (18) to 55% (19), which is largely related to different inclusion criteria and the number of STSs used. The accumulated data (20) showed a 12.2% microdeletion rate in azoospermic men and 3.4% in oligozoospermic men. These values are not comparable to those observed in our study, which shows a low frequency of Yq microdeletions in both these groups. The reason is probably the strict selection criteria applied in our study as well as geographic and ethnic differences. Within India, studies have been carried out in different regions, which showed a frequency between 5% and 9.5% (21–23). The selection criteria in these were also different from the criteria used in our study, the number of STSs used were variable. Ethnic and environmental factors could influence the differences in the frequency distribution. Our study emphasizes the diagnostic and prognostic value of Yq deletions in the clinical management of men with NOA. It supports earlier studies that the presence of a complete AZFa or AZFb deletions have a negative prognostic value for testicular sperm retrieval (24–26). One of our subjects, who had an AZFb deletion, was azoospermic and underwent sperm retrieval for ICSI; no sperm could be retrieved and the couple opted for donor insemination that resulted in a successful pregnancy. Another subject, who had a DAZ deletion, had initially severe oligoasthenozoospermia of <5 million/mL. Within a time span of 2 years the same subject reported as azoospermic. Y chromosome is known to have massive palindromes in the region of the DAZ gene, located of the Y chromosome, it is observed that the phenotype varies between severe oligoasthenozoospermia to azoospermia; hence, in this case it could be possible that due to the palindromes, the important functional copy of the DAZ gene would have been deleted. It is observed that the phenotype has also been reported by other workers (27). In patients presenting with severe oligoasthenozoospermia, who are at risk for

progressive decrease in sperm concentration over a period of time, cryopreservation of spermatozoa could avoid future invasive techniques such as testicular sperm aspiration (TESA)/ICSI (28). The study carried out by us shows Y chromosome microdeletions to be associated with a more severe testicular phenotype. Out of the six cases with microdeletions, SCO was seen in four cases and MA was seen in two cases. In patients who did not show any deletions, a large number of cases had Hypo (36%). Some of these cases also showed SCO and MA, indicating other causes such as epigenetic or testicular autocrine/paracrine regulators of spermatogenesis may be involved in the decrease or absence of spermatozoa. A more in-depth study related to autosomal and sex chromosome alterations as well as related to the DAZ gene copies need to be addressed to define the association of genetic factors in idiopathic infertility, resulting in a decrease or absence of spermatogenesis.

Chromosomal abnormalities in infertile men have been found within the range of 2.2–15.2% (average = 5.15%) compared to the normal population (0.2–0.6%). A total of 3.7% of these involve the sex chromosome and 1.3% involve autosomes (29). The most common abnormal karyotype is Klinefelter syndrome, affecting 7–13% of azoospermic males with small testes, gynecomastia, and hypogonadism. A total of 90% of the case are classical, 46 XXY, while only 10% of the cases show mosaic 47 XXY/46 XXY.

Sperm retrieval is possible despite a poor testicular histology and fertilization as well as pregnancy is reported in cases in which sperm have been utilized for ICSI (30). One out of 20 oligozoospermic males has abnormal karyotypes, mainly Robertsonian and reciprocal translocation. Clinical relevance of sex chromosomal instability with resulting 47XXY cell lines remains to be established. Nevertheless, low-level mosaicism for numerical sex chromosomal abnormalities has been reported as a frequent cytogenetic abnormality in couples undergoing ICSI (31). Our study showed a mosaic Klinefelter in all the cytogenetically abnormal azoospermic patients. Abnormal karyotype is also associated with higher pregnancy loss (32). Also, Klinefelter syndrome has been recently reported to be associated with increased mediastinal (33) cancer risk among the infertile men. A close association with osteoporosis has also been reported in this group. In view of these findings, patients with severe male infertility of unknown origin should undergo karyotyping tests, as these abnormalities can be transmitted to the male progeny and/or may result in early pregnancy loss.

To conclude, our study on idiopathic male infertility showed that patients with NOA have a more severe hormonal and testicular phenotype with an inverse relationship with inhibin B. Lower normal values of testosterone in men especially with genetically diagnosed infertility need to be followed up from the point of the long-term effect on bone health and early andropause, as both can affect the quality of life. Large numbers of infertile men need to be studied to determine the genetic implication with regard to aberrant spermatogenesis in idiopathic infertility. It is also essential, since this group of infertile men constitute those that would opt to undergo assisted reproductive technology, especially ICSI in which these aberrations can be transmitted to the male progeny; also pregnancy rates are lower in male factor infertility.

Counseling forms a strong point for this group of subjects, as an informed decision can be offered to the couple and better clinical management can be given when a comprehensive investigational approach is performed in a complex entity such as idiopathic male infertility.

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