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Chromosomal Anomalies in Patients with Azoospermia and Oligoasthenoteratozoospermia

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ABSTRACT Male factor accounts for infertility in 10% of couples of reproductive age worldwide and is treatable in many cases. The etiology of male infertility is complex and may include anatomical problems, imbalance in levels of gonadal steroids and gonadotropic hormones, and genetic causes. It is demonstrated that infertile men have an increased frequency of chromosome abnormalities and gene disorders that make a significant contribution to male infertility. The aim of this study is to investigate the contribution of chromosomal abnormalities in patients with abnormal spermatozoa. Each infertile male referred with sperm count less than 5 x 10^6/ml and increased abnormal sperm morphology. This study included 150 infertile males diagnosed to have azoospermia (AZF) (n=125), oligoasthenozoospermia (OAT) (n=22), severe oligoasthenozoospermia (SOAT) (n=2), and globozoospermia (n=1). Chromosomal abnormalities were detected in 5 (3.3%) and polymorphisms in 11 (7.3%) patients. Chromosomal abnormalities include sex chromosome aneuploidy (Klinefelter syndrome, 47,XXY), Robertsonian translocation [45,XY,t(13;14)(q10;10)], and a deletion, 46,X,del(Y)(q11.2). Polymorphisms included a pericentric inversion on chromosome 9 and increase in the length of heterochromatic segments - 1qh+, 9qh+, 15p+, 21p+, 22p+ and Yqh+.

INTRODUCTION

Infertility is defined as failure to conceive after at least one year of unprotected intercourse (Griffin and Finch 2005; Lissitsina et al. 2006). Infertility is a major health problem affecting 10-20% of couples (Ceylan et al. 2009), of which male factor infertility represents ≃ 50% of cases (Bhasin et al. 1994). The etiology of male infertility is complex and may include anatomical problems such as congenital bilateral absence of vas deferens (CABVD), cystic fibrosis, infections such as mumps and herpes, diabetes, obesity, varicocele, imbalance in levels of gonadal steroids and gonadotropic hormones, and immunologic problems such as antisperm antibodies and genetic causes (Sigman and Jarrow 1997; Sokol, 2001). In spite of this, genetic factors are considered to be significant (Samli et al. 2006).

Most infertile men have normal karyotypes, but their spermiogram are abnormal. In many cases, these patients show an increased incidence of aneuploid and diploid sperm. Previous studies for different populations have shown that the incidence of chromosomal abnormality in infertile males was between 2.2% and 19.6%. The most common chromosomal abnormality in these studies was Klinefelter's syndrome followed by Yq deletions (Nakamura et al, 2001; Poongothai et al, 2009). A 7.1% of major chromosomal abnormalities was reported in 496 infertile males, of which 21% were azoospermic (Retief et al. 1984). Bourrouillou et al., found a higher incidence (10.3%) in 952 infertile males, but in this study 40% of patients were azoospermic. These studies pointed out that the frequency of chromosomal abnormalities were high in patients with abnormal spermatozoa. Further, in azoospermic patients more than 90% of abnormalities affect sex chromosomes while autosomal chromosomal abnormalities are more frequent in patients with oligozoospermia (De Palma et al. 2005; Lissitsina et al. 2006). In this prospective study, it was proposed to analyze the various chromosomal abnormalities in infertile males with abnormal semen parameters.

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MATERIALS AND METHODS

Infertile men (n=150) were prospectively subjected for chromosomal analysis from 2006 to 2009 at the Department of Human Genetics, Sri Ramachandra University, Porur, Chennai, India. The mean age of the patients was 37.5 years (range 23-52 years). The subjects were classified into azoospermia (AZF) (83.3%), oligoasthenozoospermia (OAT) (14.6%), severe oligoasthenozoospermia (SOAT) (1.3%), and globozoospermia (0.67%) based on andrological workup. The study protocol was approved by Institutional Medical Ethics Committee and informed consent was taken from the patients prior to collection of blood samples. Chromosome investigations were performed on cultures of peripheral blood lymphocytes using RPMI 1640 medium and phytohemagglutinin (Verma and Babu, 1995). From each patient, 25 well-spread metaphases were analyzed after GTG-banding for numerical as well as structural abnormalities and karyotyped with Automated Image Analyzer, Cytovision Software, Version 4.0. The abnormalities were designated as per International System for Cytogenetic Nomenclature (Shaffer and Tommerup, 2005). The presence of heterochromatin on Y chromosome and nucleolar organizing regions on chromosomes in D and G groups were further confirmed by Q-banding and AgNOR-staining methods respectively.

RESULTS

Table 1 shows the type of abnormal spermatozoa observed in 150 infertile men after andrological workup presenting with absence of sperm or decreased sperm count (range 0-4 million/ml), reduced motility (range 6-12%) and abnormal morphology (normal ranges from 2-10%). One hundred and twenty five cases (83.3%) were referred with azoospermia, twenty two (14.6%) with oligoasthenoteratozoospermia, whereas two infertile men (1.3%) showed severe oligoasthenoteratozoospermia and one patient (0.67%) with globozoospermia.

Out of 150 infertile men studied, only five cases showed (3.3%) constitutional chromosomal abnormality. The abnormalities comprised of gonosomal aberrations in four cases of azoospermia (2.7%) and one oligoasthenoteratozoospermia patient showed Robertsonian translocation (0.6%) (Fig. 1), two (1.3%) cases of non-mosaic Klinefelter syndrome (Fig. 2) and two (1.3%) with deletion on Y chromosome of karyotype 46,X,del(Y)(q11.2) (Fig. 3) were observed.

Table 1: Spermiogram of infertile men (n=150).

Abnormal semen parameters	*Number of patients**
Azoospermia (AZF)	125 (83.3)
Oligoasthenoteratozoospermia (OAT)	22 (14.6)
Severe Oligoasthenoteratozoospermia (SOAT)	2 (1.3)
Globozoospermia	1 (0.67)

* Values in parentheses show the percentage.

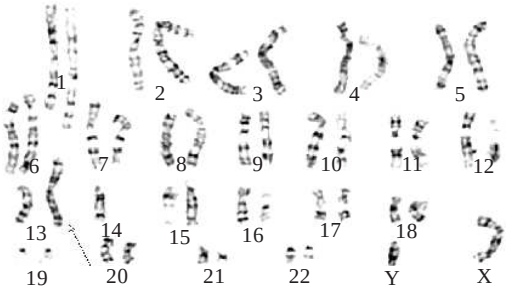

Fig. 1. Metaphase showing the presence of 45,XY, t(13;14) (q10;10)

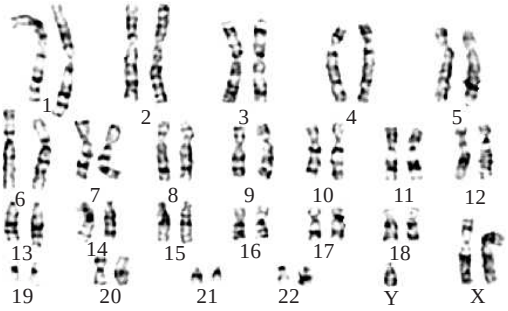

Fig 2. Metaphase showing the presence of 47,XXY

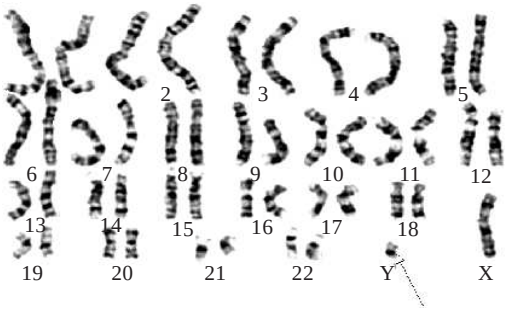

Fig. 3. Metaphase showing the presence of Yq deletion

Chromosomal polymorphisms were observed in 11 (7.3%) patients, of which nine (6%) were azoospermic, and two (1.3%) diagnosed to have oligoasthenoteratozoospermia patients (Table 2). An increase in the length of heterochromatic regions namely 1qh+, 9qh+, 15p+, 21p+, 22p+ and Yqh+ were noted in azoospermia and oligoasthenoteratozoospermia groups of infertile men and the pericentric inversion inv(9qh) was noted in one case of azoospermia. An elongation of the heterochromatic region of the Y chromosome was identified in five patients (3.3%) with azoospermia, while autosomal variants were seen in both azoospermia and oligoasthenoteratozoospermia groups. However, a normal karyotype of 46,XY was observed in 125 patients with azoospermia (83.3%), two patients with severe oligoasthenoteratozoospermia (1.3%) and in one patient with globozoospermia (0.67%).

Table 2: Chromosomal polymorphisms in infertile men

Classification	*Karyotype*	*No. of men with polymorphisms*
Azoospermia	46,X,Yqh+	5(3.33)
	46,XY,9qh+	1(0.67)
	46,XY,inv(9qh)	1(0.67)
	46,XY,15p+	1(0.67)
	46,XY,22p+	1(0.67)
Oligoazthenotera-tozoospermia	46,XY,1qh+	1(0.67)
	46,XY,21p+	1(0.67)

* Values in parentheses shows the percentage.

DISCUSSION

Male factor was reported to be responsible in 50 per cent of the infertility. (O'Connell et al. 2002). The family history, physical examination, and various laboratory tests on both partners are generally required to evaluate the etiology and treatment in infertile males (WHO, 1999). Not only defects in hormone production, testicular structure, ejaculation and/or the spermatozoa themselves can adversely affect the chances of conception, but also genetic defects can affect the fertility (Griffin and Finch, 2005). Most infertile men have normal karyotypes, but their semenograms are abnormal. In many cases these patients show an increased incidence of aneuploid sperm and diploid sperm (Egozcue et al., 2003). Recent studies have focused on the genetic basis of male infertility (Ceylan et al. 2009).

Chromosomal abnormalities in infertile men have been found within the range of 2.2-15.2% (average=5.15%) compared to the normal population. A total of 3.7% of these involve the sex chromosomes and 1.3% involves autosomes. Robertsonian translocations were found in 0.9% of the oligozoospermic and 0.3% of the azoospermic patients (De Palma et al. 2005; Lissitina Lissitsina et al. 2006).

In infertile males with severe spermatogenesis impairment, chromosomal aneuploidy seems to be more common than other abnormalities with low sperm quality (Gekas et al. 2001; Dohle et al. 2002; Vincent et al. 2002). In the present study, the incidence of constitutional chromosomal abnormalities was found to be 3.3% with sex chromosome abnormalities being detected in four patients with azoospermia (2.7%) and autosomal abnormality in one patient with oligospermia (0.6%), which was in concordance with the literature.

Sex chromosome abnormalities are the most frequent chromosome-related cause of infertility, affecting 7-13% of azoospermic males resulting in testicular failure, androgen deficiency, and infertility. The prevalence of Klinefelter syndrome among infertile men is very high, up to 10% in azoospermia and 5% in severe oligozoospermia (Huynh et al. 2002; Clementini et al. 2005). A total of 90% of the cases are classical 47,XXY, while only 10% of the cases reported the presence of mosaicism in motile sperm (Cozzi et al. 1994).

Klinefelter syndrome occurs due to chromosomal nondisjunction in sperm or egg and in few cases due to post zygotic mitotic error (Shamsi et al. 2007). The predominant sex chromosome anomaly among infertile men was attributed to Klinefelter syndrome who have the 47, XXY karyotype. Marchina, (2007) et al., did not find any cases of Klinefelter syndrome in 470 couples undergoing Intracytoplasmic Sperm Injection (ISCI). In the present study, out of 150 infertile males two azoospermic patients (1.6%) showed Klinefelter syndrome with a karyotype of 47, XXY, which is less to the results of other studies (Bhasin et al. 2000; Huynh et al.2002).

Robertsonian translocation is a chromosome defect found in oligozoospermic males (1.6% versus 0.1% in the general population) (Bourouillou et al.1985; Nielson and Wohlert, 1991). It was to be 0.63% in patients associated with infertility (Frydman et al. 2001; Gardner and

Sutherland 2004; Baccetti et al. 2005) and with miscarriages (Scriven et al. 2001). A frequency of karyotypic abnormalities in 1791 males with infertility was found to be as high as 12.67% in azoospermia and 4.6% in oligozoospermia (Van Assche et al. 1996). The frequency of autosomal abnormality was found to be only 0.6%, which is slightly higher than the general population. Hence prolonged follow up studies will be necessary to establish whether male offspring carrying the same structural chromosome abnormality inherited from their oligozoospermic infertile father will themselves be oligozoospermic and infertile.

Observations in experimental animals and electron microscopic examination of meiotic profiles in human male carriers have suggested that the trivalents formed at the pachytene stage of meiosis by the Robertsonian translocation chromosome and the two single D or G group chromosomes, may interact with the X/Y bivalent, resulting in spermatogenic impairment (Johannisson et al. 1993 ; Everett et al.1996).

Studies have indicated that deletions on the long arm of the Y chromosome involving a particular and consistent segment might lead to azoospermia and sometimes to severe oligospermia (Reijo et al.1995). The present study showed 1.3% of infertile men to have a deletion on chromosome Y. The variation in the length of the Y chromosome was due to a variation in the distal part of the long arm that is known to contain heterochromatin. An increase in the length of the heterochromatic region of the Y chromosome is the most frequent variant observed in sex chromosomes.

The relationship between many normal variants of chromosomes, such as pericentric inversions, 9qh+, Yqh+, and impaired spermatogenesis is not clear (Barch et al.1997). The most frequently occurring heterochromatic variant is inv(9) with a prevalence of 1–1.65% in the general population and 1-1.75% in infertile males. The heterochromatic variant 9qh+ was observed in 0.3-14.3% of infertile males compared to the 1.4% in the normal population. Lissitsina et al. also reported that there could be several unknown factors, which impair spermatogenesis. Several reports on male infertility mentioned the presence of chromosomal variants or polymorphisms. The overall occurrence of chromosomal variants in the present study was 7.3 per cent, which was comparable with other studies.

CONCLUSION

Cytogenetic analysis followed by genetic counseling would be helpful in infertile men with azoospermia and oligozoospermia in the determination of the genetic factors causing infertility and in the assessment of the genetic risks to the offspring through assisted reproductive techniques. In view of these findings, patients with severe male infertility should undergo karyotyping, as these abnormalities can be transmitted to the male progeny and/or may result in pregnancy loss.

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