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Chromosomal Abnormalities in Infertile Men with Azoospermia and Oligospermia

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Abstract

The present study was carried out to find out frequency of chromosomal abnormalities in infertile males with azoospermia& oligospermia.50 males referred for complaints of infertility with azoospermia & oligospermia were included in the present study. The study was carried out in the following steps. 1) Selection of patients 2) Clinical examination of patients 3) Collection of blood and karyotyping 4) Photomicrography 5) Data tabulation and Analysis. Among the total 25 azoospermic males, 8 patients showed abnormal karyotype. Among these abnormal karyotypes, 3 patients showed 47XXY karyotype, 2 patients showed 46XX karyotype, 46XY(20%)/47XXY(80%) was found in 1 patient, 1 patient showed 47,X,i (Xq)Y & 1 patient showed a 45,XY,-22 t (14/22) karyotype. Seventeen patients had normal karyotype. Among the total 25 oligospermic male, 3 patients showed abnormal karyotype. Among these abnormal karyotype, 1 patient showed mosaic Klinefelter i.e. 46XY(20%)/47XXY(80%), 1 patient showed a karyotype of 46,XY, inv(9) and one patient showed 46,XY, large Y.

Key Words: *Karyotype, Chromosome, Infertility, azoospermia, oligospermia*

INTRODUCTION

Infertility is disorder of reproduction representing a significant social, medical & economic burden for individual & the society [13]. It affects on average 25% couples worldwide.

Infertility affects 10-15% of couples of childbearing age, and nearly half of these cases are attributable to the male partner and particularly sperm related problems. Approximately 10% of infertile men are azoospermic. A large majority of these men have associated genetic disorders that ranges from chromosomal (gonosomal) aneuploidy or structural rearrangements to mutations or microdeletions. In infertile men with a chromosomal abnormality, 2.7% shows oligospermia& 10.8% shows azoospermia. At least 5% of azoospermic males have been found to have Klinefelter syndrome.

There is a complex correlation between genetics and infertility. Several factors affect gametogenesis from which, factors that lead to chromosomal abnormalities are one of the best known. Some chromosomal aberrations are inherited, while others arise *de novo*. The result can be failure or a decrease in sperm production, or the production of sperm with an unbalanced chromosomal constitution. The latter may result in unsuccessful conception or in a chromosomally unstable zygote, which in turn may lead to either fetal wastage or the birth of a chromosomally abnormal child [1].

The overall incidence of chromosomal factors in infertile males ranges from 2% to 8%, with a mean value of 5%. The chromosomal abnormalities include sex chromosomal abnormalities are predominating in azoospermic men [23].

Azoospermia is the absence of sperm in semen, may due to a physical obstruction in the post-testicular genital tract, or may have non-obstructive causes that could be genetic.

Infertility is defined as the inability of a couple to conceive after 1 year of unprotected sexual intercourse. It affects approximately 10%–15% of couples, and male factors are responsible for about 40%–50% of these cases [6]. Chromosomal anomalies are considered as important causes of male infertility. The reported frequencies of chromosomal anomalies are 10-23.62% and 1.10–13.33% in non-obstructive azoospermia and severe oligozoospermia, respectively [7], [15].

A male factor is solely responsible in about 20% of infertile couples and contributory in another 30-40%. Azoospermia, defined as complete absence of sperm from the ejaculate, is present in about 1% of all men and in approximately 15% of infertile men.

The incidence of chromosome abnormalities is about ten times higher in infertile men than in the general population.

The most common type of karyotype abnormality observed in infertility is represented by Klinefelter'ssyndrome (KS) and also Y chromosome long arm microdeletions which is described as the most frequent non-chromosomal alteration [26].

AIM OF PRESENT STUDY

The aim of this study was to determine type and rate of chromosomal abnormalities in infertile azoospermic and oligospermicmales.

MATERIAL AND METHODS

Fifty males referred for complaints of infertility with azoospermia& severe oligospermia were included in the present study. Patients wereexplained the procedure and possible outcome of the test. A written and informed consent of the patients were taken. The study was carried out in the following steps.

1. Selection of patients

   Male patients referred to the genetic division for infertility with history of inability to have an issue after one year of marriage without use of any contraceptive and/or erectile dysfunctions were included in present work. These patients were already diagnosed as azoospermia&oligospermia. Cases were classified into groups using sperm count. Azoospermia was defined as the total absence of sperm cells and oligozoospermia was defined as the sperm cell count less than 20 million/ml in seminal liquid.

2. Clinical examination of patients

   All patients were initially evaluated. Patient's detailed history, genital examination, ultrasonography and hormone analyses were performed.

3. Collection of blood and karyotyping

   Peripheral blood in 7ml sodium heparin collection tubes were taken from each patient.

   Cytogenetic analyses were performed from peripheral blood lymphocyte culture. In brief, the cultures of peripheral blood lymphocytes were treated with colcemid after 72 hr incubation period and chromosomes were analyzed by GTG banding at approximately 400–450 band resolution. At least 50 metaphases were analyzed for each patient and up 100 metaphases were analyzed in case of mosaicism.

4. Photomicrography

   Photographs of appropriate abnormal metaphases were taken for documentation

5. Data tabulation and Analysis

   The collected data was tabulated

OBSERVATIONS AND RESULTS

A total of 50 patients with infertility with azoospermia&oligospermia were evaluated retrospectively. Eight out of 25 (32%) with azoospermiapatients showed chromosomal alteration. Three out of 25 (12%) with oligospermia showed chromosomal abnormalities. Among the chromosomal abnormalities, Numerical abnormalities were present in 8 (16%) patients and Structural abnormalities were present in 3 (6%) patients. Among the 8 patients with Numerical abnormalities, 3 (6%) patients showed 47,XXY karyotype which is accepted to be a variant in the population, 2 (4%) patients were found with a 46,XX karyotype, 2 (4%) patient was found with mosaicism i.e. 46,XY(20%)/47,XXY(80%), and 1 (2%) patient showed a karyotype of 47,X,i(Xq)Y. Among the 3 patients with structural abnormalities, 1 (2%) patientshowed a 45,XY,-22 t(14/22) karyotype, 1 (2%) patient showed 46,XY, inv(9), and 1 (2%) patient showed 46,XY, large Y

Table 1: Showing Agewise Distribution of Patients Included in the Present Study

Age Group (Yrs)	No. of Patients	Percentage
20 – 25	11	12
26 – 30	30	60
31 – 35	05	10
36 – 40	04	08
Total no. of Patients	50	100

Table 2: Showing Distribution of Semen Analysis Study

Semen Analysis	Total No. of Patients (n=50)	Percentage
Azoospermia	25	50
Oligospermia	25	50

Table 3: Showing Correlation between Karyotype and Semen Analysis

Karyotype	Semen Analysis (n=50)	
	Azoospermia	Oligospermia
Normal	17	22
Abnormal	08	03

Table 4: Showing Distribution of Chromosomal Study in Present Work

Karyotype	Total No. of Patients(n=50)	Percentage
46,XY	39	78
47,XXY	03	06
46,XX	02	04
46,XY(20%)/47,XXY(80%)	02	04
47,X,i(Xq)Y	01	02
46,XY,inv(9)	01	02
45,XY,-22t(14/22)	01	02
46,XY,largeY	01	02

Table 5: Showing Distribution of Chromosomal Abnormalities

Type of abnormality	Karyotype
Numerical abnormality	47,XXY
	46,XY(20%)/47,XXY(80%)
	47,X,i(Xq)Y
	46,XX
Structural abnormality	46,XY,inv(9)
	45,XY,-22t(14/22)
	46,XY.largeY

DISCUSSION

Several disorders of spermatogenesis result in permanent and irreversible infertility. In these patients, germ cells are either absent or fail to proliferate beyond a particular stage of spermatogenesis. These disorders are associated with chromosomal abnormalities. Germinal cell aplasia and germinal cell arrest account for about 10% of men with infertility. The semen sample of these men shows azoospermia or severe oligospermia[18].

Classification of Sperm Count

Classification of Sperm Count	Sperm Count in Millions/Ml
Azoospermia	0
Severe oligospermia	<1
Moderate oligospermia	1-5
Mild oligospermia	5-20
Normal	>20

Sex chromosome abnormalities predominate in azoospermic patients (12.6%) whereas autosomal abnormalities are the most frequent in the severe oligozoospermic patients [24].

Chandley and Cooke (1994) studied 50 azoospermic and oligozoospermic men and found a deletion in the Yq region for 4 men.

Girardi et al [11] (1997) have proposed that 3-18% of men with non-obstructiveazoospermia or severe oligozoospermia may have deletions of the Y chromosome.

Chromosomal abnormalities are more frequently observed in the population of azoospermia and/or oligozoospermic males than in the general population [7].

Ng PP et al [15] (2009). reported that the incidence of sex chromosome abnormalities in azoospermia group was higher than that in the oligospermia group.

In our study, the highest frequency of abnormal karyotype was among patients with azoospermia (32%) as compared to the oligospermic subgroup (12%).

The chromosomal abnormalities found in infertile men are structural, numerical or mosaicism[9]. Sex chromosomal abnormalities predominate in male infertility [21], [23].The single sex chromosomal abnormality of 47, XXY and mosaics of 46,XY/47,XXY are relatively common and are seen more likely in azoospermic as well as in severe oligospermic males. The gonadal defect in XXY men is related to germ cell survival and sex chromosome constitution[21]. Testicular maldevelopment can be found in association with Klinefelter syndrome. Males with the latter genetic abnormality (XXY) usually have small testes and azoospermia[22].

Ceylan et al [6](2009) reported that the prevalence of KS among infertile men is very high, up to 3.3% in severe oligozoospermia and 26.7% in azoospermia.

Ambasudhan et al [2] (2003) studied 180 azoospermic / oligospermic patients and found out 6 (3.33%) patients with 47, XXY.

CuneytTuzun et al [8] (1998) studied 50 men due to azoospermia or severe oligospermia. He found 4(8%) patients with chromosome abnormalities. Among the 4 patients with abnormal chromosome, 3 patients showed 47XXY and 1 patients showed 46XY,t(1:7)(p32:o32) karyotype.

Orsolya B. et al [17] (2006) studied 53 non-obstructive azoospermic patients. He found 47XXY karyotype in 3(5.67%) patients.

Zhang Z.B. et al [28] (2012) studied 81 non-obstructive azoospermic men. He found 16(19.75%) men to have Klinefelter Syndrome with 47,XXY karyotype.

In the present study, 3(12%) patients showed a karyotype of 47XXY among 25 azoospermic patients.

Thus present study correlates with the finding of Ambasudhan et al, CuneytTuzun et al, OrsolyaBellovits et al & Zhang Z.B. et al

SayeeRajangam et al [20] (2006) found 2 patients (2.73%) with a mosaic pattern.

Ambasudhan et al [2] (2003) found 2 mosaics (1.11%).

In the present study, 46,XY/47,XXY mosaic Klinefelter was seen in 2 (4%) patient. Among these two mosaicKlinefelter, 1 patient had azoospermia and 1 patient had severe oligospermia. Thus the present study correlates with the findings of Ambasudhan et al (2003) and SayeeRajangam et al (2006).

The 46,XX maleness is characterized by testicular development despite the lack of normal Y chromosome. The frequency of XX males in the general population is very low (1 in 10,000) whereas they are found more frequently in azoospermic men [21].

Nishino et al [16] (1993) studied a 24-year-old infertile male. Semen analysis revealed azoospermia. Endocrinological examination showed elevated serum LH and FSH and low level of serum testosterone. Testicular biopsy disclosed atrophic seminiferous tubules. Abdominal computed tomography revealed no ovaries or uterus. The chromosomal analysis revealed a karyotype of 46XX. This case was diagnosed as a case of 46XX male.

Yumura Y. et al [27] (2003) reported 2 cases of XX male with chief complaint of infertility.

Yencilek F. et al [25] (2005) studied a 26 year old infertile male. He had normal external male genital phenotype and secondary sex characters. No gynecomastia was noted. At physical examination soft &atropic testes were palpated. Laboratory analysis and testes biopsy indicated non-obstructiveazoospermia. Chromosomal analysis showed 46XX karyotype.

In the present study 2(4%) patients were found with a 46,XX karyotype. In both these patients seminal studies showed azoospermia.

Thus the findings in present study correlate with the findings of Nishino et al, Yumura Y et al and Yencilek F et al.

Isochromosome is the resultant of an abnormal split of the centromere (horizontal instead of vertical) followed by duplication of one of the arm.

Badovinac et al [3] (2000) studied 782 patients with fertility problems. On chromosomal analysis, he found 2 patients with 46, X,i(Xq)/45,X karyotype.

Sayee et al[20] (2007) found 1 patient with 45,X/46,X,i(Xq) among 83 chromosomally abnormal patients.

In present study, one patient showed a karyotype 47,X,i(Xq)Y. Thus the chromosome complement revealed an isochromosome involving ‘q’ arm of ‘X’ chromosome. The semen analysis of the patient with 47, X,i(Xq) Y showed azoospermia.

In infertile males, translocations are reported in 1.2% cases. These may be Robertsonian (0.7%) or Reciprocal (0.5%). Robertsonian translocations are frequently observed in oligospermic patients (1.6%). Also 0.9% reciprocal translocations are found in azoospermic and 0.8% in oligospermic men [21].

Forejt[10] (1974) suggested that non-random association might produce interference with precocious X chromosome inactivation in the primary spermatocytes which would be required for normal spermatogenesis.

Yoshida et al [26] (1997) studied 1007 males with infertility and found out 18 (1.79%) patients with translocations.

Baschat et al [4] (1996) studied 32 patients of male infertilitry and found 2 (6.25%) patients with a translocation.

Haidl et al [12] (2000) studied 305 infertile males and found 10 (3.27%) patients with translocation.

Carp et al [5](2004) studied 458 males referred for infertility. Translocation was observed in 21 (4.58%) patients.

Quilter et al [19] (2005) found 2 (1.94%) patients of Robertsonian translocation in 103 patients.

Sayee et al [20] (2006) found 2 (2.73%) patients of reciprocal translocation among 73 infertile males.

In the present study, translocation 45, XY, -22 t (14/22) was found in 1 (2%) patient. The other significant features which were of importance in this patient were the raised FSH & LH levels, reduced testosterone and azoospermia.

Thus the present study correlates with the findings of Yoshida et al (1997), Haidl et al (2000), Quilter et al (2005) and Sayee et al (2006).

Paracentric and pericentric inversions are often reported in infertile males. Inversions of chromosome 1-3, 5-7 and 9 have been reported[26]. Chandley et al studied patients with inversion in chromosome 1 and found out extensive disturbance of synapses across the inverted region at metaphase I resulting in a loop formation. The infertility effects of chromosome I inversion could be due to germ cell maturation impairment because of the failure of synapses.

Yoshida et al [26] (1997) studied 1007 patients and found 5 patients (0.49%) with inversion.

Carp et al [5](2004) investigated 458 patients of male infertility and found 20 (4.36%) patients with inversions.

Quilter et al [19] (2005) studied 103 infertile males. They found inversion in 2 (1.94%) patients.

In the present study inversion was found in 1 patient (2%). The karyotype was 46, XY, inv (9). This patient had severe oligospermia.

Thus the present study correlates with the findings of Yoshida (1997), Quilter et al (2005).

Structural abnormalities involving Y chromosome are found to be higher in infertile males and more so in azoospermic males. Structural abnormalities like dicentric Y, a ring Y chromosome and the pericentric inversion of the Y chromosome are associated with spermatogenic failure.

Ismail et al [14] (1993) studied 100 infertile males and found out 10% males with large Y. They suggested that such Y chromosome abnormalities were frequent among azoospermic than oligospermic males.

In a study conducted by SayeeRajangam et al [20] (2006), they found out 1 patient with large Y out of 73 patients.

In the present study 1 patient showed a karyotype 46,XY with large Y. In this patient semen analysis was found to oligospermia. This study correlates with the study conducted by Ismail et al and SayeeRajangam et al.

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