Original Article

Cytogenetic abnormalities in 222 infertile men with azoospermia and oligospermia in Iran: Report and review

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BACKGROUND: Infertility affects approximately 10%-15% of couples in reproductive age. In half of the couples, causes are male-related, associated with impaired spermatogenesis. There is a complex correlation between genetics and infertility. Several factors affect on gametogenesis, from which factors that lead to chromosomal abnormalities are one of the best known. The aim of this study was to determine type and rate of chromosomal abnormalities in infertile azoospermic and oligospermic males in Iranian population.
MATERIALS AND METHODS: The records of a total of 222 participants were evaluated retrospectively.
RESULTS: As a whole we observed 13.96% chromosomal abnormality, from which 12.15% showed numerical and 1.8% showed structural abnormalities.
CONCLUSION: Comparison of our results with the review of the literature shows a higher incidence (4-fold) of gonosomal, in particular, numerical gonosomal, chromosomal anomalies. Cytogenetic analysis is strongly suggested for infertile men, particularly in those who suffer from azoospermia.

Keywords: Chromosomal abnormality, male infertility, Iranian population

Introduction

Infertility is a common clinical problem. It affects approximately 10%-15% of couples in reproductive age.[1] The prevalence varies widely, being less in developed countries and more in developing countries where limited resources for investigation and treatment are available.[2] 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.[3]

A major area of cytogenetic investigation has been on the nature and frequency of chromosome anomalies associated with azoo- or oligospermia in infertile men. In this study, we report the occurrence of chromosomal aberrations and chromosomal variants by high-resolution banding method in 222 infertile azoo- and oligospermic men.

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Materials and Methods

The study was conducted on 222 patients with infertility problems who referred for cytogenetic investigation. This project was approved by the Tehran University of Medical Sciences (TUMS) ethics committee. The referral centers were IVF wards of Mirza Kouchak Khan and Shariati

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Hospital and Sarem Medical Center. The criterion for infertility was failure of a couple to conceive after 1 year of regular unprotected intercourse. The patients mean age was 34.37 years, and cases were between 20 and 53 years old. Cases that underwent a detailed physical examination and paraclinical investigation (spermiogram, hormonal tests, sonography, and testis biopsy) were recruited into the study. The aims and objectives of the study were explained to the patients who signed written consent. Peripheral blood in 7ml sodium heparin collection tubes were taken from each patient. 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. Azoospermia group involved 132 cases, and oligospermia group involved 90 cases. Except for 4 cases with secondary infertility, the others had primary infertility.

Chromosomal analysis was carried out from cultures of peripheral blood lymphocytes by high-resolution banding (Thymidine method) according to Rooney *et al.*[4] with minor modifications. The chromosomal aberrations were recorded following the ISCN 2009 guideline.[5] At least 10 well-spread metaphases were analyzed by G-banding, and when required, C-banding (10 cells), Q-banding staining, and NOR were carried out.

Results

The chromosome abnormality rate was 13.96%; the numerical type 12.15% and structural type 1.8% [Table 1]. 27 out of 132 (20.4%) azoospermic males, showed chromosomal alterations, consisting of 25 (18.9%) and 2 (1.5%) numerical and structural abnormality, respectively. Among the 90 oligospermic males, both kinds of chromosome abnormalities were seen in equal number of 2 patients (2.2%). Most of the numerically abnormal patients had classic Klinefelter karyotype (9.45%) [Figure 1]. There were 6 mosaic cases involving X and Y chromosomes (3%). 3 (1.35%) had *47,XXY/46,XY* karyotype; whereas each of the karyotypes, *46,XY/46,XX* and *47,XXY/46,XX* was seen in only 1 patient (0.45%).

1 patient had a deleted *Yq(46,XY,del(Y)(q11.2-q11.2)*, and 2 others were balanced translocation carriers with *46,XY,t(Y;4)(q11.2; p14)* and *46,XY,t(X;14)* chromosome complements. The last patient with structural aberration had a pericentric inversion involving chromosome 1 with *46,XY, inv(1)(p22;q24)* karyotype.

Discussion

In this study as a whole, we observed 13.96% chromosomal abnormality. The prevalence of

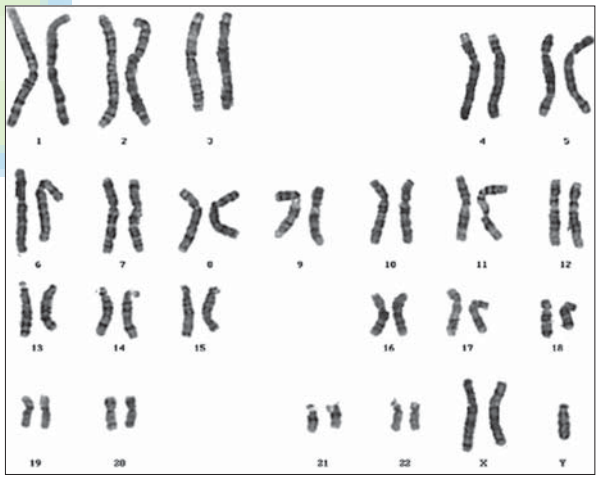

Figure 1: Klinefelter karyotype

Table 1: Chromosomal abnormalities among 222 infertile men

Karyotype	Azoospermia (n/%)	Oligospermia (n/%)	Total (n/%)
47,XXY	21(15.9)		21(9.45)
46,XY/47,XXY	2(1.5)	1(1.1)	3(1.35)
47,XXY/46,XX	1(0.75)		1(0.45)
46,XY/45,X	1(0.75)		1(0.45)
46,XY/46,XX		1(1.1)	1(0.45)
46,XY, t(X;14)(q28;24)	1(0.75)		1(0.45)
46,XY,t(Y;4)(q11.2; p14)		1(1.1)	1(0.45)
46,XY,del(Y)(q11.2-q11.2)		1(1.1)	1(0.45)
46,XY, inv(1)(p22;q24)	1(0.75)		1(0.45)
Total	27(20.4)	4(4.4)	31(13.96)

chromosome abnormality is higher in infertile men than in normal ones, and it is well-known that the sperm count is inversely related to the existence of chromosomal anomaly.[6] Evaluation of 22 similar studies from the literature including a total of 10,408 cases showed 6.90% chromosomal anomaly rate [Table 2].[7-27] In our study, 13.96% of all cases revealed chromosomal alteration. Chromosomal abnormalities are more frequently observed in the population of azoo-and/or oligozoospermic males than in the general population.[28] In our study, the highest frequency of abnormal karyotype was among patients with azoospermia (20%) as compared to the oligospermic subgroup. PY Ng *et al.* reported that the incidence of sex chromosome abnormalities in azoospermia group was higher than that in the oligospermia group.[26] In the present study, chromosomal abnormalities were detected in 20.4% of 132 azoospermic cases and 4.4% of 90 oligozoospermic cases. The most common type of karyotype abnormality in infertile cases is represented by Klinefelter's syndrome (KS). The incidence of KS was 9.45% in our study, which is similar to other studies. KS is the most common abnormality of sexual differentiation, and occurs in approximately 1 in 1000 live births.[29] KS is a form of primary testicular failure with testicular hypotrophy and elevated gonadotropin plasma levels, and it represents the most common form of male hypogonadism.[30] Ceylan *et al.* reported that the prevalence of KS among infertile men is very high, up to 3.3% in severe oligozoospermia and 26.7% in azoospermia.[25] It has been always assumed that more than 90% of non-mosaic *47,XXY* males are azoospermic.[31] In our study, we detected that 21 cases had non-mosaic *47,XXY* karyotype that all of them belonged to azoospermic subgroup.

Pericentric inversion is one type of chromosomal rearrangement, which has been categorized as a minor chromosomal rearrangement, not expected to associate with abnormal phenotype.[6] However, pericentric inv (1) is a different situation associated with infertility. Chandley *et al.* in their case report study of an oligospermic male with pericentric inv (1) in Pachytene analysis in microspread preparations showed an absence of full loop formation in the inversion bivalent and only the rare occurrence of a partial loop. The majority of the cells exhibited extensive asynapsis across the inverted segment, or a normal looking synaptonemal complex indicative of heterologous pairing along the length of the inversion. Crossing over has been reduced in the No 1

Table 2: Review of the literature in male infertility

Author	Year	No. of cases	Gonosomal chromosome abnormality		Autosomal chromosome abnormality	Total (n/%)
			Numerical (n/%)	Structural (n/%)		
Mau *et al.*	1997	150	6 (4.0)	-	12 (8.0)	18 (12.0)
Tuerlings *et al.*	1998	1792	24 (1.3)	6 (0.3)	42 (2.3)	72 (4.0)
Gunduz *et al.*	1998	102	13 (12.7)	-	3 (2.9)	16 (15.6)
Meschede *et al.*	1998	432	2 (0.4)	-	7 (1.6)	9 (2.1)
Pina-Neto *et al.*	2006	165	10 (6.0)	3 (1.81)	3 (1.81)	16 (9.6)
Peschka *et al.*	1999	781	7 (0.8)	4 (0.5)	19 (2.4)	30 (3.8)
Vutyavanich *et al.*	2007	130	4 (3.07)	-	2 (1.53)	6 (4.6)
Nakamura *et al.*	2001	1790	80 (4.4)	19 (1.0)	126 (7.0)	225 (12.6)
Dohle *et al.*	2002	150	8 (5.3)	1 (0.6)	7 (4.6)	16 (10.6)
Morel *et al.*	2004	335	2 (0.5)	-	-	9 (2.6)
Rao *et al.*	2004	251	8 (3.1)	2 (0.7)	18 (7.1)	28 (11.1)
Clementini *et al.*	2005	2078	6 (0.2)	36 (1.7)	-	42 (2.0)
Balkan *et al.*	2008	80	7 (8.75)	-	2 (2.5)	9 (11.25)
Martinez Garza *et al.*	2008	82	6 (7.31)	1 (1.21)	2 (2.43)	9 (10.97)
Nagvenkar *et al.*	2005	88	2 (2.2)	3 (3.4)	5 (5.6)	10 (11.3)
Samli *et al.*	2006	819	36 (4.3)	14 (1.7)	17 (2.0)	67 (8.1)
Meza-Espinoza *et al.*	2006	227	36 (15.0)	5 (2.0)	2 (0.8)	43 (18.9)
Mohammed *et al.*	2007	289	19 (6.5)	3 (1.0)	1 (0.3)	23 (8.0)
Ceylan *et al.*	2009	60	10 (16.6)	4 (6.66)	-	14 (23.26)
PY Ng *et al.*	2009	295	10 (3.38)	7 (2.37)	8 (2.71)	25 (8.47)
Akgul *et al.*	2009	179	16 (8.93)	2 (1.11)	3 (1.68)	21 (11.74)
Kleiman *et al.*	1999	133	5 (3.7)	3 (2.25)	3 (2.25)	11 (8.27)
Total		10408	317 (3.04)	113 (1.08)	282 (2.70)	719 (6.90)
Our survey	1998	222	27 (12.16)	3 (1.35)	1 (0.45)	31 (13.96)

bivalent with only a rare chiasma was seen in the inverted region at metaphase I. Therefore, the cause of infertility is impaired spermatogenesis regardless of breakpoint positioning.[32] In this study, the patient with pericentric inv (1) was in azoospermic subgroup.

Fertility effects of a balanced X-autosome translocation vary depending on the sex of the carrier.[33] In female carriers, gonadal dysgenesis may occur, and ~9% may have multiple anomalies and/or mental retardation.[34] In male carriers, azoospermia is the most common finding, although a few cases have been reported with severe oligozoospermia.[35] The cause of the spermatogenic failure in carriers of an X-autosome translocation is unknown, but spermatogenesis generally is much more sensitive to meiotic disruption than oogenesis due to a number of meiotic cell cycle checkpoints.[36] Rao *et al.*, in their survey, reported an azoospermic patient with *46,XY t(X;15)(q28;q22)* karyotype.[17] We also detected 1 case with *46,XY, t(X;14)(q28;q24)* karyotype from azoospermic subgroup.

Y chromosome is necessary for male development due to its gene content. SRY and AZFs are also very important. Chromosomal translocations between Y and autosomes are categorized in 3 groups: (1) Involved autosome is an acrocentric and mostly are not problematic, because break point is within the heterochromatin region. (2) Translocations between Y and non-acrocentric autosomes are rare and classically *de novo*. They usually lead to abnormal phenotypes such as infertility and hypogonadism. (3) The last group is completely rare, and their characteristic is transfer of TDR to an autosome. Kleiman *et al.* reported a patient with *46,XY, t(Y,4)(q11;q12)* karyotype in azoospermic males.[27] In our study, 1 case showed *46,XY, t(Y;4)(q11.2; p14)* karyotype who belonged to oligospermic subgroup. In microscopic analysis, it seems that it is a balanced rearrangement; therefore, impaired spermatogenesis is probably due to an inappropriate formation of sex- vesicle and an incomplete formation of X-Y synapses. Because the source of this disorder is the inheritance of a gamete with chromosomal defect from one parent, its occurrence in other family members is possible. Thus, genetic counseling is necessary in these patients.

Kleiman *et al* reported that Cytogenetic analysis of infertile men revealed that 0.5% had macroscopic deletions of the distal long arm of the Y chromosome (Yq).[27] Infertile men with deletion in proximal region of Yq11 have sertoli cell-only syndrome. Those who have deletion in middle of Yq11 show stopped spermatogenesis and males with deletion in distal part manifest severe oligospermia. In this study, 1 severe oligospermic male (0.45% of total) showed *del(Y)(q11.2-q11.2)* karyotype. According to his phenotype and the results of testis biopsy, it seems that he belongs to the latter group. In appearance, he was tall with completely defective teeth. It can partly be due to GCY (Growth Control Y chromosome Influence) gene, which is located in Yq11 and is involved in height and tooth size determination.

In the present study, chromosomal abnormalities in mosaic form were also observed, which consisted of *47,XXY/46,XX; 46,XY/47,XXY; 46,XY/45,X* in azoospermic subgroup and *46,XY/47,XXY and 46,XX /46,XY* in oligospermia cases.

The results of other studies in the literature revealed a mean of 2.70% autosomal and 4.12% gonosomal chromosomal anomaly rate [Table 2]. In our study, those values were 0.45% and 13.51%, respectively. In other surveys among gonosomal chromosomal anomalies, 1.08% was structural and 3.04% was numerical, whereas those values were 1.35% and 12.16% in our study. Comparison of our results with the review of the literature shows a higher incidence (4-fold) of gonosomal, in particular, numerical gonosomal, chromosomal anomalies. In Table 2, in studies with a large sample size but low incidence of total chromosomal abnormality,[10,12,18] patients are mostly oligospermic rather than azoospermic, but in surveys with small sample size[25] or a sample size similar to our study,[23] which showed high incidence of total chromosomal abnormality, cases are totally azoospermic or have the same number with oligospermia cases. On the other hand, Morel *et al.*,[16] in their study in the review of the literature, reported that frequency of KS in azoospermia (10.17) is about 19-fold more frequent than in oligospermia (0.57).

In our study, most of the patients belonged to azoospermia subgroup and KS was the most frequent chromosomal abnormality (9.45% of 13.96%), which just observed in azoospermia subgroup. Thus, it seems that high incidence of chromosomal abnormality (13.96%) in our study is due to the number of azoospermic cases.

In conclusion, the results of this study and the review of the literature showed that chromosomal aberrations occur frequently in infertile men, which emphasize the importance of cytogenetic investigation and the relevance of its findings in the patient's management in the fertility clinics.

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