

Chromosomal Abnormalities in Infertile Men Referred to Iran Blood Transfusion Organization Research Center

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Abstract

Introduction: The prevalence of somatic chromosomal abnormalities in infertile male individuals has been reported to vary in different literatures. The aim of this study was to investigate the frequency of chromosomal aberrations among infertile men referred to the Cytogenetic Laboratory of Iran Blood Transfusion Organization Research Centre (IBTO).

Materials and Methods: Chromosomal analysis was performed on phytohemagglutinin (PHA)-stimulated peripheral lymphocyte cultures of 1052 infertile men using standard cytogenetic methods. The study took place during 1997 to 2007.

Results: Total chromosome alterations were revealed in 161 (15.30%) infertile men. The most prevalent chromosomal abnormality in the infertile men was 47, XXY, that was seen in 94 (58.38%) men while one of them had a mosaic karyotype: mos 47, XX[54]/47,XXY[18]/46,XY[9]. In 37 (22.98%) cases, structural aberrations were detected. There were 30 (18.63%) cases of sex reversal.

Conclusion: Cytogenetic studies of these patients showed increased chromosomal abnormalities in infertile men in comparison with that of the normal population, justifying the need for cytogenetic analysis of men with idiopathic infertility.

Keywords: Chromosomal abnormalities, Cytogenetic, Infertility, Klinefelter's syndrome, Male infertility, Sex reversal.

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Introduction

Infertility is a significant problem, affecting up to 15% of couples of reproductive age (1). The genetic causes of infertility can be divided into Y chromosome deletions (specifically deletions in the AZF a, b, or c regions), single gene disorders (particularly those relating to the CFTR gene), multifactorial causes and autosomal chromosome abnormalities.

The prevalence of somatic chromosomal anomalies in the infertile male population has been reported to be varied in different populations and studies (1-3). But the frequency of chromosomal

abnormalities increases as the sperm concentration in the ejaculate decreases (1).

In patients with sperm counts below 10 million/ml, this rate is estimated to be 5 - 7%, with the percentage of cytogenetically abnormal causes rising up to 10 - 15% in azoospermic men (2).

In Iran, like many other countries, there is a considerable number of infertile men. However, because of the high cost of diagnostic procedures and lack of equitable access to advanced diagnostic facilities many of these men are not referred for a genetic evaluation.

On the other hand, because of the recent advances in assisted reproductive techniques such as intracytoplasmic sperm injection (ICSI), there is always the risk of transmitting chromosomal aberrations to the offsprings. Therefore, we aimed to evaluate the prevalence of chromosomal aberrations in infertile men in order to justify the inclusion of karyotyping in the work-up of every man with idiopathic infertility.

Materials and Methods

This study was performed on 1052 infertile men who were referred to the Cytogenetic Laboratory of Iran Blood Transfusion Organization Research Centre (IBTO) for chromosomal work-up during 1997 to 2007. The patients included infertile men with azoospermia, oligospermia and men with normal sperm counts but with idiopathic infertility.

Cytogenetic Analysis: Chromosomal analysis was performed on phytohemagglutinin (PHA)-stimulated peripheral lymphocyte cultures using standard cytogenetic methods (4, 5). Briefly, blood cells were cultured in RPMI 1640 (GIBCO, USA), supplemented by 20% (v/v) fetal bovine serum (GIBCO, USA) and 10 µg/ml phytohaemagglutinin (GIBCO, USA) at 37 °C. After 68 - 72 hours, colcemid (Sigma, USA) was added to the cells. The cells were incubated at 37 °C for about 10 mins. The suspension was centrifuged and the pellets were resuspended in 5 - 10 ml KCl (0.075 M) for about 20 mins at 37 °C. After centrifugation, the cells were resuspended in a fixative [methanol: acetic acid, 3:1 (Merck, USA)]. The fixative was replaced for a minimum of three times. Using a Pasteur pipette, drops of the solution were poured onto a slide for morphological studies. The chromosomes were viewed under a phase-contrast microscope to assess metaphase quality and the nuclei. The chromosomes were GTG-banded after aging.

Thirty metaphases were analyzed per individual and in suspected cases of mosaicism; the number of analyzed metaphases were increased to 100. A 400-band stage resolution was applied for the minimum and a 550 -700-band stage for a more detailed structural analysis.

Results

Cytogenetic analysis was carried out in 1052 patients out of whom 161 (15.30%) cases had abnormal karyotypes. The most frequent abnormality was Klinefelter's syndrome (47, XXY) which was detected in 94 (58.38%) patients. One of the individuals with the syndrome had a mosaic karyotype: mos 47,XX[54]/47,XXY[18]/46,XY [9].

Twenty-seven cases were found to have 47,XXY karyotype among a total of 85 cases with azoospermia and one case of oligospermia, all of whom had been referred to the laboratory as suspected cases of Klinefelter's syndrome.

The second cause of infertility was structural aberrations in 37 (22.98%) cases_ balanced translocation being the most frequent chromosomal abnormality_ followed by deletions in Y chromosome (q11.1) (Table 1).

The most frequent structural chromosomal rearrangement was found among patients with

Table 1. The structural chromosomal abnormalities detected among infertile men attending IBTO.

Karyotypes	n
46,X;del(Y)(q11.23)	3
46,X;del(Y)(q11.22)	3
46,X;del(Y)(q11.1)	2
46,XY;del(5)(p15.3)[8]/46,XY[116]	1
46,XY;t(2;12)(q31;q15)	2
46,XY;t(12;22)(q11;p11.2)	1
46,XY;t(2;6)(q37.3;p21.3)	3
46,XY;t(1;3;9;7)(q21;q26.2;q34;q11.2)	2
46,XY;t(1;21)(q21;p22.3)	2
46,XX;t(1;18)(q32;q23)	1
46,XY;t(14;17)(q32.3;q11.1)	1
46,XY;t(16;17)(q32.3;q11.1)	1
46,XY;t(5;12)(p14;q21.2)	1
46,XY;t(3;19)(p21;q13.1)	1
46,XY;t(9;15)(p24;q13)	1
46,XY;t(1;22)(p34.1;q24.1)	2
46,X;add(Y)(P?)	1
46,XY;add(22)(p?)	1
46,XY;inv(Y)(p11.2;q11.2)	6
46,XY;inv(12)(p13.3;q13.1)	1
46,XY;Fra(16)(q22)[16]/46,XY;del(16)(q22)[3]/46,XY[55]	1
Total	37

azoospermia and patients who had been referred to the Center for infertility work-up (14 and 12 cases, respectively).

The second most prevalent abnormality was sex reversal in 30 (18.63%) patients.

Discussion

The percentage of chromosomal abnormalities among infertile men varies in different studies. For example, in a cytogenetic study on a large Dutch infertile population (1792 infertile men) as low as 72 cases (4%) with chromosomal abnormalities were reported and Klinefelter's syndrome (either pure or mosaic form) was found in just 12 patients (0.6%) (6). In a study including 150 infertile couples in Germany, 13% had chromosomal anomalies and four (2.6%) patients had Klinefelter's syndrome (7). In another study in Germany on 781 infertile couples, the rate of chromosomal aberrations was only 1.9% for the male subjects including sex chromosome abnormalities (0.6%) (8). Low rates of chromosomal abnormality among European countries could be due to fair and easy access of patients to clinical genetics laboratories.

In a study done in China, chromosomal abnormalities were found in 102 (20.86%) out of 489 patients with azoospermia, among them 86 (84.31%) suffered from sex chromosome abnormalities and 73 (14.9%) from Klinefelter's syndrome (9). Nearly similar results were obtained in a study done on subfertile men in Hong Kong, China. The corresponding figure for chromosomal anomalies in the groups with non-obstructive azoospermia, very severe oligospermia and severe oligospermia was 21.1% (10).

There are also a few published articles regarding cytogenetic studies in non-European countries all showing rather high rates of chromosomal abnormalities among infertile populations. For example, in a study on 179 men with infertility in Turkey, 21 (11.49%) cases had chromosomal abnormalities, including 13 (7.2%) cases with Klinefelter's syndrome (9). In another study on 102 infertile men in Turkey, 16 (15.7%) cases had chromosomal abnormalities (11). Among 1000 infertile men from India, about 14% had a chromosomal abnormality (12) which is very close to the findings of the present study.

In general, the findings of the present study are in accordance with most investigations from non-EU countries, which confirm the XXY aneuploidy to be the most frequent chromosomal abnormality in infertile men. The prevalence of sex chromosome anomalies showed a significant difference between the cases with azoospermia and oligospermia (85 vs. 1 case, respectively).

Nowadays, it is possible to conceive pregnancy by the use of ICSI, from even male patients with Klinefelter's syndrome; Tachdjian et al. reported 36 successful pregnancies in men with Klinefelter's syndrome. The resultant 36 pregnancies produced 32 karyotypically normal neonates (13). Therefore, diagnosis of cases with Klinefelter's syndrome, especially in younger ages, could be helpful and provide the chance to freeze some of their sperm for later use in assisted reproductive techniques.

Male infertility and chromosomal anomalies are often closely related and reciprocal translocations are the most frequent (1 in 600) structural chromosomal anomalies in humans, (14). Chromosomal reorganizations are about 10 times more frequent among infertile men in comparison with the general population (15). In the present study, we found balanced translocation to be the most frequent structural aberration among these patients. While pregnancy is possible for this group of cases, prenatal diagnosis for the resultant embryos is highly recommended as translocation carriers are at a high risk for producing offspring with partial trisomy/ monosomy of the relevant chromosomal fragments.

The high rates of chromosomal anomalies among infertile men, strongly suggests the need for routine cytogenetic analysis prior to implementation of assisted reproduction techniques. The present results alongside those in previous studies stress the importance of cytogenetic evaluations, especially karyotyping, in both male and female partners before ICSI is tried. Genetic counseling, possibly followed by prenatal diagnosis, should be offered if a chromosomal anomaly is detected in infertile couples.

This is one of the few studies on a large population of infertile men and may be the largest study on Iranian patients in this regard, and hence may present the prevalence of chromosomal abnormalities in the Iranian infertile men.

Conclusion

This 10-year-old cytogenetic study on infertile men confirmed previous reports that chromosomal abnormalities are one of the possible causes of male infertility and a risk for transmission of chromosomal abnormalities to their offspring. Therefore, the need for karyotyping in cases with idiopathic infertility, especially prior to use of any assisted reproductive techniques, seems to be justified.

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