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Full Length Research Paper

Cytogenetic studies among Iranian infertile men: The first 20-year long-term report

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Chromosomal aberrations have been postulated to be one of the principal genetic factors in male infertility and occur in about 2 to 3% of unselected patients with proven sub-fertility. This rate is estimated to be 5 to 7% in patients with oligospermia, increasing to 10 to 15% in patients with azoospermia. The aim of this study was to report the frequency of all chromosomal aberrations among Iranian infertile men. In this 20-year retrospective study, we investigated 829 men which were referred to our department due to infertility. Karyotyping was performed on peripheral blood lymphocytes according to standard methods. Out of 829 patients, 557 patients (67.19%) had normal karyotype and 272 patients (32.81%) showed abnormal chromosomes. Klineferlter syndrome, found in 195 patients (23.52%), was the most frequent aberration in our study. The remaining 77 cases (9.29%) showed a variety of abnormal karyotypes.

Key words: Karyotyping, chromosome abnormality, male infertility.

INTRODUCTION

Infertility and childlessness is a major health and family problem in the world especially in the third world countries. It is a distressing condition that adds to the psychological trauma of majority of couples, which some-times causes separation. It has been suggested that infertility affects about 15 to 18% of all couples attempting pregnancy, with male-factor identified in about half of the cases (Bhasin et al., 1994; de Kretser, 1997; McLachlan and de Kretser, 2001; Mau-Holzmann, 2005; Nagvenkar et al., 2005; Pasinska et al., 2006).

The women were thought to be the main cause of the childless partnerships in the past, but recent investigations showed that in infertile couples, the male and female can equally be responsible (each 40%) and both of them can be responsible (20%) (Foresta et al., 2001; Huynh et al., 2002; Zhang and Lu, 2004).

Numerous factors contribute to male infertility; genetic factors include chromosomal aberrations and genetic syndromes cause gene defects (Gorduza et al., 2003; Quilter, 2005). Other factors included cryptorchidism, testicular trauma, systemic diseases, systemic infections, hormonal milieu, genital injury, genital infections, spermatic duct obstruction, chemical and physical agents, varicocele, retrograde ejaculation, antisperm antibodies, and testicular cancer. Apart from these, in 30 to 40% of male infertile cases that are referred to as idiopathic, a genetic abnormality is suspected (Griffin and Finch, 2005).

A marked decline in male reproductive relevance and an increase in sub-fertile males have been demonstrated worldwide. Both genetic and environmental factors are considered to be responsible for this decline (Dada et al., 2004). The cause of male infertility is unknown in more than 50% of cases. A few research investigations have focused on the possible genetic etiologies (Foresta et al., 2001; Dada et al., 2004). Male infertility has been linked with numerous parameters including sperm number, motility and morphology. It has been demonstrated that 5 to 7% of patients with sperm count below 10 millions/ml have chromosomal aberrations, increasing to 10 to 15% in patients with azoospermia (Retief et al., 1984; Ravel et al., 2006). In about 60% of infertile men, no cause is found for low sperm counts or inadequate production of sperm with normal motility, morphology and function

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(McLachlan and de Kretser, 2001).

There are numerous animal models of single-gene defects associated with specific impairment of spermatogenesis, and, although such evidence is lacking in humans, it seems extremely likely that single-gene and polygene defects will be found to be an important cause of infertility (MaLachlan and de Kretser, 2001). An autosomal recessive pattern of transmission is a possibility in families with a history of involuntary infertility (Lilford et al., 1994). It is a known fact that in a range of causes of male infertility, chromosome aberrations with 10 to 15% are one of the major ones (Penna Videau et al, 2001; Patsalis et al, 2002). Five percent (5%) of these are numerical or structural chromosomal abnormalities. About 80 to 85% of these cases are due to sex chromosome anomalies (Zhou et al., 2009), and about 2% are mosaics with autosomal abnormalities (Siffroi et al., 2000; Visootsak et al., 2001; Huynh et al., 2002). It has been revealed that between 10 to 20% of infertile men have microdeletions in the euchromatic region of the long arm of the chromosome Y. These are three regions on the chromosome Yq11 and are called azoospermia factors a, b and c (Siffroi et al., 2000; Silber and Repping, 2002; Patsalis et al., 2002; Vicdan et al., 2004; Marchina et al., 2007; Balkan et al., 2008). Kalantari et al. (2003) suggested that “variants of the Y chromosome” have no influence on the sperm count and fertility in men.

In addition to the recognizable structural abnormalities of the sex chromosomes and autosomal chromosomes, specific gene mutations and nonspecific genetic syndromes may be associated with male infertility. Although the exact mechanism is not clear, it has been suggested that a wide variety of chromosomal anomalies exert an adverse effect on spermatogenesis, resulting in azoospermia or severely impaired semen parameters (Tuzun et al., 2008).

The aim of this retrospective study was to ascertain chromosomal aberrations in Iranian males who have been referred to our department, due to infertility, for a period of 20 years.

MATERIALS AND METHODS

Our survey included 829 men with diagnosis of infertility that were referred to the Genetics Group, Cancer Institute of Iran, over a period of 20 years (from 1986 to 2005).

Our cases were initially admitted to Infertility Clinics and were physically examined carefully by urologists, endocrinologists, and immunologists; all necessary laboratory tests such as semen analysis, hormonal screening, and sonographies were carried out. Finally, those who passed this filter and were highly suspicious of having chromosome abnormalities were referred to us.

Chromosomal analysis was performed on phytohemagglutinin (PHA) stimulated peripheral lymphocyte cultures using standard techniques (Moorhead et al., 1960; Gosden et al., 1992; Barch et al., 1997). Chromosome staining and banding techniques were as described by de Grouchy and Turleau (1984) and Benn and Perle (1992). From each patient, 30 well-spread metaphases were analyzed by GTG-banding (Seabright, 1971), from two independent blood samples. In cases of suspected mosaicism, 200 cells were counted again from the two independent blood samples. If an abnormal karyotype was present, additional cytogenetic studies were carried out, using other procedures such as Q-banding (Casperson et al., 1970) and high resolution banding according to the method of Rybak et al. (1982). All karyotypes were interpreted in accordance with the recommendation of the International System for Human Cytogenetic Nomenclature (Mitelman, 1995; Shaffer et al., 2009).

RESULTS AND DISCUSSION

Out of 829 referred infertile men, 557 patients (67.19%) had normal karyotype, and 272 patients (32.81%) showed some kind of constitutional chromosome aberrations. Klinefelter syndrome, which was found in 195 patients (23.52%), was the most frequent anomaly in our study. The remaining 77 cases (9.29%) showed various abnormal karyotypes (Table 1). Klinefelter variant (48,XXYY) was seen in four patients (0.48%). Different mosaics of Klinefelter syndrome were seen in 25 patients (3.03%). Eight persons (0.96%) were observed to have different deletions and nine cases (1.08%) with translocations and isochromosomes. Five cases (0.60%) showed sex reversal with the 46,XX karyotype.

Male infertility may be caused by a variety of chromosomal abnormalities, including aberrations in the sex chromosomes and autosomes, gain or loss of an entire single or more chromosomes, resulting in aneuploidy or structural anomalies, as in balanced and unbalanced translocations. The frequency of abnormal karyotypes in various studies showed a wide range of 2.2 to 15.7% for infertile men (Yoshida et al., 1995; Gunduz et al., 1998; Tuerlings et al., 1998; Nakamura et al., 2001; Vincent et al., 2002; Duzcan et al., 2003; Clementini et al., 2005a, b; De Braekeleer et al., 2006; Salahshourifar et al., 2007; Mohammed et al., 2007; Vutyavanich et al., 2007; Balkan et al., 2008; Riccaboni et al., 2008; Akgul et al., 2009). Some authors who had investigated chromosomal anomalies, specifically among patients with severe oligospermia and azoospermia, have shown higher figures such as 20.86% (Zhou et al., 2009) and 21.1% (Ng et al., 2009).

Sex chromosome aberrations are the most frequent chromosome related cause of infertility (Radojcic et al., 2000; Penna Videau et al., 2001; Rajangam et al., 2007). Klinefelter syndrome is the most common one and is associated with severe spermatogenic failure causing a

Table 1. Karyotypes of all referred infertile men.

Karyotype	Number	Percent
47,XXY	195	23.52
48,XXYY	4	0.48
mos48,XXYY/47,XXY/47,XYY	1	0.12
mos48,XXXY/47,XXY/46,XY	4	0.48
mos47,XXY/46,XY	9	1.10
mos45,X/46,XY	7	0.85
mos47,XXY/46,XX/46,XY	7	0.85
mos46,XX/46,XY	5	0.60
mos45,X/46,X,del(Y)(q10)	2	0.24
mos47,XXY/45,X/46,XY	4	0.48
mos46,XY,t(7;13)/46,XY	1	0.12
47,X,i(X)(q10)Y	3	0.36
46,X,add(Y)(p11.3)	7	0.85
46,X,del(Y)(q11)	6	0.72
46,X,r(Y)	2	0.24
47,X,t(X;Y)(q10;q10)	2	0.24
46,X,t(Y;14)	2	0.24
45,XY,t(14;22)	1	0.12
47,XYY	2	0.24
46,XX(sex reversal)	5	0.60
46,XY,inv(9)(p11q13)	3	0.36
Sub total	272	32.81
46,XY	557	67.19
Total	829	100

marked reduction in testicular size and azoospermia resulting in childlessness (Pandiyan and Jequier, 1996).

In the present study, 195 cases (23.52%) with classic Klinefelter syndrome, 25 patients (3.03%) with five various forms of mosaic Klinefelter syndrome, and four cases (0.48%) with 48,XXYY were the most prevalent abnormality as was seen in previous literatures (Kalantari et al., 2003; Foresta et al., 2005; Etem et al., 2009; Mahjoubi et al., 2010). Males with a 47,XYY karyotype are usually fertile, but there were a few reports of infertile patients with 47,XYY syndrome (Hens et al., 1988; Foresta et al., 2005; El-Dahtory and Elsheikha, 2009). We also had two cases (0.24%) with 47,XYY karyotpe among our sample.

Some researchers have reported male infertility among patients with deletion of chromosome Y (Siffroi et al., 2000; Salahshourifar et al., 2007; Rosenbusch, 2010), with ring chromosome Y (Tuzun et al., 2008), and with isochromosome X (Pasinska et al., 2006). Similarly, in this investigation, we found six patients (0.72%) with 46,X,del(Y)(q11), and two cases (0.24%) with mosaic del(Y); two patients (0.24%) with 46,X,r(Y); and three cases (0.36%) with 47,X,i(X)(q10)Y karyotypes.

Although, some authors have shown male infertility amongst patients with either mos46,XX/46,XY or mos45,X/46,XY (Clementini et al., 2005; Kayed et al., 2006; Mahjoubi et al., 2010), this study also showed five patients (0.60%) with mos46,XX/46,XY and seven cases (0.85%) with mos45,X/46,XY karyotypes.

In the present study, we found 5 males (0.60%) with 46,XX karyotype. The clinical manifestations of male patients with sex reversal syndrome are azoospermia associated with one or more of the short stature, gynecomastia, external genitalia, and pelvic cyst. Most sex reversal males originate from a crossing over between Xp and Yp during paternal meiosis, so that the SRY gene is translocated on the X chromosome. Therefore, these patients carry the SRY gene, but are azoospermic (Hackstein et al., 2000).

Inversion of chromosome 9 is commonly seen in normal humans and the frequency has been reported to be 1 to 3% in the general population, and some authors account the inversion 9 (inv(9)) as a normal variant (Nielsen and Wohlert, 1991; Teo et al., 1995; Rao et al., 2006; Meza-Espinoza et al., 2008). Capkova et al. (2004) investigated chromosomal abnormalities in couples with reproductive disorders, and showed that structural aberrations, including inversion 9, were more frequent among infertile couples. Some authors reported inv(9) among infertile men (Sasagawa et al.,1998; Davalos et al., 2000), suggesting that these inversions can have a role in the causation of infertility, especially in cases with

de novo inversions. Khaleghian and Azimi's suggestion (2006) further confirmed this. We also had three cases (0.36%) with 46,XY,inv(9) karyotpe among our sample. Several authors have reported male infertility among patients with reciprocal balanced autosomal translocation t(1;5), t(1;7) (Tuzun et al., 2008), t(1;19), t(15;16) (Pasinska et al. 2006), t(9;15), t(13;18) (Pandiyan and Jequier, 1996; Hellani et al., 2006; Martin, 2008; Rosenbusch, 2010), and also among male patients with Robertsonian translocation carrier (Roux et al., 2005), t(14;21) (Pasinska et al., 2006), and in t(15;15) (Etem et al., 2009). We found one case (0.12%) with t(14;22) and one mosaic patient (0.12%) with t(7;13). Some investigators have shown the reciprocal balanced translocation between autosomes and Y chromosome (Rosenbusch, 2010). We also found two cases (0.24%) with 46,X,t(Y;14), and two patients (0.24%) with 47,X,t(X;Y)(q10;q10).

Many researchers have reported a lower frequency of chromosome anomalies among the infertile men. In the present investigation, we found 32.81% of our referred male patients with chromosome abnormalities. This was much higher than other reports. The reason is that our patients were a highly selected group; our patients had passed through many filters. They were physically examined and tested by urologists, endocrinologists, immunologists, and all necessary laboratory tests such as semen analysis, hormonal screening, and sonographies were carried out on them. Through these analyses, if the diagnosis was chromosome abnormality, they were then referred to us.

Conclusion

Among many etiologic factors, genetic factors and chromosomal abnormalities play a primary role in male infertility. Although there are many microdeletions of chromosome Y and some other gene mutations which require molecular studies, along with Shah et al. (2003) and Tovar et al. (2009), our studies confirm that especially in the poor and undeveloped countries, which lack expensive molecular cytogenetic techniques, routine peripheral blood chromosome analysis remains the first choice in assessing the genetic characteristics of infertile males; this allows detecting numerous chromosomal aberrations. For instance, in Iran, up to now, FISH and molecular cytogenetic techniques are not available on routine basis, for general population, in public and university hospitals, all over the country, and patients have to go to private sector, which are very expensive.

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