J Assist Reprod Genet (2010) 27:17–21
DOI 10.1007/s10815-009-9366-y

GENETICS

Cytogenetic abnormalities detected in patients with non-obstructive azoospermia and severe oligozoospermia

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Received: 22 April 2009 / Accepted: 4 November 2009 / Published online: 9 January 2010

Abstract
Purpose To find the frequency and types of major chromosomal abnormalities with nonobstructive azoospermia and severe oligozoospermia to give appropriate genetic counseling before assisted reproduction techniques in Isparta (South of Turkey), and to investigate the general characteristics in this infertile male population.
Methods and patients A total of 115 infertile males (92 were azoospermic, 23 severe oligospermic) were studied for the cytogenetic evaluation prior to use of assisted reproduction techniques. Also, 60 fertile males as a control group were studied. Karyotyping was performed on peripheral blood lymphocytes according to the standard methods. Levels of luteinising hormone, follicle-stimulating hormone (FSH), testosterone and prolactin were obtained and a testicular sonography examination was conducted.
Results The total prevalence of chromosomal abnormalities was found to be 4.3% (5/115), including 4 patients with Klinefelter's Syndrome and 1 patient with gonadal dysygenesis (46XX). All of them were azoospermic males, corresponding to a frequency of 5.4% (5/92 patients). Oligozoospermic males and control males had no chromosomal abnormalities. There was a significant difference in serum FSH, LH, mean testicular volume and smoking when comparing patients (both azoospermic and oligozoospermic) and control groups ($p<0.05$). Also, there was a significant difference in serum FSH, LH and mean testicular volume when compared with azoospermic and oligozoospermic patients ($p<0.05$)
Conclusions The occurrence of chromosomal abnormalities among infertile males strongly suggests the need for routine genetic testing and counseling prior to the employment of assisted reproduction techniques.

Keywords Male infertility · Chromosome · Azoospermia · Oligozoospermia · Cytogenetic abnormality

Capsule A select population of infertile azoospermic males was found to exhibit a high incidence of chromosomal abnormalities relative to oligospermic and control males warranting genetic screening for this group of patients.

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Introduction

Chromosomal abnormalities are one of the most important causes of male infertility [1, 2]. The numerical and structural chromosomal abnormalities are seen frequently in azoospermia and oligospermia cases with unknown etiology [3]. In the cytogenetic analysis of 94,465 newborn male infants, the chromosomal abnormality ratio is reported as 0.38% (0.14% gonosomal, 0.25% autosomal) [4]. It could be approximated throughout the literature that the overall incidence of a chromosomal factor in infertile males ranges between 2% to 10% [4, 5]. It was reported that the chromosomal abnormality ratio as 6% in oligospermia cases and 19% in azospermia cases [6]. The risk of unbalanced chromosomal arrangements during spermatogenesis is seriously high in this infertile population with genetic abnormality [7]. Assisted reproductive techniques gave the chance of having a child to infertile males with azospermia and oligospermia. Using the ICSI in this group with high genetic abnormality ratio may

increase the inheritance of paternal genetic disorders to offspring [8, 9].

The main purpose of this study is to investigate the prevalence and types of major cytogenetic abnormalities in azoospermic and severe oligozoospermic patients using standard cytogenetic methods. Also, it is to investigate the general characteristics such as serum hormonal levels, smoke and alcohol habits, testis volume in this infertile males.

Materials and methods

Infertile men (n=115) were prospectively recruited for chromosomal analysis from February 2005 to April 2007 at Süleyman Demirel University IVF Centre, Isparta. The patients were selected prior to ICSI treatment because of severe male-factor infertility. The mean age patients were 32.3±6.7 years (range 19–55). All of them underwent an andrological work-up, which included medical history, physical examination, hormonal estimation, testicular ultrasonography and semen analysis according to World Health Organization recommendations and standards [10]. Cigarette and alcohol consumption behavior of patients were registered. Using blood samples obtained during the morning, serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone were measured by electrochemiluminescence immunoassays using Beckman Coulter DXI800 (USA) according to the manufacturer's instructions. Testicular volume was determined in all patients using testicular ultrasonography. High-frequency US was performed by one experienced examiner using 7.5-MHz transducers (LoGIQ 200 Proseries, South Korea). The ultrasonographic testicular volumes were calculated as the length × width × depth × 0.71 [11]. There were 92 men with non-obstructive azoospermia and 23 with oligozoospermia with a sperm count of <5× 106/ml. The diagnosis of non-obstructive azoospermia was based on the finding of azoospermia in the presence of small volum testes and/or elevated serum FSH and histopathology of a previous testicular biopsy (if available). Forty normozoospermic male donors with normal semen parameters and proven fertility were included as controls.

Informed consent was taken from the patients and donors prior to collection of heparinised blood samples. Chromosome investigations were performed on cultures of peripheral blood lymphocytes using standard techniques [12]. The samples were cultured for 72 h, using RPMI-1640 medium including 10% fetal calf serum and 2% phytohemagglutinin (PHA). At the end of 72 h, the cultures were harvested and G-T-G banding was performed**.** At least 25 metaphases were analyzed for each patient and Up 100 metaphases were analyzed in case of mosaicism. To characterize the polymorphisms, specific techniques such as C-banding and NOR staining were additionally applied. Statistical analysis was performed using the Graph pad Instant Tm program on a computer (Dr Granger, LSU Medical Center). Statistical evaluations were performed using unpaired t test, the Mann–Whitney U-test and Wilcox on matched pairs test where appropriate. P values of 0.05 or less were considered statistically significant.

Results

Characteristics of the azoospermic, the oligozoospermic and the control patients were summarized in Table 1. There was a significant difference in serum FSH, LH, mean testicular volume and smoking habits when compared with the patients and the control group (Table 1). Alcohol consumption rate was higher in the azoospermic (10.8%) and the oligozoospermic group (8.7%) than the control group (3.3%). But, these differences were not statically significant (p=0.2, for both groups). Also, there was a significant difference in serum FSH, LH levels and mean testicular volume when compared with the azoospermic and the oligozoospermic patients.

Mean sperm count was 2.5±1.4 million/ml (range 0.1–4.8 million/ml) with reduced motility (mean 8.5±5.4%; range 1–19%) and morphology (mean normal form 5.1± 2.6; range 1–10%) was seen in oligozoospermic patients groups.

Characteristics of the patient's detected chromosomal abnormality were summarized in Table 2. Among the 115 infertile men studied, 5 showed chromosomal abnormality corresponding to a frequency of 4.3%. All of the cases with chromosomal abnormality were azoospermic (5/92; 5.4%). All of chromosomal abnormalities in azoospermia cases were found to be gonosomal. Four patients were diagnosed as a mosaic form Klinefelter's Syndrome (KS) and a patients was diagnosed as a 46, XX male. All of the azoospermic patients with chromosomal abnormality had the higher serum FSH and LH levels and the lower testicular volume. In the oligozoospermia cases, no chromosomal abnormalities were detected. A normal karyotype of 46, XY was observed in all the control groups.

Discussion

Patients in azoospermia and oligozoospermia groups had the higher FSH and LH levels and lower testicular volume. Serum FSH and LH levels and testicular volume correlate with the testicular function including sperm density, total sperm count [13]. Because our azoospermic patients are non-obstructive, they have worst hormonal levels and

Table 1 General findings in 92 azoospermic, 23 oligospermic and 40 control men

Patients group	age (Years) (mean±SD)	FSH (MIU/mL)	LH (MIU/mL)	testosterone (Ng/ml)	testis volume (Ml) right Left	smoker/total patients Alcohol/ total patients (%)
Azoospermia	32.1±7.2	19.5±11[ab]	10.8±5.3[de]	6.4±4.9	9.2±4.5[gh]	53/92(57.6%)[l]
					8.7±3.7[ii]	10/92(10.8%)
Olizoospermia	33.3±6.4	13.4±12.3[c]	7.1±5.6[f]	5.3±3.1	13.2±4.2[j]	13/23(56.5%)[m]
					12.3±4.5[k]	2/23(8.7%)
Control	32.7±6.5	4.6±1.2	4.3±1.1	5.7±2.3	16.9±4	8/60(13.3%)
					15.8±4.1	2/60(3.3%)

[a] p=0.01, compared to oligospermia group
[b] p<0.0001, compared to control group
[c] p<0.0001, compared to control group
[d] p=0.005, compared to oligospermia group
[e] p<0.0004, compared to control group
[f] p<0.0001, compared to control group
[g] p=0.002, compared to oligospermia group
[h] p<0.0001, compared to control group
[ı] p=0.001, compared to oligospermia group
[i] p<0.0001, compared to control group
[j] p=0.01, compared to control group
[k] p=0.01, compared to control group
[l] p<0001, compared to control group
[m] p<0001, compared to control group

Table 2 Chromosomal anomalies detected in the 115 patients

Case no	age (Years)	FSH (mIU/mL)	LH (mIU/mL)	testis volume (Ml)	smoke/alcohol consumption	karyotype	sperm count
Case 1	25	17.4	10.2	4.2/4	+/+	46, XY 46, XX 47, XXY Mosaic	azoospermia
Case 2	45	21.3	13.2	7/7.5	+/−	46,XX	azoospermia
Case 3	32	16.2	11.5	5/5	−/−	46, XX 46,XY 47,XXY 45,X Mosaic	azoospermia
Case 4	34	18.6	10.5	11/10	−/−	46,XY 47,XXY Mosaic	azoospermia
Case 5	36	17.3	11.7	9/8.5	−/−	46,XY 47,XXY Mosaic	azoospermia

smallest testicles (Table 1). These findings are consistent with those of previous reports [14, 15]. Serum FSH, LH levels and testicular volume may have prognostic implications on testicular function, but we don't know whether these parameters have any prognostic implications on cytogenetic abnormality of infertile patients. Our study has showed that patients with chromosomal abnormality are prone to have smaller testicles and higher serum FSH and LH levels (Table 2). The patients with chromosome abnormalities featured similar testicular volume and serum FSH and LH levels with those non-obstructive azoospermic patients in Table 1. The higher serum levels of FSH and LH together with smaller testicles are just characteristics of patients with non-obstructive azoospermia. Such phenotypes did not show any value to predict chromosome abnormalities in our study. Thus, these parameters should not be used to include and/or exclude patients from a cytogenetic examination.

Also, our patients have higher cigarette consumption rate as compared to the control group. The fact that cigarette smoke contains known mutagens and carcinogens, there has been concern that smoking may have adverse effects on male reproduction. Some authors show that smoking deteriorates the sperm quality [16, 17]. Also, an increased incidence of smoking consumption has been reported in infertile patients in the literature [18]. Vutyavanich T et al. reported that the prevalence of smoking was 40% in the azoospermic men, 32.5% in the oligozoospermic men, and only 10% in control fertile men. They have also reported that 20% of the azoospermic and oligozoospermic men reported regular use of alcohol. This increased incidence of smoking may be one of the causes of the infertility in the patients or it is likely that the patients may have increased cigarette consumption psyhologically after they had found out that they were infertile. This situation should be investigated in large series.

The occurrence of karyotypic abnormalities among infertile men depends on a number of factors; the most important of these is the criterion for selection of patients based on the sperm counts. The prevalence of chromosome abnormalities is higher in infertile men and it is well-known that the sperm count is inversely related to the existence of chromosomal anomaly. The first study, emphasizing the relation between some chromosomal abnormalities and male infertility, included 6982 cases, and the frequency of chromosomal abnormality was reported as 5.3% [19]. The frequency in whole population was given as 0.6%. In the same study, gonosomal and autosomal chromosomal abnormality ratios were found to be 15- and 6-fold, respectively [6, 20]. In our study among the 115 infertile men studied, 5 showed chromosomal abnormality corresponding to a frequency of 4.3%. The chromosomal abnormalities were more frequently observed in the population of azoo-and/or oligozoospermic males than in the general population [21]. Lissitsina et al. reported that the incidence of sex chromosome abnormalities in azoospermia group was higher than that in the oligozoospermia group [22]. In the presented study, chromosomal abnormalities were detected in 5.4% of 92 azoospermic cases and no patients of 23 oligozoospermic cases. The chromosomal abnormality ratio in azoospermia was given as 12.5–31% [4, 6, 23–26]. Although our results reflect a regional pattern of those referrals, comparison of our results with the review of the literature shows a relatively smaller incidence of chromosomal anomalies in azoospermia in our center. The chromosomal abnormality frequency in males with oligospermia was reported as 1.8–6.9% in several studies [4, 6, 24–26]. In our study, any chromosomal abnormality wasn't detected in all the oligozoospermia cases. Because of the small sample size in the oligospermia group, further studies with large patients groups focusing on this issue may be suggested to clarify exact chromosomal abnormality incidence in our region.

The most common type of karyotype abnormality in infertile cases is represented by KS. KS is the most common abnormality of sexual differentiation, and occurs in approximately 1 in 500 live births. 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 [27]. Ferlin et al. reported that the prevalence of KS among infertile men is very high, up to 5% in severe oligozoospermia and 10% in azoospermia [28]. It has always been assumed that more than 90% of non-mosaic 47, XXY males are azoospermic. In their series, 74.4% of mosaic 47,XXY/46,XY patients were azoospermic, whereas the remaining had severe oligospermia [28]. KS was the most frequent chromosome-related cause of infertility in our study group. In our study, we detected that four cases had mosaic KS, and a patient had 46, XX. XX man described as a Klinefelter variant is a rare condition. All of the patients with chromosomal abnormality had azoospermia. Among azoospermia groups they comprised 5.4%.

There are some other cytogenetic studies conducted on infertile patients from different part of Turkey [29–31]. Akgül et al. have found that chromosomal abnormalities were detected in 17.4% of 86 azoospermic cases and 6.8% of 73 oligozoospermic cases revealed 11.7% of all cases in a regional study from Turkey. Samli et al. have detected that chromosomal abnormality was detected in 47 (12%) of 383 non-obstructive azoospermia cases and in 20 (4%) of 436 oligospermia patients. Balkan M et al. have found that the total prevalence of chromosomal abnormalities was found to be 11.2% (9/80; 52 were azoospermic, 25 oligozoospermic and 3 asthenozoospermic), including seven patients with KS. All of the patients with KS had azoospermia. Comparison of our results with the other studies carried out in this field in Turkey shows a relatively

lower incidence of chromosomal anomalies in our center (Table 2). Although our results reflect a regional pattern of those referrals, combined with the previous results from Turkey, further studies focusing on this issue may be suggested to clarify this relatively lower incidence.

In conclusion, the results of this study showed that infertile men had a higher prevalence of the chromosomal alterations. Therefore, the higher incidence of the chromosomal anomalies among the infertile males strongly suggested karyotyping and counseling prior to the employment of the assisted reproduction techniques.

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