GENETIC ASPECTS OF INFERTILITY IN MEN WITH AZOSPERMIA OR OLIGOSPERMIA

Ali Shaltout[1] and Faeza El-Dahtory[2]

[1]Departments of Pediatrics and Genetics, Faculty of Medicine.

[2]Genetics Unit, Children Hospital.

Mansoura University, Egypt

ABSTRACT

***Aim of the work**: To determine the frequency of chromosomal abnormalities and microdeletions in the AZF in a sample of infertile men with idiopathic azoospermia and oligozoospermia. **Materials and Methods:** study a group of 236 infertile men with oligospermia or non-obstructive azospermia from Genetics Unit of Mansoura University, Children Hospital. The age ranged from 21 - 46 years. 89 had oligospermia, and 147 non-obstructive azospermia. All patients underwent a genetic study which included karyotype analysis and AZF microdeletion investigation using polymerase chain reaction (PCR) technique. **Results:** Genetic abnormalities were found in 45/236 (19.06%) of the studied patients. Chromosomal abnormalities were found in 29/236 (12.29%), and AZF microdeletions in 20/236 (8.47%) of patients. **Conclusion**: Our findings suggest that the genetic screening should be advised to infertile men before starting assisted reproductive treatments as results may help determine the prognosis, as well as the choice of an assisted reproduction technique.*

Key words: male infertility; chromosome abnormalities; oligospermia; azospermia.

Introduction

Infertility affects 15% of couples worldwide, and in roughly half of these cases, the defect can be traced to the male factors (1). Several factors have been implicated in male infertility such as hormonal abnormalities, erectile dysfunction, infections, antisperm antibodies, exposure to chemical agents and radiations, testicular cancer, varicose, genetic factors and others (2-5).

The main genetic cause of male infertility is chromosomal abnormalities, which account for almost 5% of infertile males, and the prevalence increases to 15% in the azospermic males (6). Men with non-obstructive azospermia have high prevalence of aneuploidy, particularly in their sex chromosomes (7). The second most common genetic cause of male infertility is microdeletion in the azoospermia factor (AZF) region of the Y chromosome (8). Micro deletions in this region cause defect in spermatogenesis that leads to development of azospermia and oligozospermia (9). Three major loci have been identified in the AZF and named AZFa, AZFb and AZFc regions. The three loci contain 16 coding genes that play a role in the process of spermatogenesis such as regulation of gene expression, RNA processing and trafficking (10).

In this study, we examined the frequency of chromosomal abnormalities and microdeletions in the AZF in a sample of infertile Egyptian men with idiopathic azospermia and oligospermia using karyotyping and polymerase chain reaction (PCR) techniques. In addition, we compared our results with numbers identified in other countries and populations.

Materials and methods

Two hundred and thirty six men with idiopathic infertility and thirty age-matched fertile controls were included in this study. The age ranged 21 - 46 years. Informed consent was obtained from patients and controls. The study was approved by the Mansoura University Ethics Committee. The diagnosis of azospermia and oligospermia was made on the basis of semen analysis according to WHO guidelines (1992). Each patient was carefully examined to rule out other causes of infertility and a detailed history was taken. Peripheral blood cultures were setup for chromosomal analysis according to the chromosomal culture method described by Rooney and Czepulkowski (11). about 1 ml of blood was mixed with 5 ml of RPMI medium, 1 ml of fetal bovine serum, 0.1 mg/ml of Phytohemagglutinin (PHA) and was incubated at 37°C. After 72 hour of incubation, the Colcimid (1 mg/ml) was added and incubated for another 1.5 hour. The cells were then harvested by hypotonic treatment (1.5 hour with 0.075M KCl at 37°C), fixed and washed thrice with fixative solution (methanol and acetic acid in a ratio of 3:1), and then metaphases were spread and stained using standard G-banding technique. For each case, 50 spread metaphases were analyzed with cytovision system.

PCR analysis

PCR screening was done for AZF microdeletions. Peripheral blood sample was collected from patients and DNA was isolated using phenol chloroform extraction method. Each of these patients were examined for 6 AZF loci which mapped to interval 5 and 6 of the Y chromosome. The STS primers used were – for AZFa: sY84; for AZFb: sY127, sY134; for AZFc: sY254. Fertile male and female samples were used as positive and negative control and water was used as blank. This primer set was suggested by Simoni et al (12). Samples were subjected to PCR amplification using 35 cycles of 95°C for 1 min, 56°C for 30 s and 72°C for1 min. Initial denaturation was done for 5 min at 95°C and final extension time of 7 min at 72°C was given. The PCR products were analysed on a 1.8% agarose gel containing ethidium bromide (0.5mg/ml).

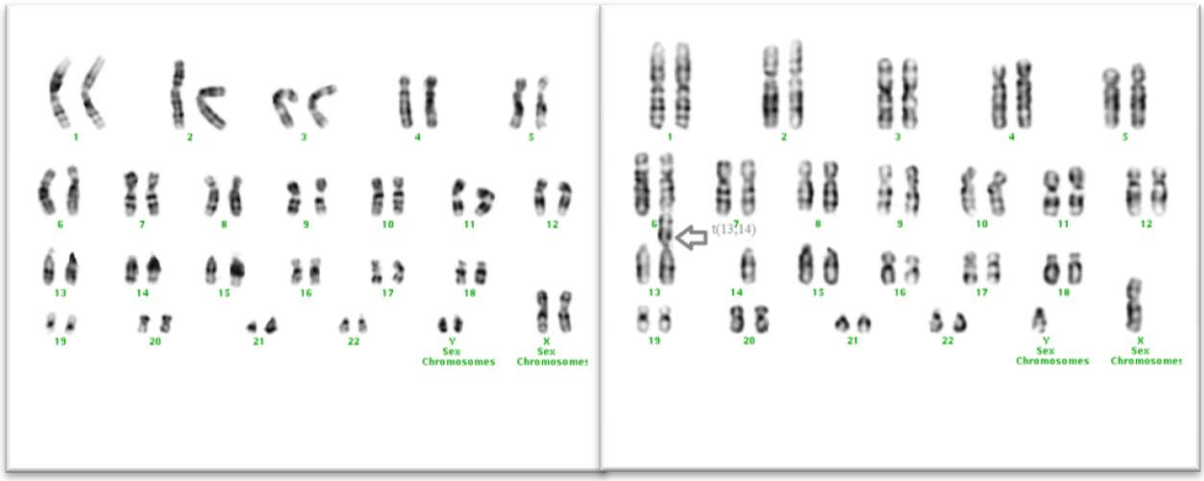
Results

We examined 236 men with infertility, 147 azoospermic and 89 oligospermic infertile men for chromosomal abnormalities. Chromosome abnormalities were found in 29/236 (12.29%): 14 men had 47,XXY, 2 were mosaic form 46,XY/47,XXY, 1 had 48,XXXY, one had 48,XXYY, 2 had 47,XYY, one had 45,X and two had 46,XX, while, there were six men had autosomal abnormalities: Two had 45,XY,t(13:14), one had 46,XY,t(1;5), one 46,XY,t(1:15), one 46,XY,t(3:16) and one had 45,XYp-,t(10:21)(q26:q11) (Table 1) and (figure 1).

Table 1. Chromosomal abnormalities in 29 azoospermic and oligozoospermic men.

Number of patients (%)	Karyotype	Sperm
14 (48.27)	47,XXY	Azo
2 (6.89)	47,XXY/46,XY	1 Azo / 1 oligo
1 (3.44)	48,XXXY	Azo
1 (3.44)	48,XXYY	Azo
2 (6.89)	47,XYY	1 Azo / 1 oligo
1 (3.44)	45,X	Azo
2 (6.89)	46,XX	Azo
2 (6.89)	45,XY,t(13;14)	Azo

1 (3.44)	46,XY,t(1;5)	oligo
1 (3.44)	46,XY,t(1:15)	Azo
1 (3.44)	46,XY,t(3:16)	Azo
1 (3.44)	45,XYp-,t(10:21)(q26:q11)	Azo



(a) (b)

Fig. 1. (a) karyotyping of the individual with numerical abnormalities in the form of 48,XXYY. And (b) karyotyping of an individual with structural abnormalities in the form of 45,XY,t(13;14).

AZF microdeletions showed in 20/236 (8.47%) of patients. They thowed microdeletions of one or more genes on the Y chromosome. Out of the 20 patients 7 had microdeletions in AZF b (sY-127, 134), two had microdeletions in AZF b (sY-127, 134) and AZF c (sY- 254), two had microdeletions in AZF a (sY-84) and AZF b (sY134), two had microdeletions in AZF b (sY-127) and AZF c (sY-254), one had microdeletions in AZF b (sY-134) and AZF c (sY-254), one had microdeletion in AZF b (sY-127), one had microdeletion in AZF b (sY-134), one had microdeletion in AZF c (sY-254) and three had microdeletions in all AZFa,b,c. (table 2) and (figure 2) .

Table 2: Azoospermia factor microdeletions in 20 infertile men with azospermia.

Number of patients (%)	AZF deletion	STS deletion
7 (35)	AZF b	sY-127, 134
2 (10)	AZF b, c	sY-127,134,254
2 (10)	AZF a,b	sY-84,134
2 (10)	AZF b,c	sY-127,254
1 (5)	AZF b,c	sY-134,254
1 (5)	AZF b	sY-127
1 (5)	AZF b	sY-134
1 (5)	sY-254	AZF c
3 (15)	sY-84,127,134,254	AZF a,b,c

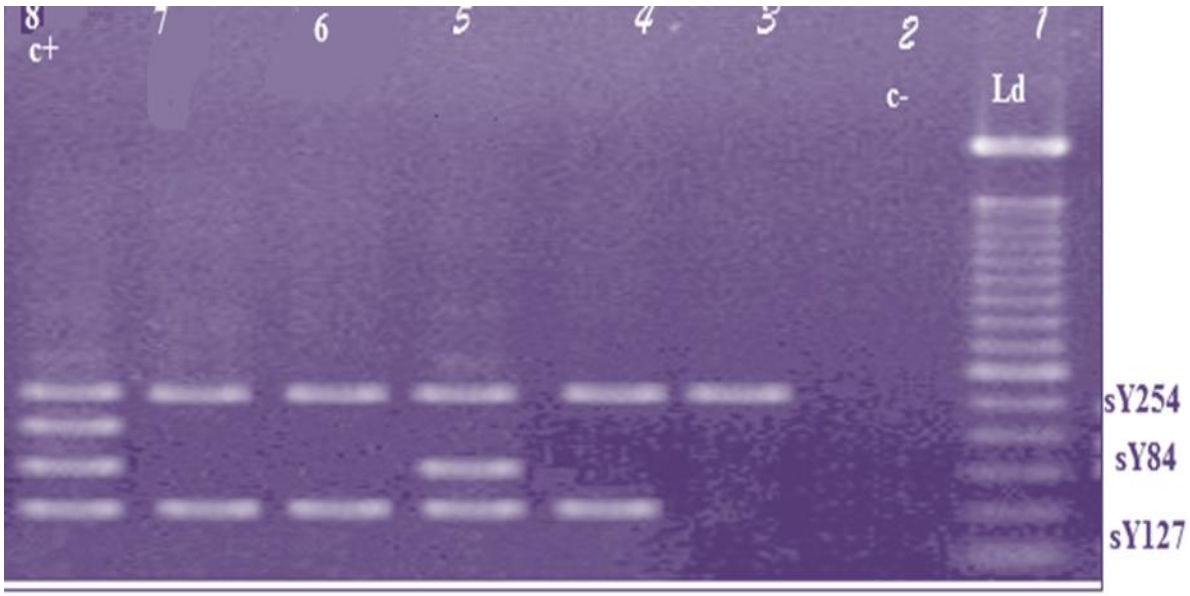


Fig.2. Agarose gel photograph showing deletion in the AZF gene. Lane 1, molecular weight marker Msp1 digest 100 bp; Lane 2, negative control (female sample); Lane 3, AZFa,b,c (sY-84,127,254) deletions in patient with azospermia (46,XX karyotype); Lane 4,6 and 7, (sY-254,84) deletions in three patients; Lane 5, (sY-84) microdeletion in a patient and Lane 8, of normal fertile man (positive control).

Discussion

The prevalence of chromosomal aberrations ranges from 4.3% to 17.5% in azoospermic and oligozoospermic infertile men attending reproductive centers (13, 14-18). In our study it was agree with these ranges (12.29 %), the most common karyotypic abnormality in men with severe male factor infertility is Klinefelter syndrome, affecting 7%–13% of azoospermic men. In most cases, KS develops because there is an extra copy of the X chromosome in each of a male's cells (47, XXY). Because there are extra X chromosome genes, this interferes with male sexual development. The testes don't work normally and they produce lower levels of testosterone (19). Other karyotypic abnormalities that have been identified include Robertsonian translocations, chromosomal inversions, and non-Klinefelter sex chromosome abnormalities (20). Dana Mierla et al.(21), performed karyotyping for 850 infertile men, (12.70%) had chromosomal abnormalities, finding sex chromosome anomalies in 11(1.29%) patients and autosomal chromosome anomalies in 20 (2.35%) : 6 azoospermic patients with Klinefelter's syndrome (47,XXY), 2 patients with 47,XYY syndrome and 100 patients with structural chromosomal abnormality. Similarly, we found chromosomal abnormalities in (12.29%) of our patients, but at the reverse we found autosomal chromosome anomalies at a lower rate than sex chromosomal abnormalities. We found:14 azospermic men with Klinefelter syndrome (47,XXY), 2 were mosaic form 46,XY/47,XXY, 1 had 48,XXXY, one had 48,XXYY, 2 had 47,XYY, one had 45,X and two had 46,XX, while, there were six men had autosomal abnormalities: Two had 45,XY,t(13:14), one had 46,XY,t(1;5), one 46,XY,t(1:15), one 46,XY,t(3:16) and one had 45,XYp-,t(10:21)(q26:q11). Translocation carrier subjects have an increased chance for creating unbalanced gametes and for abnormal sperm production. The frequency of abnormal sperm has been reported to be 3.4%–40% in Robertsonian translocation carrier men and 47.5%–81% in reciprocal translocation carrier men (22). Similarly, we found two (6.89%) patients with Robertsonian translocation of karyotyping 45,XY,t(13q;14q) and 4 (13.78%) patients with reciprocal translocation.

The spermatogenesis gene complex called "azoospermia factor" (AZF) on Yq. Yq part mainly divided in important AZFa, AZFb and AZFc regions. Deleted in azoospermia gene-DAZ' that exists in AZFc region, is expressed specifically in testis. DAZ is also the most frequently deleted site in non-obstructive severe or mild oligozoospermic infertile men.

Deletions in the AZFa and AZFb regions have been associated with azoospermia (23). The frequency of AZF microdeletion observed in this study was about 8.47% among azoospermic males. This frequency is similar to what reported in patients from China (8.6%), India (7.6%) and Netherland (8.1%) (26,24,32) while it is slightly lower (Table 3) than that detected in patients from Mexican (12%), USA (11%), Japan (11.7%) and Tunisia (11.8%) (34,321,28,29). However, very low frequency of AZF microdeletions was reported in studies from Algeria (2%) and Turkey (1.3%) (27,30). The variation in the detected frequencies of AZF microdeletions among azospermic infertile males could be due to method of detection of deletions, inclusion criteria and sample sizes. In our sample, no microdeletions were detected among 89 oligospermic infertile males. Similar findings were reported in studies from India, Algeria and Turkey (24, 27, 29) (table 3). The result is also consistent with the majority of the studies.

Table 3. The frequency of AZF microdeletions among azoospermic infertile males in selected populations

Population	Frequency of AZF microdeletion	Reference
Egyptian	8.47%	This study
Indian	7.6%	(24)
Spanish	14%	(25)
Chinese	8.7%	(26)
Algerian	2%	(27)
Japanese	11.7%	(28)
Tunisian	11.8%	(29)
Turkish	1.3%	(30)
USA	10.4%	(31)
Netherlander	8.1%	(32)
Brazillian	6.6%	(33)
Mexican	12%	(34)

We found Y chromosome microdeletion in 8.47% of our study subjects, and the AZFb deletion was the most common, at 7/20 (35%) (Table 2) while the AZFc deletion was the

most common in the literature (35-37). The complete removal of the AZFa and AZFb regions are associated with severe testicular phenotype, Sertoli cell-only syndrome, and spermatogenic arrest. The specificity and the reported genotype/phenotype correlation confers to Y deletion analysis a diagnostic and a prognostic value for testicular sperm retrieval (38). We found three azospermic patients with the complete removal of AZFa, AZFb and AZFc regions, one had 45,X karyotype and two had 46,XX karyotype. Thus, we recommend that Y chromosome microdeletion tests, in addition to karyotype analysis, be performed before IVF procedures in couples who are candidates for IVF.

Conclusion: Our findings suggest that the genetic screening (karyotyping and AZF gene deletion) should be advised to infertile men before starting assisted reproductive treatments as results may help determine the prognosis, as well as the choice of an assisted reproduction technique.

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