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Original Article

Genetic screening for AZF Y chromosome microdeletions in Jordanian azoospermic infertile men

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Abstract: The azoospermia factor (AZF) region of the human Y chromosome contains essential genes for spermatogenesis. Microdeletions in AZF region has been shown to cause male infertility. The aim of this investigation was to determine the frequency of AZF microdeletions in Jordanian infertile males. A sample of 100 infertile males (36 with azoospermia and 64 with oligozoospermia) was screened for microdeletions using 16 AZF markers and polymerase chain reaction (PCR) technique. Two subjects were found to have microdeletions in AZFc region and one subject has microdeletion that includes AZFb and part of AZFc and AZFa. The three deletions were found in azoospermic subjects (8.3%). No microdeletions were found in oligozoospermic group. The frequency of AZF microdeletions in Jordanian azoospermic infertile males is comparable to that observed in other populations (1%-15%). The results suggest the importance of AZF microdeletion analysis for genetic counseling prior to providing assisted reproduction technique.

Keywords: AZF, microdeletion, Jordan, infertility, male

Introduction

Infertility is defined as failure to conceive after one year of regular unprotected sexual intercourse [1]. 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]. Thus, male infertility can be considered as multifactorial syndrome that has a collection of different conditions with a variety of etiologies. However, in about 40% of male cases, causing factors of infertility are unknown or called idiopathic [6].

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 azoospermic males [7]. Men with non-obstructive azoospermia have high prevalence of aneuploidy, particularly in their sex chromosomes [8]. The second most common genetic cause of male infertility is microdeletion in the azoospermia factor (AZF) region of the Y chromosome [9]. Microdeletions in this region cause defect in spermatogenesis that leads to development of azoospermia and oligozoospermia [10]. 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 [11].

In this study, we examined the frequency of microdeletions in the AZF in a sample of infertile men with idiopathic azoospermia and oligozoospermia using polymerase chain reaction (PCR) technique. In addition, we compared the detected microdeletion frequencies with numbers identified in other countries and populations.

Methods

Subjects

Unrelated 100 infertile men with oligozoospermia (n = 64, sperm count; < 15 million/ml) and

Table 1. Sequence tagged sites (STS)- PCR multiplexing groups, locations and their amplified sizes

Multiplex set	Name of STS	Region	Amplified fragments (Bp)
1	ZFY	IC	495
	SY 86	AZFa	320
	SY 127	AZFb	274
	SY 254	AZFc	400
2	SRY	IC	472
	SY 84	AZFa	362
	SY 134	AZFb	301
	SY 255	AZFc	126
3	SY 152	AZFc	125
	DAZ	AZFc	1300
4	SY 142	AZFb	180
	SY 164	AZFb	590
	SY 277	AZFc	275
5	SY 82	AZFa	280
6	SY 81	AZFa	200
7	CDY	AZFc	132

Table 2. Pattern of microdeletions in the 3 azoospermic infertile males

Region	Phenotype STS	Patient 1	Patient 2	Patient 3
IC	ZFY			
	SRY			
AZFa	SY 81			
	SY 82			
	SY 86			
	SY 84			DeL
AZFb	SY 127			DeL
	SY 134			DeL
	SY 142			DeL
	SY 164			DeL
	RBMI			DeL
AZFc	SY 152	DEL	DeL	DeL
	DAZ	DeL	DeL	DeL
	SY 255	Del	DeL	DeL
	SY 277	DeL		DeL
	CDY	Del		

azoospermia (n = 36) were participated in the study. The subjects were recruited from infertility clinics in Middle of Jordan. Subjects with chromosomal abnormalities or known causes of infertility were excluded from study. A structured questionnaire was used to collect information about subject's medical history and demographic characteristics. Informed consent was taken from all subjects according to Institutional Review Board at Jordan University of Science and Technology.

DNA extraction

Genomic DNA was extracted from blood specimens collected from subjects in EDTA tubes using commercially available kit (Promega, Madison, USA). The concentration of DNA in the samples was measured spectrophotometrically and stored at -35°C until used.

Detection of AZF microdeletions

Microdeletions in AZF region were detected using polymerase chain reaction (PCR) technique as previously described [12, 13]. Sixteen sequence tagged sites (STS) in the AZF region were used as markers for analysis of microdeletion. The STS markers were ZFY and SRY for internal control (IC) region; SY 81, SY 82, SY 84 and SY 86 for AZFa; RBM1, SY 127, SY 134, SY 142 and SY 164 for AZFb; DAZ, CDY, SY 152, SY 255 and SY 277 for AZFc. STS PCR multiplexing groups and their amplified fragments are shown in **Table 1**. PCR was carried out in 30 µL reaction mixture containing 10 ng of DNA, 1 µM of each primer and ready to use PCR master mix (Promega, Madison, USA). The PCR conditions were: denaturation at 95°C for 6 minutes followed by 35 cycles of 95°C for 60 s, 62°C for 60 s and extension at 72°C for 90 s, and a final extension at 72°C for 8 minutes.

Results

In this study 100 infertile males with azoospermia (n = 36) and oligozoospermia (n = 64) were screened for AZF microdeletions in the Y chromosome. The mean age of subjects was 32.9 ± 7 years (range 23-43).

None of the patients showed complete microdeletion of all AZF regions. However 3 patients with azoospermia out of 36 had partial microdeletions (8.3%). Distribution of deleted markers in the three patients is shown in **Table 2**.

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

Population	Number of azoospermic males	Frequency of AZF microdeletion	Reference
Jordanian	34	8.3%	This study
Indian	119	7.6%	[15]
Spanish	57	14%	[24]
Chinese	137	8.7%	[16]
	945	11.5%	[13]
Algerian	49	2.0%	[21]
Japanese	60	11.7%	[18]
Tunisian	76	11.8%	[19]
Turkish	52	1.3%	[12]
USA	385	10.4%	[23]
Netherlander	37	8.1%	[14]
Brazillian	60	6.6%	[22]
Mexican	50	12%	[11]

Two subjects had microdeletion in AZFc region while one had a microdeletion that involved AZFb region and part of AZFa and AZFc regions.

Discussion

In this study, we examined microdeletions in AZF region of Y chromosome in 100 male with unknown cause of infertility. The results showed that 3 out of 36 azoospermic infertile males had microdeletions in AZF region, whereas no microdeletions were detected in oligozoospermic infertile males.

The frequency of AZF microdeletion observed in this study was about 8.3% among azoospermic males. This frequency is similar (**Table 3**) to what reported in patients from China (8.6%), India (7.6%) and Netherland (8.1%) [14-16] while it is slightly lower (**Table 3**) than that detected in patients from USA (11%), Japan (11.7%) and Tunisia (11.8%) [17-19]. However, very low frequency of AZF microdeletions was reported in studies from Algeria (2%), Slovakia (4.6%) and Turkey (1.3%) [12, 20, 21]. The variation in the detected frequencies of AZF microdeletions among azoospermic infertile males could be due to method of detection of deletions, inclusion criteria and sample sizes. In our sample, no microdeletions were detected among 64 oligozoospermic infertile males. Similar findings were reported in studies from India, Algeria and Turkey [12, 15, 21]. The result is also consistent with the majority of the studies that observed higher frequency of microdeletions in infertile males with azoospermia than those with oligozoospermia [12, 13, 15, 18, 19, 21-24].

In the current study, the distribution of microdeletions detected in AZFc region was higher than the other AZF regions. This result is consistent with most of the literature that indicate that the prevalence of microdeletions in AZFc is high compared to AZFb and AZFa regions [12, 13, 15, 18, 19, 21-23]. AZFc region contains the DAZ family genes that encode proteins with RNA-binding motive and involved in the regulation of RNA metabolism during testicular development [25, 26]. Deletions in DAZ gene cluster have been shown to be associated with a variety of spermatogenic alterations [25, 26]. The reasons behind high frequency of microdeletions in AZFc could be due to the presence of repetitive sequences of the genes in this region that predispose it to intrachromosomal recombination.

In conclusion, AZFc Y chromosome microdeletion among infertile azoospermic males is common. This highlights the importance of examination of AZF microdeletions and genetic counseling prior to providing IVF service in Jordan.

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Disclosure of conflict of interest

Nothing to declare.

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