Original Article

AZF, SRY Microdeletions and Hormonal Disturbances among Azoospermic Iraqi men

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Abstract

Y chromosome microdeletions and hormonal disturbances were implicated in men infertility. This study was conducted to investigate the AZF and SRY regions microdeletions and hormonal disturbance in 43 azoospermic and 20 healthy and fertile men. Twelve (27.9%) of azoospermic men have shown deletions with undetected chromosomal abnormalities. Five of these deletions have been detected in men with a history of post pubertal mumps. AZFc is recorded as the most frequent deleted region in azoospermic men. FSH and prolactin elevation levels were also detected in patients but with undistinguishable correlation parallel to the detected microdeletions.

Keywords: Azoospermia, AZF, FSH, LH, Testosterone
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Introduction

Genetic abnormalities are considered to make an important contribution to unexplained spermatogenesis failure. From several survey, up to 15% of numerical and constitutional chromosome abnormalities have been detected in infertile men (Rao et al., 2004). Azoospermia was first associated with a possible genetic cause by Tiepolo and Zuffardi, 1976 (Tiepolo and Zuffardi, 1976). They detected deletion in the distal portion of the long arm of Y chromosome in the infertile men. They proposed according to these results that spermatogenesis may associated to a specific genes which carried by the distal portion of the long arm of Y chromosome and called them azoospermic factors-AZF. Later on, a substantial data have been published implicated these genes to male infertility (Bhasin, 2007; Ferlin et al., 2007). Up to now, more than five loci have been identified on Yq11 and their deletions are associated with infertility (Kleiman et al., 2007). AZFa, AZFb and AZFc were localized closed to centromere, Y chromosome RNA-recognition motif (YRRM/RBM) and deleted azoospermia (DAZ) were localized more proximal to centromere (Ferras et al., 2004).The deletion of these

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genes have been found to be associated with a wide range of spermatogenic defects responsible for azoospermia and oligospermia (Maduro et al., 2003). Sex determining region (SRY) another region found to have a major role in male infertility. SRY was mapped to the short arm. Deletion of each AZF region has been found to have different phenotypic effects (Foresta et al., 2001). The frequencies of the sequence-tagged sites (STS) and gene deletions among different studies are quite wide and vary between 1-55% (Kamp et al., 2001). AZFa deletion has been found to associate with Sertoli cell-only syndrome (Ferras et al., 2004), AZF b and AZFc deletions were associated with azoospermia and oligospermia (Lin et al., 2007). It has been found that similar deletions of AZF regions cause quantitative loss in spermatogenesis and among all AZF regions. Deletion of AZFc has been found to be the most frequent abnormality followed by AZFb (Kuroda-Kawaguchi et al., 2001). Also deletions in AZF genes are thought to be pathogenetically involved in some cases of male infertility associated with azoospermia or oligospermia (Krausz et al., 2001). The current study aims to detect the deletion of the regions AZFa, AZFb, AZFc, RBM and DAZ of the Y chromosome in azoospermic men.

Materials and Methods

Forty three men who were investigated for infertility at the Kamal Alsamarai Hospital, Alkadimyah Teaching Hospital and private clinics in Baghdad,Iraq from July 2008 to Jan 2009 and 20 healthy and fertile men were included in this study. Relevant information including the patient's age, medical history, job, social habits and diagnosis were extracted from the patients according to a comprehensive questionnaire. The seminal fluid analysis was done according to the procedure described by the World Health Organization (WHO, 2000). A minimum of two separate samples were required before the diagnosis of abnormality of the seminal fluid was made. In addition, blood samples were obtained for DNA extraction, chromosome analysis and hormonal evaluations. Hormonal levels were determined using by ELISA using Anthos 2020 system (Biochrom, Cambridge,UK) and according to the manufacturer's instructions. Normal reference ranges for men were: Follicle Stimulating Hormone-FSH 1.7-12mIU/L, Luteinizing Hormone-LH 1.4-10mIU/L, Prolactine-PRL 1.5-7.5 ng/mL and Testosterone 3-9 ng/mL. Chromosome analyses were performed in the Genetic Engineering Department, Genetic Engineering and Biotechnology Institute-Baghdad University. Briefly, 70 hours cultures of peripheral blood lymphocytes were treated with 0.1 μg/mL of colcemid (Sigma-UK) for 2-hrs of incubation period, treated with hypotonic solution and fixed with a fixative. The metaphase chromosomes were spread and stained using standard G- banding techniques. At least 20 metaphases per subject were analyzed. DNA was extracted from blood using DNA extraction kit (Promega-Canada) according to the manufacturer's instructions. PCR was carried out using master mix from Promega PCR kit (Promega- Canada) and primers for the regions SRY, SY254, SY84, SY127,DYZ1 and RBM1/DXY233(Table 1). Thermo cycling for PCR was carried out at 95 °C for 3 min; followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, extension at 72 °C for 1.5 min and a final extension at 72 °C for 10 min. PCR products were separated on 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized using UV illuminator. In each PCR assay, samples from one normal fertile man, without Y chromosome microdeletions, were used as normal controls.

Table 1: Primer sequences and PCR product sizes.

STS	Forward primer (5'—3')	Reverse primer (5'—3')	AZF interval	Product in bp
SRY	GAATATTCCGCTCTCCGGA	GCTGGTGCTCCATTCTTCAG		472
SY84	GTGACACACAGACTATGCTTC	ACACACAGAGGGACAACCCT	AZFa	320
SY127	GGCTCACAAACGAAAAGAAA	CTGCAGGCAGTAATAAGGGA	AZF	274
SY254	GGGTGTTACCAGAAGGCAAA	GAACCGTATCTACCAAAGCAGC	AZFc	400
DYZ1	AATTTGAGCATTCGTGTCCATTCT	AATGCCCTTGAATTAAATGGACT	AZFc	1024
RBM1/DX3Y	GCCGCAAACAATATCCAATA	CATCCAAAAAGTCACGAGCA	AZFb	411

Results

No chromosomal abnormalities were detected in all examined metaphases from azoospermic samples. FSH and prolactin elevation were detected in significant levels in azoospermic (12.3 ± 2.04 mIU/L, 10.35 ± 1.5 ng/mL respectively). On the other hand testosterone showed normal levels (Table 2). Twelve (27.9%) of 43 azoospermic men have shown deletions.Four of them (33.3%) shown with AZFc deletions (deletions of SY254 /DAZ) and 8 (66.7%) was associated with AZFa and SRY deletions. Y chromosome deletions were not detected in fertile control. Also RBM1 deletion was not detected in any infertile men (Figure 1).

Table 2: Hormonal profile of the azoospermic, oligospermic men and control.

Subgroups	Mean ± SD			
	FSH- mIU/L	LH- mIU/L	Prolactin- ng/mL	Testosterone- ng/mL
Azoospermic	*12.30 ± 2.04	8.62 ± 2.9	*10.35 ± 1.5	5.57 ± 2.8
Control	8.45 ± 2.21	8.18 ±1.82	6.0 ±1.5	7.9 ± 1.32

* $p < 0.05$ -SD

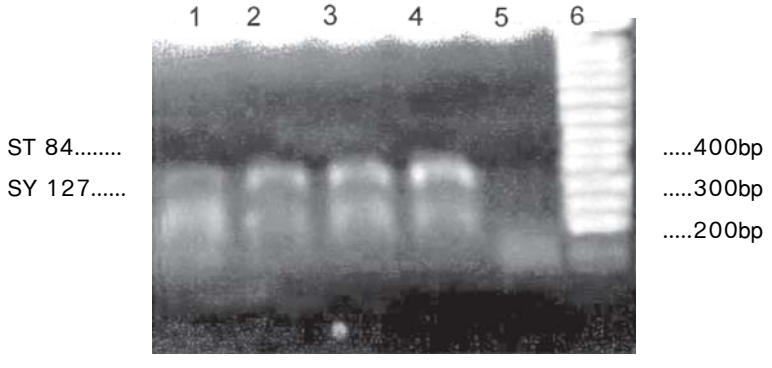

Figure 1 Electrophoresis of PCR reactions of SY sites. Five microliters from each reaction was mixed and electrophoresed through 1.0% agarose gel. Lanes 1,2,3,4: SY 84 (320bp) and SY 127 (274bp) (Bands), Deletions of SRY and SY254 / DAZ (No bands), Lane 5: Deletions of SRY, SY254, SY84 and SY127. Lane 6: Marker fragments 1200bp-100bp.

Discussion and Conclusion

Microdeletions of the Y chromosome represent the most frequent cause of male infertility (Krausz et al., 2001). The frequency of these deletions have shown to associate with the type of spermatogenesis defects and increased with severity of defects (Fernandes et al., 2002). Such deletions have been implicated on more than 15% of cases of azoospermia (Ozdemir et al., 2007). In the present study, 27.9% of the azoospermic men who were cytogenetically normal shown to have a microdeletion of Y chromosome spanning the three AZF loci and SRY and SY254 (AZFc), SY84(AZFa) and SRY were the most frequent deleted sites among azoospermic men. Five of 43 azoospermic (11.6%) had a history of post pubertal mumps, three of them shown to have a deletion in SY254-AZFc- and the other two shown to have a deleted SRY. Also two of these patients with SRY deletions were with small testes. Based on this we can explained the detected microdeletions by previous post pubertal mumps (41.6%) and by unknown reasons in 59.4%. This make AZFc (SY254) and SRY reliable markers to identify azoospermia caused by mumps. Also FSH and prolactin elevation levels were undistinguishable parallel to the detected microdeletions which suggest that hormonal imbalance is independent extra factor in men sterility. Y chromosome microdeletion frequencies were reported in a wide variation ranging from 0.1% to 25% based on the sample size in the studies, ethic variations and the type and number of primers used (Rao et al., 2004; Ozdemir et al., 2007). Most of these reports were observed the correlation between azoospermia and AZF microdeletions. Such deletions have been also detected in oligospermia (Dada et al., 2003a), asthenooligospermia (Griffin and Kinch, 2005), Sertoli cell-only syndrome (Kamp et al., 2001) and normal fertile men (Chang et al., 1999). It is well known that the deletions of the AZF regions cause spermatogenic impairment and that the complete deletion of any of them is usually associated with the total depletion of spermatogenic cells (McElreavey et al., 2006). The AZFa deletion was found to associate with complete absence of germ cells and presence of Sertoli cells in the seminiferous tubules (Ferras et al., 2004; Kamp et al., 2001) while AZFb and AZFc deletions are associated with developmental arrest of germ cells at pachytene stage or at spermatid stage respectively (Dada et al., 2004). Also AZFc was found to associate with hypospermatogenesis, maturation arrest and a variety of testicular phenotypes (Dada et al., 2003b). The high percentage of the AZFc distributed among azoospermia in our study suggesting that it is possible that AZFc is predominant in Iraqi azoospermic. Also the correlation found in this work between the azoospermia and mumps infection suggesting that SY254 of the AZFc and SRY were fragile sites toward mumps virus. In the light of the above, further studies using more sites and large sample size need to done to expand the view of these correlations.

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