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RESEARCH ARTICLE

Prevalence of Y chromosome microdeletion in azoospermic infertile males of Iraqi population

ANWAR M. AL-JANABI[1]*, ALI IBRAHIM RAHIM[2], SHEHAB A. FARIS[2], SALIH M. AL-KHAFAJI[2] and DHAFER JAWAD[3]

[1]*Department of Clinical Biochemistry, College of Medicine, University of Kufa, P.O. Box (21), Najaf, Iraq*
[2]*Department of Anatomy and Histology, College of Medicine, University of Kufa, P.O. Box (21), Najaf, Iraq*
[3]*Department of Pathology, College of Medicine, University of Kufa, P.O. Box (21), Najaf, Iraq*
*For correspondence. E-mail: anwar.aljanabi@uokufa.edu.iq.

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Abstract. In human gamete development, the important period is spermatogenesis, which is organized by specific genes on Y chromosome. In some cases, the infertile men have shown microdeletions on Y chromosome, which seemed as if the structural chromosome variance is linked to the reduction of sperm count. This study aimed to determine the frequency and patterns of Y chromosome microdeletions in azoospermia factor (AZF) of Iraqi infertile males. Here, 90 azoospermic infertile males as a study group and 95 normal fertile males as control group were investigated for the microdeletion of AZF loci using numerous sequence-tagged sites. Of these 90 infertile male patients, 43 (47.8%) demonstrated Y chromosome microdeletions, in which AZFb region was the most deleted section in azoospermia patients (33.3%) followed by deletions in the AZFc region (23%), while there were no microdeletion in the AZFa region. The largest microdeletion involved in both AZFb and AZFc was detected in six azoospermic patients (6.7%). The present study demonstrated a high frequency of Y chromosome microdeletions in the infertile Iraqi patients which is not reported previously. The high frequency of deletions may be due to the association of ethnic and genetic factors. PCR-based Y chromosome screening for microdeletions has a potential to be used in infertility clinics for genetic counselling and assisted reproduction.

Keywords. azoospermia; azoospermia factor; Y chromosome; microdeletions; Iraq.

Introduction

Infertility is a public health issue, affecting about 15% of couples who are planning to have a child; the male factor is found in about 50% of the cases (Ambasudhan *et al.* 2003; Pryor *et al*. 1997). The cause of infertility may be due to the well-known risk factors, such as varicocele, genital infection, anatomical abnormalities, immunological reasons, chromosomal abnormalities and others (Hamada *et al*. 2011).

The molecular basis of male infertility which leads to impaired spermatogenesis is still obscure and yet to be examined. Our previous cytogenetic studies showed that the chromosomal abnormalities in Iraqi men suffering from infertility is about 12.5% (Yasseen and Alkhafaji 2002), compared to 6% elsewhere (Dohle *et al*. 2012). The high percentage of male infertility may be due to the mutations and possibility of the effect of depleted uranium or chemicals, which is widely used in the indicated area. The presence of an essential spermatogenesis factor called azoospermia factor (AZF) was discovered early in 1976 from a *de novo* Yq deletions in azoospermic patients (Tiepolo and Zuffardi 1976). Deletions of the Y chromosome regions that contain the AZF are considered as the most common genetic defect in male infertility (Kent-First *et al*. 1999). Three regions on Y chromosome long arm (AZFa, AZFb and AZFc) are frequently deleted in males with severe spermatogenic failure (Vogt *et al*. 1996). The frequency of these microdeletions in severe oligospermic and azoospermic males were between 1 and 50% (Foresta *et al*. 1998).

In fact, the previous studies reported a variation of 5–10% frequency of Y-chromosome microdeletions in azoospermic males, and 2–5% in severe oligozoospermic males (Hopps *et al*. 2003; Guney *et al*. 2012). The Y-chromosome microdeletions have been reported in population with ethnic differences; we conducted this project to assess azoospermic

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infertile males in the local public using some sequence-tagged site (STS) marker sequences present on the long arm (Yq) of Y chromosome (AZF sub regions).

Materials and methods

The current case–control study was performed in the Fertility centre of Al-Sadder teaching Hospital in Najaf, Iraq. The study population was composed of 90 infertile azoospermic patients, with age ranging from 25 to 45 years, and 95 normal fertile males as controls, with age ranging from 24 to 43 years. Semen examination was performed for all participants at least twice a month, after three days of abstention. The samples of semen collected were examined after centrifugation for 10 min at 1000×g. The mean values of seminal fluid analyses were registered and used as an average results according to the reference values that published by the World Health Organization (WHO 2010), where the sperm count more than 15 million sperms/mL was considered as normal and the absence of sperms as azoospermia. The specimens were taken after informed consent obtained from all participants. The consent protocol included in this study was approved by the Medical Ethics Committee, Faculty of Medicine, University of Kufa.

During the two years period (September 2016 to October 2018), a total of 185 male patients and healthy control were examined for this study. All patients were diagnosed by urologist and tested for seminal fluid analysis. Culture media preparation, chromosome cytology, Giemsa stain have been published elsewhere (Hopps *et al*. 2003; WHO 2010; Guney *et al*. 2012).

In the current study, 5 mL of whole blood was collected from all participants by vein puncture. The collected blood was divided in two parts, the 1st part was used for genetic analysis, which included only 1 mL of whole blood collected in a tube with EDTA and was used for DNA extraction. The 2nd part was left for 15 min in room temperature to coagulate and centrifuged for 10 min at 3000×g to separate the serum for biochemical analysis. Measuring the concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone were done using the Mini Vidas kits (Biomerieux) according to the manufacturer's procedures.

Y chromosome microdeletions detection by multiplex PCR

After the extraction of DNA, according to the procedure published previously (Al-Khafaji *et al*. 2015), individual patients and normal control groups were scanned for six regions of AZF loci. The STS primers sY86 and sY84 were used for AZFa; sY127 and sY134 were used for AZFb; sY255 and sY152 were used for AZFc, as well as the STS primer sY14 was used by (SRY) sex determining region as internal control (table 1). These primer sets were recommended by Simoni *et al*. (1999), and was approved by the European Molecular Genetics Quality Network (EMQN), and European Academy of Andrology (EAA) (Vogt *et al*. 1996; Krausz *et al*. 2014). The amplification was performed in a 25 μL final volume, containing 10–100 ng DNA template, 15 pmol of each primer, and master mix containing 1.5 μL of 20 mM $MgCl_2$, 2.5 μL of 10 mM dNTPs, 0.3 μL of 5 U/μL *Taq* polymerase with 2.5 μL of 10× *Taq* Buffer (Promega, USA). The reaction volume was adjusted by adding water. PCR conditions applied were as follows: initial denaturation at 94°C for 6 min, then 35 cycles started with denaturation at 94°C for 45 s, followed by annealing at 60°C for 45 s, after which extension at 72°C for 45 s, and a final extension at 72°C for 5 min were done. The DNA fragments were separated on 3% agarose gel, and stained with ethidium bromide. The products were photographed under UV light, after resolving bands by agarose gel electrophoresis.

Results

Hormonal assay and the results revealed no significant difference between patients group and normal control subjects ($P > 0.05$) (table 2). All 185 males had a normal 46, XY karyotype, of which 90 were azoospermic and the other 95 were normal healthy control. Subjects who participated in this study were investigated for screening Y chromosome microdeletion. However, the results showed no

Table 1. Primer sequences and PCR product sizes.

STS	Forward primer	Reverse primer	AZF interval	Product size (bp)
SRY	5-GAATATTCCCGCTCTCCG GA-3	5-GCTGGTGCTCCATTCTTGAG-3		472
SY84	5-AGAAGGGTCTGAAAGCAGGT-3	5-GCCTACCTGGAGGAGGCTTC-3	AZFa	324
SY86	5-GTGACACACAGACTATGCTTC-3	5-ACACACAGAGGGACAACCCT-3		326
SY127	5-GGCTCACAAACGAAAAGAAA-3	5-CTGCAGGCAGTAATAAGGGA-3	AZFb	274
SY134	5-GTCTGCCTCACCATAAAACG-3	5-CTCGTCATGTGCAGCCAC-3		301
SY255	5-GTTACAGGATTCGGCGTGAT-3	5-CCGTGTGCTGGAGACTAATC-3	AZFc	123
SY152	5-AAGACAGTCTGCCATGTTTCA-3	5-ACAGGAGGGTACTTAGCAGT-3		125

Table 2. Serum concentrations of FSH, LH and testosterone in male patients with azoospermia and healthy fertile males.

	Azoospermic males (n = 90) Mean ± SD	Fertile males (n = 95) Mean ± SD	P value
FSH (mU/mL)	4.55 ± 2.1	6.33 ± 1.9	0.22
LH (mU/mL)	4.48 ± 2.09	3.4 ± 1.8	0.12
Testosterone (mU/mL)	4.64 ± 2.63	5.05 ± 2.2	0.08

n, Number; $P < 0.05$ significant.

microdeletions in any of the control males but were found in 43 of 90 (47.8%) azoospermic infertile males (figure 1).

The most common deleted region was AZFb, where the incidence of microdeletions was found at a ratio of 33.3% (30/90) followed by AZFc region, with a frequency 21 of 90 (23%), while no microdeletion was detected in AZFa. The largest microdeletion was detected in two complete AZF regions (b and c), in six out of 90 male patients with a ratio of 6.7%.

Discussion

Spermatogenic failure is responsible for more than half the cases of infertility. The pathogenesis of this condition is poorly understood. The molecular studies have revealed the number of aetiopathogenetic factors, which include microdeletions of the long arm of the Y chromosome (Yq) as linked to male infertility. Chromosome deletions are evolving as a major genetic defect for male induced infertility, and the occurrence of Y chromosome microdeletions rises with severe spermatogenic defect (Suganthi *et al*. 2014). In fact, the literature showed variation in frequencies of the Y chromosome microdeletions in different regions of the world. In the current study, we evaluated the prevalence of Y chromosome microdeletions in azoospermic infertile males in Iraq, and compared it with the results reported in the literature from around the world and different regions in Iraq. Accordingly, we have studied 90 patients to relate our data with the previous available reports. In the current study, seven STSs suggested by the EMQN and EAA were examined (Simoni *et al*. 2004; Krausz *et al*. 2014). Indeed, the frequency of microdeletion in a sample of the infertile male is not related significantly to the number of STS loci analysed (Simoni *et al*. 1998). Kent-First and his colleagues analysed a high number of STSs in different regions of Y chromosome; they found if any of the STS is statistically associated with male infertility (Kent-First *et al*. 1999). It seems that the selection criteria of patients have a more profound cause on the rate of finding microdeletions than the numbers of STSs analysed. In the present study, we examined 185 males (90 azoospermic patients referred to infertility centre Al-Sadder teaching Hospital in Najaf, Iraq, parallel with 95 normal males) to match our results with the previous available reports. The results of six STS used in the current study revealed classical microdeletions detected in 43 of the 90 (48.3%) azoospermic infertile males. The most common deleted region was AZFb region, where the incidence of microdeletions was found at 33.3% (30/90), followed by AZFc region, with a frequency of 21 of the 90 (23%), while no microdeletion was detected in AZFa. The incidence of the AZF microdeletions showed a high frequency, in fact the high occurrence of the AZF microdeletions in the current study is in accordance with some previous studies reported from the Iraqi population. Khalaf *et al*. (2012) have detected a high frequency of 65% in the azoospermic group: Hanoon *et al.* (2017) revealed the large frequency of Yq microdeletions (65%) using 7STS markers. In another two studies, performed by Ghorbel *et al*. (2012a,b), a lower frequency was reported in oligozoospermic and azoospermic groups, 2.7% and 1.3%, respectively. The results of our study conducted on the Iraqi population demonstrated a much higher rate of Y chromosome

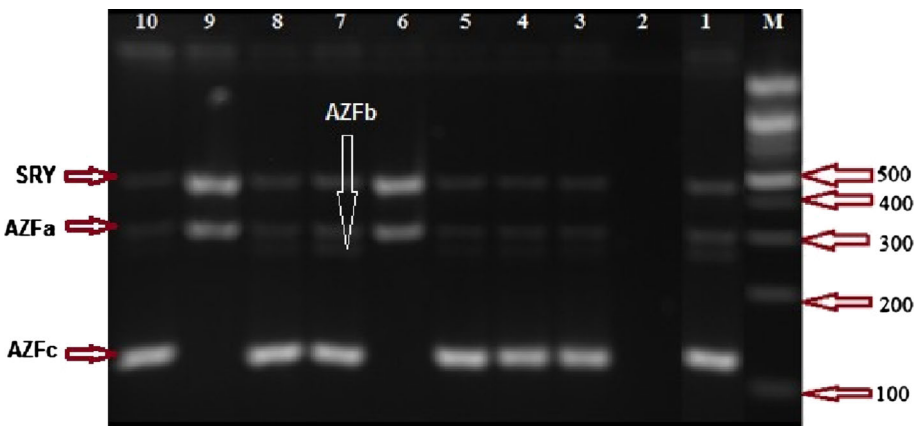

Figure 1. Multiplex PCR for SRY (472 bp); AZFa, sY84, sY86 (324 bp, 326 bp); AZFb, sY127, sY134 (274 bp, 301 bp); AZFc, sY255, sY152 (123 bp, 125 bp); lane 2, one female subject included as a negative control; lane M, 100-bp DNA ladder.

microdeletions in azoospermic subjects compared to that observed by other neighbouring counties' population. However, this rate is higher than the frequencies recorded from Turkey (3.3 and 6%) (Sargin *et al*. 2004; Cavkaytar *et al*. 2012), Kuwait (7.75%) (Alkhalaf and Al-Shoumer 2010), Iran (1.74%) (Saliminejad *et al*. 2012) and Egypt (36.7%) (Elhawary *et al*. 2010). Our previous cytogenetic studies have shown that the chromosomal abnormalities in Iraqi men suffering from infertility is about 12.5% (Yasseen and Alkhafaji 2002), compared to 6% elsewhere (Dohle *et al.* 2012). The high percentage of male infertility may be due to the mutations and possibility of the effect of depleted uranium or chemicals, which has shown to be used widely in the area. The discrepancy in the results may explain in part by population under study, ethnic differences, sample size, and lastly, the specific STS markers' application.

Finally, we concluded from this study that Y chromosome microdeletion is associated with azoospermia in infertile men, therefore performing STSs marker test in Iraqi azoospermic patients is recommended.

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