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Screening for Deletions in the AZF Region of Y Chromosome in Infertile Jordanian Males

Osamah BATİHA[1*] Mohammed A. AL-GHAZO[2] Ahmed M. ELBETİEHA[3] Saied A. JARADAT[4]

[1] Department of Biological Sciences. University of Windsor, Windsor, Canada

[2] Department of General Surgery and Urology, Jordan University of Science and Technology

[3] Department of Biology, Jordan University of Science and Technology.

[4] Princess Haya Biotechnology Center, Jordan University of Science and Technology

[*]Corresponding Author
e-mail:obatiha@gmail.com

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Abstract

Infertility is a serious problem that affects between 10%-15% of couples all over the world in which half of these cases are attributed to males. Deletions in the azoospermia factor (AZF) of the Y chromosome have been investigated and shown to be associated with azoospermia and severe oligozoospermia in many populations. However, the percentages of microdeletions were somehow variable. The purpose of this study was to investigate the association between microdeletions in the AZF region of the Y chromosome and infertility among male Jordanians.

A total of 65 oligoozospermic and non-obstructive azoospermic infertile men (Study group) and 20 fertile men (control group) were investigated for microdeletions in the AZF using multiplex PCR. We found one out of 65 (1.5%) had Y microdeletions. Microdeletions were detected in the AZFb and AZFc regions. The percentage of Y chromosome microdeletions in Jordanian infertile males is considered low compared to that from other countries. We also report a molecular genetic characterization of a sex-reversed 46,XX male, a very rare genetic abnormality in humans.

Keywords: Y chromosome microdeletions, male infertility

INTRODUCTION

Infertility is defined as the inability to conceive a child after twelve months of unprotected intercourse. It affects around 15% of couples [1], in which, male factors contribute to around 50% of them with sperm defects or dysfunction was found to be the common reason [2,3]. Genetic factors are contributing to 60% of the failure to produce mature sperm (i.e. azoospermia) or the formation of low number of sperms (i.e. oligozoospermia) [4]. Chromosomal abnormalities, both the number and the structure, are well known cause of male infertility, however, In addition to that, it has been shown that genes on the Y chromosome play an important role [1]. Deletions of the azoospermia factor (AZF) of the Y chromosome represent the most frequent molecular genetic cause of azoospermia and severe oligozoospermia [5]. These microdeletions were mapped to three non-overlapping regions, named as AZFa, AZFb, and AZFc of the Y chromosome. AZFc was shown to contain the most frequently deleted gene cluster, known as the DAZ gene [1,4].

In recent years, reproductive assisted treatments such as testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) have permitted the use of sperm from azoospermic or oligozoospermic patients to achieve successful in-vitro fertilization and pregnancies [6]. However, deletions in the Y chromosome might be spread to the male offspring, causing the persistence of infertility problems in the next generations. Therefore, we aimed to screen for Y chromosome microdeltions to determine their incidence among Jordanian infertile men.

METHODS

Blood samples from infertile men from various Jordanian fertility clinics were used in this study. Azoospermic and oligoozospermic men seeking evaluation for infertility were tested for Y-chromosome microdeletions via multiplex PCR. Semen analysis was done on semen samples following a 2-5 day period of sexual abstinence. Written informed consent was obtained from all study subjects. The hospital ethics committee approved the study.

The clinical history and investigations included gonadal anomalies, testicular volume, varicocele, epididymal and prostate abnormalities, levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone and potential testis tumors. Exclusion criteria included vas deferens obstruction, history of chemotherapy or radiotherapy, history of cryptoorchidism, varicocele, and endocrinopathy.

A total of 65 samples were collected from Azoospermic and oligospermic infertile men. Seven cases of infertility associated with varicocele were excluded. A control group of 20 males, who fathered at least two children, were included in the study. Infertile men were designated the numbers S1 to S65, while controls were given the numbers N1 to N20.

Genomic DNA was extracted from fresh whole blood collected in EDTA anticoagulant tubes using Genomic DNA Purification Kit (Promega System, Wizard Genomic DNA, USA). Y chromosome microdeletions were detected by polymerase chain reaction (PCR) amplification with a specific sequence tag site (STS) using 18-20 sets of primers (Promega

versions 1.1 and 2.0 Detection System). The four multiplexes and the STS markers included in the test are summarized in Table1.

Cytogenetic analysis was performed from phyto-haemagglutinin stimulated lymphocyte cultures by routine laboratory protocols. For microscopic analysis, metaphase chromosomes were stained with trypsin-Giemsa technique. For chromosome analysis, 10 to 20 cells were analyzed; and two to five metaphases were karyotyped.

Fluorescent In-Situ Hybridization (FISH) for SRY gene analysis was carried out using Vysis SRY Probe LSI SRY Spectrum Orange/CEP X Spectrum Green (Abbot molecular).

RESULTS

The median age of the study group was 39 years (range 21-54 years) and 37 years (range 23-42) for the control group. Of the study group, 37 were azoospermic and 28 were oligoozospermic. Only one case (S53) of microdeletions was identified among the study group (1.5%), while no microdeletion cases were detected among the control group.

The deletion in S53 was among the *AZFb* and *AZFc* regions, encompassing SYPR3, SY127, SY208 and SY242 STS markers (Figure1 and Figure 2). The first two markers (sYPR3, sY127) were in the AZFb region, while the other markers (sY242, sY208) were in the AZFc region. No deletion was detected in AZFa region.

The presence or absence of STS markers for sample 53 is summarized in Table 1.

Sample 34 showed deletion in all twenty markers that were analyzed, involving all regions (*AZFa*, *AZFb, AZFc*, *AZFd*) as shown in Figure1 and Figure 2, except the one specific for the SRY gene. To further characterize the genetic makeup of this patient, another blood sample was collected for karyotype analysis. The result showed a 46, XX karyotype (Figure 3). Further analysis using FISH techniques with two different colored probes showed that the SRY gene had translocated to the short arm of one of the X chromosomes (Figure 4).

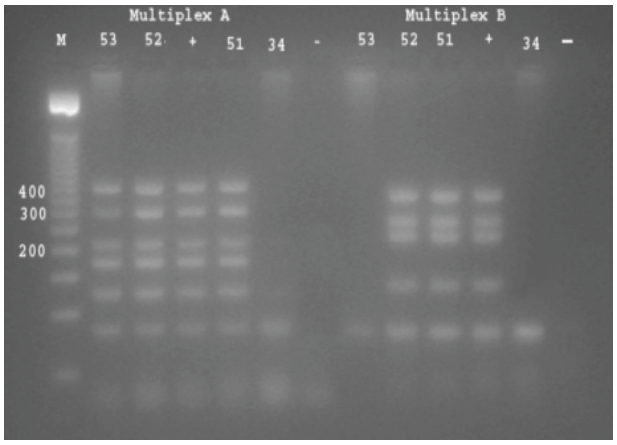

Figure 1. Multiplexes (A and B) PCR analysis for sample 53 and sample 34. Lane M; Marker, lane (+); Positive Control, lane (-); Negative Control. Sample 53 Shows deletions for markers SMCY, DYS218, and two DAZ loci in Multiplex B. Sample 34 shows deletions of all markers except the control ones.

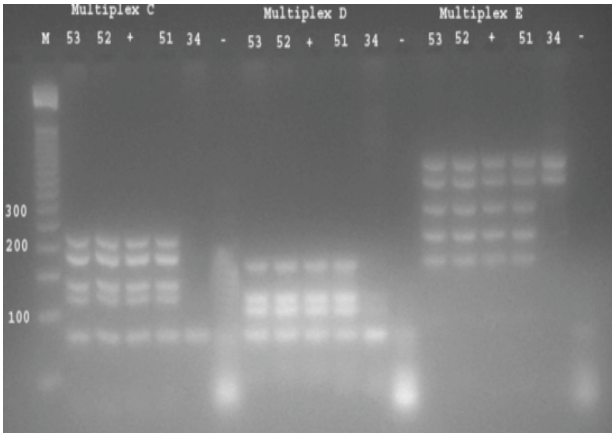

Figure 2. Multiplexes (C, D and E) PCR analysis of sample 53 and 34. Lane M; Marker, lane (+); Positive Control, lane (-); Negative Control. Sample 53 Shows deletions for markers SMCY, DYS218, and two DAZ loci in Multiplex B. Sample 34 shows deletions of all markers except the control ones.

Table1. The STS markers, their loci and the expected PCR products used in this study. The presence or absence of these STS markers in samples 53 and 34 is also shown.

STS	LOCUS	Product size	Multiplex	Sample 53 Presence/ Absence (+/-)	Sample 34 Presence/ Absence (+/-)
SY254	DAZ	370	A	+	-
SY157	DYS240	285		+	-
SY81	DYS271	209		+	-
SY130	DYS221	173		+	-
SY182	KAL-Y	125		+	-
Control	SMCX	83		+	+
SYPR3	SMCY	350	B	-	-
SY127	DYS218	274		-	-
SY242	DAZ	233		-	-
SY239	DAZ	140		-	-
Control	SMCX	83		+	+
SY128	DYS219	228	C	+	-
SY121	DYS212	190		+	-
SY145	DYF51S1	142		+	-
SY255	DAZ	126		+	-
Control	SMCX	83		+	+
SY152	DYS236	285	D	+	-
SY133	DYS223	177		+	-
SY153	DYS237	139		+	-
SY124	DYS215	109		+	-
Control	SMCX	83		+	+
Control	ZXY/ZFY	490	E	+	+
SY14	SRY	400		+	+
SY134	DYS224	303		+	-
SY86	DYS148	232		+	-
SY84	DYS273	177		+	-

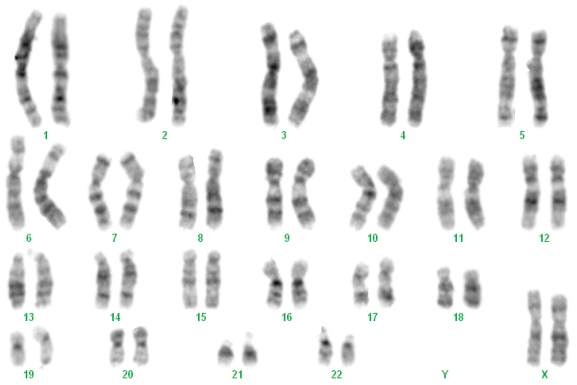

Figure 3. Karyotyping analysis for sample 34 showing 46, XX genotype.

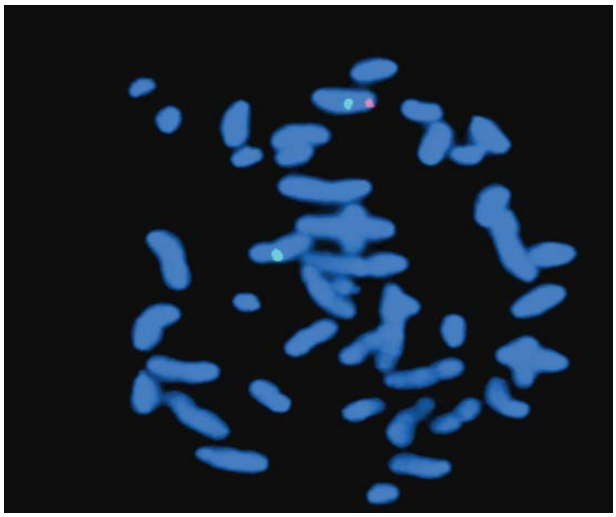

Figure 4. Fluorescent In-Situ Hybridization analysis for sample 34 showing 2 green signals specific for X-chromosome sequence and one red signal specific for SRY gene confirming the 46, XX genotype and showing the translocation of the SRY gene.

DISCUSSION

The rate of microdeletions in AZF regions on Y chromosome in this study was 1.5%. This is consistent with the findings of other studies, in which the reported range is between 2% and 15% [7-11]. This wide variation in microdeletion frequencies might be ascribed to the population variances, study design, inclusion criteria and type and number of STS tested.

The analysis of the deletion of STS loci in infertile men is challenging, because deletions of some of these loci were found to be inherited while others where found as de-novo mutations [12]

Sample No. 53 was oligozoospermic and found to carry a deletion of markers sYPR3, sY127 in the AZFb regions, and markers sY242, sY208 in the AZFc. The first marker amplifies region within the SMCY gene, while the second marker amplifies the DYS218 locus, the other two markers amplify regions within the DAZ gene cluster. SMCY has a homologue on the X chromosome (SMCX), and codes for H-Y HLA antigen that is expressed in many tissues [12]. Therefore, deletion in this gene may not be strongly associated with infertility. On the other hand, DAZ is known to play important roles in male germ line maintenance, and deletion of this gene has been strongly associated with azoospermia [13, 14]. Studies have shown a significant association between spontaneous recurrent pregnancy loss (RPL) and Y chromosome deletions [15,16]. However, a recent study came out to contradict these results and showed no association between Y chromosome microdeltions and RPL [17]. Nevertheless, in this later study, males were normospermic, and this may explain why no Y chromosome microdletions were found.

One of the most important finding in this study was with Sample 34, which initially showed azoospermia and normal male phenotype. Microdeletion analysis revealed that all of the 20 STS markers were deleted except the sY14, which is specific for a region within the SRY gene. The SRY gene is normally located on the short arm of the Y chromosome close to the PAR boundary. It has been proposed to be the master regulator of testis determination [18]. Karyotyping analysis for this patient revealed a 46, XX genotype. Fluorescent In-Situ Hybridization has been used to locate Y chromosome sequences. We have shown that the SRY gene was translocated to the distal tip of the short arm of an X chromosome. The clinical appearance of sample 34 is compatible with the characteristics of classical XX male patients. XX maleness, known as de la Chapelle syndrome or sex reversed XX males. Sex reversed XX males is a rare abnormality of sex determination that has an incidence of 1 in 200000 new born males and accounts for approximately 2% of male infertility cases [19]

The presence of SRY marker (sY14) in sample 34 most probably has led to a male phenotype, but the complete absence of the AZF regions might have contributed to his azoospermia.

The results of this study concur with the results of other studies in suggesting that genetic testing is desirable before embarking on ICSI [20]. In addition to other clinical and genetic examinations, it is necessary for infertile men with azoospermia or severe oligozoospermia to be screened for Y choromosome microdeletios before embarking on such procedures to avoid transferring infertility problems to their offspring.

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