Y Chromosome Microdeletions in Infertile Men from South West of Iran

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***Abstract*—**Today, with advances in assisted reproductive techniques, many infertile couples are able to have children. However, there is always risk of passing genetic abnormalities associated with infertility from parents to children. Therefore, detection of microdeletions of Y chromosome in patients with spermatogenesis failure seems very important. The purpose of this study was to determine the frequency of microdeletions in Y chromosome in infertile men with non-obstructive azoospermia or severe oligozoospermia in South West of Iran, Shiraz. 60 infertile men with severe oligozoospermia and non-obstructive azoospermia were examined. Multiplex PCR was applied to detect the microdeletions. Frequency of microdeletions in men with severe oligozoospermia and azoospermia was 8.3%. All deletions were observed in AZFc region. This study emphasizes that analysis of microdeletions should be carried out for all patients with idiopathic azoospermia and severe oligospermia who are candidates for intra cytoplasmic sperm injection.

***Keywords*—**Infertility, Azoospermia, Severe oligozoospermia, Y chromosome Microdeletions**.**

I. Introduction

ABOUT 14 percent of young couples suffer from infertility which is defined as the inability to conceive after one year of unprotected intercourse. In approximately 40 to 50 percent of the cases, male infertility is the cause [1]. Genetically, after Klinefelter syndrome, microdeletions of long arm of Y chromosome are the most frequent cause of male infertility [reviewed in 2]. Azoospermic factor (AZF) region of chromosome Y comprises of three regions, AZFa, AZFb, AZFc. Due to presence of palindromic sequence in these regions and their homologous recombination [3], deletions with different scales may occur. Deletions in these regions happen with different frequency (80% for AZFc, 1-5% for AZFb, 1-3% AZFbc, 0.5-4% AZFa) and can lead to spermatogenesis failure and absence of sperm in azoospermia or reduction of sperm count in severe oligozoospermia (Less than 5 million per milliliter) [4]. AZFa contains USP9Y and DDX3Y genes which are both removed when complete deletion of AZFa region occur [reviewed in 5]. AZFb and AZFc together comprise 24 genes which most of them present in several copies. Depending on the scale of the deletions, different genes are removed from these regions. Although these deletions are normally associated with spermatogenesis failure and reduced count of sperm, fertilization may occur depending on the status of female fertility or using assisted reproductive techniques (ART). Complete deletion of AZFa leads to Sertoli cell only syndrome (SCOS) and azoospermia [4]. AZFb complete deletion is also associated with spermatogenesis failure and azoospermia. In all cases with complete deletion of AZFa and in complete deletion of AZFb (other than a few cases) retrieving testicular spermatozoa [using testicular sperm extraction (TESE)] for intracytoplasmic injection (ICSI) is impossible [reviewed in 5]. However, with complete deletion of AZFc, there is a chance for retrieving sperm after TESE because this deletion shows a variety of phenotypes and reported in males with azoospermia and also severe oligospermia. Therefore, detecting these microdeletions can help to decide whether TESE must be applied or not. Moreover, microdeletions can pass to offspring when fertilization occur using assisted reproductive techniques (ART) which in turn can lead to the infertility of the offspring. Many studies have investigated the microdeletions of the Y chromosome all over the world [reviewed in 6], [7] including several areas of Iran [8]-[12] and their frequency has been determined to vary from 2 to 24%. Different Y chromosome background, selecting different ethnic groups, difference in the pathological criteria for selecting patients, and methodological issues can be considered as the sources of the differences observed in the frequency of Y chromosome microdeletions. In this study, we investigated Y chromosome microdeletions frequency among infertile males suffering from azoospermia or oligospermia who referred to the Dr Rostami's infertility center in the South West of Iran.

II. Materials and Methods

In this study, 60 infertile male [50 non-obstructive azoospermic patients and 10 patients with severe oligospermia (sperm concentration of less than 5×10^6)] from Dr Rostami's infertility center were screened for Y chromosome microdeletions. After signing the consent form, blood samples were obtained from patients and stored in -20° C until used. Genomic DNA was extracted from blood samples by the boiling method. Thermo Scientific™ NanoDrop spectrophotometer was applied to determine the concentration and purity of the extracted DNA. Two sets of Multiplex PCR (A and B) were applied to detect the AZFa, AZFb, AZFc microdeletions using primers (table 1) suggested by the

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European Academy of Andrology/European Molecular Genetics Quality Network (EAA/EMQN) [2],[5].

Sequence of the PCR primers

Multiplex Primers	
A and B	*SRY*-F: 5′-GAA TAT TCC CGC TCT CCG GA-3′
	SRY-R: 5′-GCT GGT GCT CCA TTC TTG AG-3′
A.	sY86-F: 5′-GTG ACA CAC AGA CTA TGC TTC-3′
	sY86-R: 5′-ACA CAC AGA GGG ACA ACC CT-3′
A.	sY127-F: 5′-GGC TCA CAA ACG AAA AGA AA-3′
	sY127-R: 5′-CTG CAG GCA GTA ATA AGG GA-3′
A.	sY254-F: 5′-GGG TGT TAC CAG AAG GCA AA-3′
	sY254-R: 5′-GAA CCG TAT CTA CCA AAG CAG C-
B.	sY84-F: 5′-AGA AGG GTC TGA AAG CAG GT-3′
	sY84-R: 5′-GCC TAC TAC CTG GAG GCT TC-3′
B.	sY134-F: 5′-GTC TGC CTC ACC ATA AAA CG-3′
	sY134-R: 5′-ACC ACT GCC AAA ACT TTC AA-3′
B.	sY255-F: 5′-GTT ACA GGA TTC GGC GTG AT-3′
	sY255-R: 5′-CTC GTC ATG TGC AGC CAC-3′

Both sets of multiplex PCR were carreied out using Amplicon Multiplex PCR MasterMix (containing HotStart Taq DNA Polymerase, multiplex buffer with 1.5mM $MgCl_2$ and dNTP mix). PCR conditions were as follows: initial activation for 5 min at 94° C, followed by 35 cycles of 60 Sec denaturation (94° C), 35 Sec annealing (58° C), and 30 Sec extension (72°C), and 1 cycle of final extension at 72°C for 10 min. PCR products were run on 1.5% and 2% agarose gel for multiplex A and B, respectively.

III. Results and Discussions

This study revealed that 8.3 % of infertile patients with either non-obstructive azoospermia or severe oligospermia carried microdeletions. The frequency of Y chromosome microdeletions was 4% in azoospermic cases and 30% in oligospermic patients. All of these microdeletions were detected in the AZFc region. Both STS markers, sY254, sY255, were absent in patients with microdeletions showing the complete deletion of AZFc. There is a considerable variation in the frequency of Y chromosome microdeletions reported in various investigations including the ones performed in different parts of Iran. In West Azarbaijan, 15.4% and 30% Y chromosome microdeletions were observed in infertile male with severe oligospermia and azoospermia, respectively [8]. The frequency of 24% of these microdeletions was reported in [9]. However, 2.13% and 1.8% Y chromosome microdeletions were detected in azoospermic and oligospermic cases in [10] and [12], respectively. Different patients' selection criteria and composition of the study population [5], various diagnostic protocols and inaccurate or wrong diagnostic [10], may result in frequency variations in different reports. There is also heterogeneity in selecting PCR markers both in type and number in various investigations. In order to obtain a standard protocol to detect these microdeletions, European Academy of Andrology (EAA) and European Molecular Genetics Quality Network (EMQN) has approved to publish several papers with an accurate and valid guideline for screening Y chromosome microdeletions [2], [5], [13]. It has been recommended that two sets of multiplex PCR, each containing STS marker primers for detecting all three AZF regions, is accurate enough to detect the complete deletion of each AZF region. According this protocol, Primers of sY86 and sY84 are used for AZFa, sY134 and sY127 primers for AZFb and sY255 and sY254 primers for AZFc [2], [5], [13], [14]. Therefore, in this study, we used exactly the same primers recommended in the EAA/EMQN protocol. Interestingly, the frequency of Y chromosome microdeletions in our study is much higher compared to studies [10], [12] carried out in different parts of Iran which applied the same STS markers approved by EAA/EMQN. It can be concluded that Y chromosome microdeletions detection is necessary for infertile male suffering from azoospermia and oligospermia in the South West of Iran before any decision for TESE and ICSI. Sperm retrieval is inevitably impossible in cases with complete deletion of AZFa or AZFb. Therefore, other solutions can be applied for infertile males with those deletions. Moreover, there is always risk of transmission of the microdeletions to any male child and genetic counseling is necessary prior to the treatment. The authors would like to

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