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Y-chromosomal microdeletions of the Azoospermia Factor (AZF) region in infertile men in IRAN

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Abstract: To assess for the first time the occurrence of Y chromosomal microdeletions of the Azoospermia Factor c (AZFc) region in Iranian men and to correlate them with clinical parameters. **Materials and Methods**: In a retrospective study, we analyzed 100 infertile men and 100 controls with normal spermatogenesis. AZFa, AZFb, AZFc region were analyzed by multiplex polymerase chain reaction (PCR). **Results**: No AZFa, AZFb or AZFc deletions were found in the control group. Nine patients in the group of infertile men were found to have deletions as following: two AZFb, six AZFc, and one AZFab. The relative distribution of these patterns was significantly different compared with that found in the German population. Extension analysis confirmed that the deletions occurred according to the current pathogenic model. **Conclusions**: Our results suggest that the frequency of Y chromosome AZF microdeletions is elevated in individuals with severe spermatogenic failure.
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1. Introduction:

It is estimated that 5–10% of healthy men suffer from infertility. Spermatogenic failure is the most common form of male infertility, and the significant role of the Y chromosome in male infertility is increasingly identified (Okabe, 1998). The male-specific region of the Y chromosome (MSY) be composed of long, Y-specific repeats called amplicons. Homologous recombination between amplicons has been shown to produce deletions, commonly resulting in spermatogenic failure. Three azoospermia factors (AZFa, AZFb, and AZFc) have been mapped to Yq11, and the AZFc region completely include of amplicons is particularly sensitive to deletions, the most commonly known genetic factor that leads to azoospermia or oligozoospermia (Vogt, 1996). Although it is difficult to establish exact genotype/phenotype correlations in patients with Y chromosomal microdeletions, deletions of AZFa or AZFb and deletions involving more than one region (AZFbc or AZFabc) have more severe effects on spermatogenesis than deletions of the AZFc region [Simoni, 2004; Hopps, 2003].

Y chromosome microdeletions have been identified in different populations with different frequency of deletions that ranges from 1.3% to 55%. Multi-region involvement (AZFbc or AZFabc) and deletions of AZFa were recorded at very low frequencies in the German population [Maurer, 2001]. The published information for Asia shown a certain changeability in the deletion frequency depending on the selection criteria of the patients. When patients with azoospermia or severe oligozoospermia are considered together, the frequency of microdeletions varies from 5 % in Eastern Uttar Pradesh in India [Ambasudhan, 2003], to 7.6 % in Japan [Kato, 2001], 8.5 % in Calcutta, India [Thangaraj, 2003], 9 % in China [Chiang, 2004] and 10.6 % in Taiwan, China [Lin, 2000]. Interestingly, much higher frequencies of AZFa deletions (17.2 % in India) and AZFbc deletions (51.7 % in India and 36.6 % in China) have been recorded, compared with those in Europe [Thangaraj, 2003; Chiang, 2004].

These studies suggest that geographical and ethnic differences might influence the frequencies of AZF deletions of the AZF region, as well as the deletion patterns and, possibly, the phenotypic expression. The purpose of this study was to investigate the occurrence of Y chromosomal microdeletions of the AZFc region in the Iranian men.

2. Material and methods:

2. 1. Study group:

The study population consisted of an unselected group of 100 infertile men and 100 fertile men. Informed consent was obtained from each subject. All subjects and controls were of IRAN ethnic origin.

The subjects were divided into two groups 70 oligozoospermic men (sperm concentration < 20 × 106/mL but > 1 × 106/mL) and 30 severely oligozoospermic men (sperm concentration < 1 × 106/mL). All patients were examined by a clinician for size, volume and consistency of the testis, varicocele and secondary sexual characteristics. Hormone profiles (serum follicle-stimulating hormone [FSH], luteinizing hormone [LH] and testosterone) of all patients were collected according to seminal. The control population consisted of 100 men with either known fertility (at least one child, n = 76) and normospermic men (> 20 million sperm/mL, n = 24). The seminal analysis was done according to the World Health Organization criteria.

2. 2. AZF DNA analysis by sequence tagged site (STS) polymerase chain reaction (PCR)-based strategy

Genomic DNA was prepared from peripheral blood samples using standard procedures. and amplified in multiplex polymerase chain reaction (PCR). Each of these subjects was tested for four AZF loci: the STS primers used for AZFa (sY84) AZFb (sY87) and AZFc (sY254, sY255). The internal control used was SRY14, samples from normal fertile men, without Y chromosome microdeletions and from healthy women, were used as normal controls, blank served as negative control. A total of 50-100 ng of genomic DNA was used as template in 25 µL reaction mix, 1× amplification buffer, 1-1.5 mmol/L MgCl2, 1 mmol dNTPs, 10-20 pmol of each primer and Taq DNA polymerase (1 unit). After an initial denaturation step of 5 min, each PCR reaction was carried at the annealing temperature specific for each primer pair, ended by an elongation step of 10 min and cooling to 4 ºC. The PCR products were separated on 1-2% agarose gels stained with ethidium bromide, on the basis of the size of the product obtained. In case of any failure in amplification in of samples, two additional PCRs were performed to confirm the absence of the unamplified STSs.

3. Results

One hundred subjects were screened for the presence of Yq microdeletions. Among them, 70 were azoospermic and 30 were oligospermic. We did not screen the subjects with asthenozoospermia for microdeletions (Table 1).

Table1. Frequncy of Y chromosome microdeletion

Phenotype	n	Deletion(%)	region
Azospermia	**70**	**6(6%)**	**AZFc,AZFb ,AZFc+AZFb**
Oligosperima	**30**	**1(1%)**	**AZFc**
Total	**100**	**7(7%)**	

Seven subjects were found to have microdeletions. One of these seven men (case 23) had severe oligozoospermia, and the rest had azoospermia. Microdeletions were not observed in control samples. All men with microdeletions had normal serum testosterone levels. Four patients had high serum FSH levels. One patient (case 96) showed increased serum FSH and LH levels; the LH level was normal in the others. The deletions were present in 6 azoospermic men (6/70, 8.56%): four of them were idiopathic and two was cryptorchid.

The overall frequency of microdeletions in infertile men was 7% (7/100). In azoospermic men, five deletions of the AZFc were detected: tree of them had cryptorchidism. Two deletions involving the AZFc regions had Sertoli-cell-only syndrome (SCOS) in the testicular biopsy. The further characterization of the microdeletions by extension analysis showed AZFb deletion in case 87. The microdeletion patterns observed were AZFc in five patients (cases 23,32,54,67 and 84) (Figure1), AZFbc in one patients (cases 96). No microdeletions were found in the AZFa region.

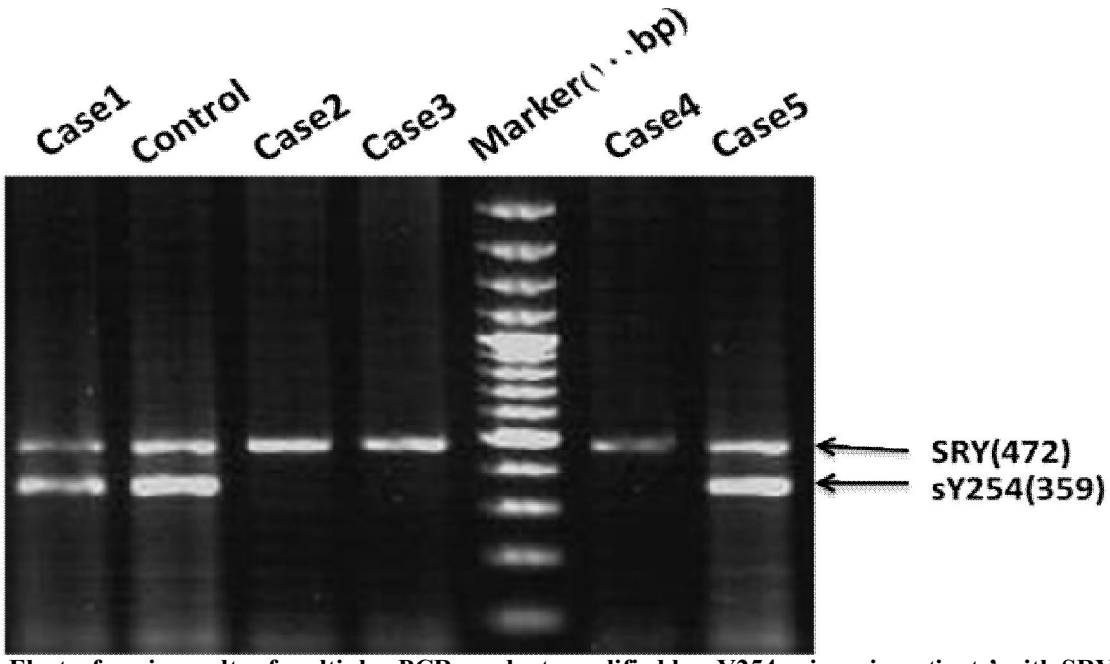

Fig 1. Electroforesis results of multiplex PCR product amplified by sY254 primer in patients' with SRY control. Arrows represent the deleted subjects.

4. Discussion

A number of genes on the Y chromosome and autosomes regulate spermatogenesis and Y chromosome deletions are emerging as a prevalent cause of male factor infertility [Krausz, 2000]. The frequency of Y chromosome deletions increases with the severity of spermatogenic defect [Pryor, 1997]. The reported incidence of Y chromosome deletion varies among the studies: approximately 15% of azoospermic and 5%–10% of oligozoospermics men. The frequency of deletions was reported to be in a range of 0.5%–34.5% in various studies [Foresta, 2001]. As previously stated [Simoni, 1998] the reason for this, might be the criteria for selecting patients, STS markers used, rather than the number of markers (STSs) used for screening protocol. It has been proposed that ethnic differences might be associated with the frequencies of Y chromosome microdeletions [Krausz, 1999]. Microdeletions in the Swedish population were reported only in immigrant populations of infertile patients [Osterlund, 2000] and with a very low frequency in Croatian infertile men [Medica, 2005]. Certain microdeletions pattern may be related to the origin of population [Peterlin, 2004]: SY240, SY129 in the Japanese population [Kato, 2001], SY269, GY6 in the Italian population [Foresta, 2009], SY100 in the French population [Krausz, 1999]. In our study, AZFc SY255 and SY254 were lost in 6/7 (85.7%), AZFb SY87 was lost in 1/7 (14.2%) of deleted patients.

In the present study, we report the analysis of Y microdeletions in the Iranian population. We found that 7 of the 100 infertile Iranian subjects tested harbored microdeletion in the AZF region (7%). In three of the subjects, testicular histology was available and two subjects had SCOS. All the subjects had AZF region deletion and showed an azoospermic phenotype, which is in accordance with the suggestion that deletions in these regions have an adverse prognosis for finding sperm in the testicular biopsies [Hopps, 2003]. Our results are similar to the published data; the deletions of such AZF region are associated with SCOS and spermatogenic arrest [Kamp, 2004]. The deletions found in the present study concern the AZFc and AZFb + AZFc regions. No deletions were found in the AZFa region. The frequency of AZF deletions in severe oligozoospermia was found to be lower than those in azoospermia. In the literature, the vast majority of deletions were found in azoospermic men with deletion frequencies up to 7%.

In our study, AZFc SY255 and SY254 were lost in 6/7 (85.7%), AZFb SY87 was lost in 1/7 (14.2%) of deleted patients. Our study shows that the microdeletion frequency (7%)found in 100 iranian infertile men is comparable to the frequencies reported in the literature in a selected group of infertile men with severe spermatogenesis failure. In conclusion, the incidence of Yq deletions in the studypopulation of infertile Iranian men falls within the range published in other countries.We estimate that an efficient test to detect Yq microdeletions in our population must analyze with more STS markers.

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