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Original Article

Frequency of Y Chromosome Microdeletions in Azoospermic and Oligospermic Iranian Infertile Men

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ABSTRACT

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Background and Aims: Azoospermia factor (AZF) region of the Y-chromosome has several genes which are responsible for normal spermatogenesis. Microdeletions of these genes are associated with azoospermia and oligospermia. These microdeletions are too small to be detected by karyotyping. They can be easily identified using polymerase chain reaction. The aim of this study is to determine the frequencies of Y-chromosome microdeletions in azoospermic and oligospermic Iranian infertile men and compare them with other studies in different ethnic groups.

Materials and Methods: At first, karyotype analysis was performed in 80 infertile men and 30 healthy age-matched counterparts as control group using standard cytogenetic methods. Second, genomic DNA was extracted from all cases and genetic screening was conducted for Y chromosome microdeletions by multiplex polymerase chain reaction for AZF genes on both infertile and control men using 6 STS markers on the long arm of the Y chromosome.

Results: Totally**,** 49 infertile men were azoospermic and 31 were oligospermic. Y-chromosome microdeletions in the AZFc region were detected in 4 of azoospermic patients. Y-chromosome microdeletions was not detected in any of the oligospermic patients and the control group.

Conclusions: This finding recommends that genetic counseling and screening before starting assisted reproductive techniques such as in vitro fertilisation and intracytoplasmic sperm injection can prevent unnecessary treatment and transmission of genetic defects to offspring.

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Introduction

Infertility is a major health problem today affecting 10-15% of the couples. In 40-50% of the cases, the male partner has quantitative or qualitative abnormalities of sperm production [1]. The known causes of male infertility are quite numerous and are divisible into several major categories. Male infertility has been associated with diverse genetic and non-genetic conditions [2]. Genetic abnormalities such as Y-chromosome microdeletions, Chromosomal abnormalities and single gene defects are the most common causes of male infertility [3]. Cytogenetic and molecular studies on azoospermic and oligospermic men with idiopathic infertility have confirmed the presence of genes that are responsible for spermatogenesis. The position of spermatogenesis locus was traced in the long Y arm (Yq11). This area of chromosome Y has been called azoospermia factor (AZF) [4]. In early 1967, this azoospermia factor was demonstrated by Tiepolo and Zuffardi and considered as an essential factor for performing spermatogenesis [5]. AZF region is divided into the AZFa, AZFb and AZFc sub regions that are in proximal, middle and distal of Yq11, respectively [6]. Each of these subregions acts at a different point in spermatogenesis process. Based on testis pathology, microdeletion in AZFa is related to deletion of germ cells and the presence of sertoli cells in seminiferous tubule that are characterized by sertoli cell-only syndrome and is associated with azoospermia. Additionally, microdeletion in AZFb is related to inhibiting the growth of germ cells in the pachytene stage leading to containing the process of meiosis division. Microdeletion in AZFc is related to stopping the germ cells in spermatid phase. Moreover, it is associated with hypospermatogenesis, maturation arrest and severe decrease in sperm count.

Therefore, eliminating a specific locus of AZF would have certain phenotypic results [7, 8]. Y chromosome microdeletions have been identified in 10-15% of azoospermia and 5-10% of idiopathic severe oligospermia [9]. In other words, after Klinefelter syndrome, Y-chromosomal microdeletions are the second most frequent genetic cause of azoospermic and oligospermic men. Although microdeletions are relatively frequentative among men with idiopathic azoospermia, oligospermia, the incidence can vary considerably depending on the selection criteria of the patients [6]. Utilizing recent assisted reproductive technologies (ART) such as *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) could treat infertility. Applying ART in men with Yq microdeletions could increase Infertility risk and other diseases in their male offspring.

Therefore, evaluating Y chromosome microdeletions in infertile male population before using ART is recommended. The present study was conducted to investigate the frequency of Y chromosome microdeletions using multiplex polymerase chain reaction (PCR) in men with idiopathic azoospermia or oligospermia.

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Materials and Methods

Eighty men with idiopathic infertility and 30 male age-matched normal controls were selected for the molecular AZF screening program and were analyzed. The infertility group included 49 males with azoospermia and 31 with oligospermia. Karyotype analysis was performed in all cases by using standard cytogenetic methods. The karyotype was normal in all cases. Genomic DNA was extracted from peripheral blood leukocytes of patients and 30 healthy controls using salting out method. PCR-based studies for Y chromosome microdeletions on both infertile and control men were carried out using 6 STS markers on the long arm of the Y chromosome. Each patient was examined for 3 AZF loci mapped to interval 5 and 6 of the Y chromosome. The ZFY gene was used as internal control. The following STS markers were used: sY84, sY86 (AZFa); sY127, sY134 (AZFb); and sY254, sY255 (AZFc). The primers set used in this study (table 1) were suggested by Simoni et al. and are recommended by the European Academy of Andrology [6]. It enables the detection of over 95% of deletions in the AZF locus and allows minimal standardization and comparison of the data on AZF deletions from diverse laboratories in different countries. Two multiplex reactions were designed for the analysis of the three AZF deletion regions. Both multiplexes contain 4 fragments, i.e. the three AZF and one control fragment ZFY. Briefly, 50–100ng of genomic DNA was used as template in 25mL reaction mix, 1.4ml amplification buffer, 5mM $MgCl_2$, 1.2mM dNTPs, 0.28µM of each primer, and 0.4U/µl of *Taq* DNA polymerase. After an initial denaturation step at 95°C for 5 minutes, each PCR reaction was carried at the specific annealing temperature for each multiplex set. The PCR products were separated on 3.5% agarose gels stained with ethidium bromide on the basis of the size of the product obtained. Whenever failure of amplification in any sample was detected, two additional PCRs were performed to confirm the absence of the unamplified STSs.

Table 1. Primer sequences and product sizes of 6 Y STSs that used in this study. Multiplex A: sY84, sY134, sY255 and ZFY. Multiplex B: sY86, sY127, sY254 and ZFY

Primer Name	Sequence	AZF Region	Product Size (bp)
sY84	F: 5'-AGA AGG GTC TGA AAG CAG GT-3' R: 5'-GCC TAC TAC CTG GAG GCT TC-3'	AZFa	213
sY86	F: 5'-GTG ACA CAC AGA CTA TGC TTC-3' R: 5'-ACA CAC AGA GGG ACA ACC CT-3'	AZFa	320
sY127	F: 5'-GGC TCA CAA ACG AAA AGA AA-3' R: 5'-CTG CAG GCA GTA ATA AGG GA-3'	AZFb	274
sY134	F: 5'-GTC TGC CTC ACC ATA AAA CG-3' R: 5'-ACC ACT GCC AAA ACT TTC AA-3'	AZFb	301
sY254	F: 5'- GGG TGT TAC CAG AAG GCA AA-3' R: 5'- GAA CCG TAT CTA CCA AAG CAG C-3'	AZFc	400
sY255	F: 5'- GTT ACA GGA TTC GGC GTG AT-3' R: 5'- CTC GTC ATG TCA TGT GCA GCC AC-3'	AZFc	126
ZFY	F: 5'-ACC RCT GTA CTG ACT GTG ATT ACA C-3' R: 5'-GCA CYT CTT TGG TAT CYG AGA AAG T-3'	Internal control	495

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Results

Among the 80 infertile men, 49 were azoospermic and 31 were severely oligospermic. PCR microdeletion analysis was executed in all 80 infertile patients and the control group. PCR amplification produced a band of the expected size for all six loci investigated in normal fertile men. The most frequent microdeletions were detected in the AZFc region. Y-chromosome microdeletions in the AZFc region were traced in 4 of 80 cases (5%) (Fig. 1). These patients had complete deletion of AZFc loci, and the deletions were detected completely within the AZFc region in the interval 6 of the Y chromosome. No deletions were found in AZFa and AZFb regions among all infertile men. None of oligospermic patients had Y chromosome microdeletion. To explore the possible contribution of Y chromosome deletions in idiopathic infertility of Iranian males with azoospermia or oligospermia, 30 normal fertile males with normospermia as a control group were analyzed for whom no deletion was found. The total frequency of the microdeletions was 5% that comprised 4 cases in azoospermic but none in the oligospermic and the control groups.

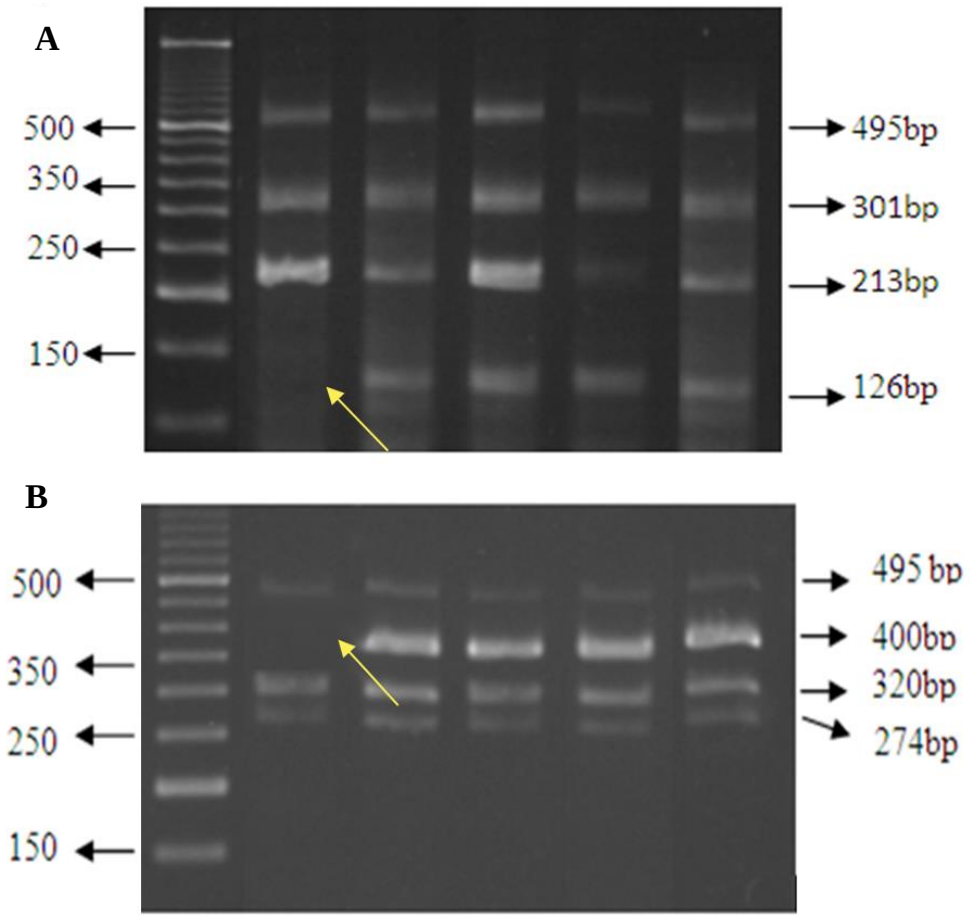


Fig. 1. Y chromosome microdeletion in AZFc region.

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Discussion

A widespread variety of genes on the Y chromosome and autosomes conduct spermatogenesis. Y chromosome deletions are known as a significant cause of male infertility factors. The frequency of the Y chromosome deletions increases by the severity of the spermatogenic imperfection [1].

Recent advances in molecular biology propose that Y-chromosome microdeletions are the second most prevalent genetic motive of spermatogenic failure and male infertility after Klinefelter's syndrome. The phenotype of azoospermia or sever oligospermia can be associated with a diversity of spermatogenic imperfections [7]. While according to former scientific consensus Y chromosome was believed to be poor in terms of gene contents, discovering the genetic complexity of the AZF region, which is divided into AZFa, AZFb and AZFc locuses, proved that its q arm constitutes most of heterochromatic regions. Molecular mapping analysis has shown that at least some but not all of these loci harbors are responsible for spermatogenesis [4]. Each AZF locus of the Y chromosome is associated with distinct phases of spermatogenesis and deletion of each locus disrupts spermatogenesis at a particular phase. Firstly, the presence of Sertoli cells within the seminiferous tubules along with an entire absence of germ cells is associated with AZFa deletion.

Secondy, the deletion of AZFb is associated with germ cell development arrest at the pachytene stage, and third, AZFc deletion is related to germ cell improvement arrest at the spermatid stage and maturation arrest. These deletions have been reported in 2.7–55.5% of infertile men [12,13,14]. Typically, around 15% of azoospermic and 5–10% of oligospermic men show Y chromosome deletions. However, these Y chromosome microdeletions could not be anticipated cytogenetically, based on the clinical findings, and through the semen analysis. Thus, PCR-based screening of AZF regions for microdeletions on the Y chromosome is essential. Formerly, the diagnosis of a genetic etiology had little clinical importance. Nowadays, the assisted reproductive technology helps in surmounting this infertility problem, yet there is still the transmission of the genetic defects, like microdeletion, to their offspring. Consequently, this diagnosis presents the vital information to counsel these couples efficiently, particularly in regards to the birth of an infertile male offspring who might also have the same or secondary larger deletions with more severe testicular phenotype [1]. In this study, Y-chromosome microdeletions were detected in four cases (5%; 4/80). In all four cases, Y-chromosome microdeletions in the AZFc region have been found. The AZFc region is the most dynamic region on the Yq according to different studies.

Several researches have established the frequency of AZF microdeletions amongst infertile men and proved AZF microdeletions bearing a substantial impact on azoospermia and oligospermia in infertile men globally. Similar studies in other regions of Iran have confirmed our findings, where these results prove that Y

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chromosome microdeletions are present in Iranian infertile men with azoospermia and severe oligozoospermia. In addition, most of these results demonstrate that the AZFc region is the most important region on the Yq to the extent by which it can cause azoospermia and oligospermia. Also, other studies have mainly reported a frequency of less than 10% for Y chromosome microdeletions [16, 17, 18, 19, 20]. The most frequent microdeletions were detected within the AZFc region Özdemir. et al. [21] reported a 2.7% prevalence for Yq microdeletions among 1696 cases with primary male infertility from Turkey while Kim [22] stated an alternation of about 10% for microdeletions among 1,226 azoospermic/oligospermic infertile men in Korea, a level much higher than the other report. In another study, Kovacheva1 et al. [23] observed a frequency of Yq microdeletions of 5.5% among 142 Bulgarian males with azoospermia or drastic oligospermia. The results of this study are consistent with those of ours. Our results indicate that the frequency of microdeletions in the Y choromosome of azoospermic men is higher than that of oligospermic infertile men. Johnson et al. [24] observed a frequency of 4% in the prevalence of Yq microdeletions and identified most microdeletions in AZFc, which resembles our results. Han, et al. [25] also observed that among the 78 infertile men with Y-microdeletions, the most frequent microdeletions are detected in the AZFc region. The occurrence of the deletions in this study is lower than that some of the previously submitted works. The limited population or the smaller number of PCR primers included in this study and the patient selection criteria might be responsible factors for this discrepancy. In fact, no deletion was observed in oligozoospermic cases. Probably, this variability is related to differences in the selection of patient groups, ethnic differences, and sample sizes. Other reasons might include the heterogeneity of STSs used in distinctive studies [4].

Conclusion

On balance, this study shows that the frequency of Y chromosome microdeletion in investigating Iranian patients is almost identical to that of reported in other countries. The findings suggest that genetic counseling and screening before starting assisted reproductive techniques such as IVF and ICSI can prevent unnecessary treatment and transmission of genetic defects to offspring.

Conflict of Interest

The authors have no financial conflict of interest.

Acknowledgment

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