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Y chromosome Microdeletions in Infertile Men with Severe Oligozoospermia

Dr.Kyumars Safinejad*[1], Leila Yadegar[2], Dr. Massoud Houshmand[3, 4], Dr. Reza Mirfakhraie[5], Dr. Esmaeel Mohammadi Pargoo[6]

[1]Department of Biology, Broojerd Branch, Islamic Azad University, Broojerd, Iran
[2] Qom University of medical sciences, Qom, IRAN
[3] National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
[4]Department of Genetic, Special Medical Center, Tehran, Iran
[5]Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences and Health Services, Tehran, Iran
[6]Department of Genetic, Special Medical Center, Tehran, Iran

ABSTRACT

This study was designed to determine the correlation between Y chromosome azoospermia factor (AZF) subregions microdeletions and severe oligozoospermia in Infertile Men. Subjects included 89 infertile men with severe oligozoospermia who had been referred to the Royan-2 infertility center for assisted reproduction. DNA was isolated from blood samples. Polymerase chain reaction (PCR) amplification of 6 loci spanning the AZFa, AZFb and AZFc subregions of the Y chromosome using sY84, sY86, sY127, sY134, sY254 and sY255 was performed. Microdeletions of the Y chromosome were found in 26 of the patients (29%). Microdeletions in the AZFa subregion (61%) were encountered more often than in AZFb (21%) and in AZFc (18%) in the patients. Our study showed that men with severe oligozoospermia should be evaluated for Yq11 microdeletions before deciding to operate varicoceles or else scheduling them for assisted reproductive techniques and there is a specific correlation between Y chromosome AZF subregions microdeletions and severe oligozoospermia.
KEY WORDS: Yq11, AZF microdeletions ,subregions, assisted reproduction, PCR

INTRODUCTION

Y chromosome microdeletions(YCM) are the most frequently observed structural abnormalities in the male-specific region of the Y chromosome(Georgiou et al., 2006), and of primary spermatogenesis failures, 15% are related to at least 6 known major YCM patterns(Cram et al., 2006). Microdeletions are present in 5% to 10% of infertile men (Ferlin et al.,2006) .Specifically, they have been reported in 6% to 16% of azoospermic men, and 4% to 5.8% of those with severe oligzooospermia (Georgiou et al., 2006, Katagiri et al.,2004).

Limited studies in the Middle East have been done; YCMs were reported in 3.2% of men with idiopathic azoospermia or oligozoospermia in Saudi Arabia, in 3.3% of those in Turkey, and in 2.6% in Kuwait(Hellani et al.,2006, Mohammed et al.,2007, Sargin et al.,2004). In Iran, studies on small numbers of patients showed that 5% to 24.2% of infertile men with idiopathic severe spermatogenesis impairment had these genetic aberrations(Omrani et al.,2006, Akbari Asbagh et al.,2003).

Deletions in the Y chromosome are mostly de novo(Foresta et al.,1998).However, several cases of natural transmission of the microdeletion have been reported to date(Chang et al.,1999, Rolf et al.,2002, Kuhnert et al.,2004, Saut et al.,2000, Krausz et al.,2006, Calogero et al.,2002) .

Since Tiepolo and Zuffardi reported cytologically detectable deletions of the proximal Yq in azoospermic men(Tiepolo et al.,1976), a tremendous amount of research has been done to scrutinize the mechanism of developing and characteristics of these deletions .In 1996, Vogt and colleagues identified 3 recurrently deleted subregions in Yq11. These were termed the AZF and the 3 subregions were named as AZFa, AZFb, and AZFc (Vogt et al.,1996).

Deletion of AZFa is associated with lack of germ cells or Sertoli cell only syndrome. Deletion of AZFb is associated with spermatogenesis arrest and finally deletion of AZFc gene products is associated with failure of maturation process of post-miotic germ cells. Although this hypothesis remains controversial, it is accepted that

***Corresponding Author:** Dr Kyumars Safinejad ,Department of Biology, Broojerd Branch, Islamic Azad University, Broojerd, Iran

the completion of spermatogenesis requires multiple genes, not only on the Y chromosome but elsewhere as well. Recently another AZF subregion named AZFd, localized between AZFb, and AZFc has been described, which has complicated the issue (Ma et al.,2000). With the advent of PCR and construction of a Y-chromosome sequence-tagged site (STS) map, microdeletions were detected at a frequency of 0.4-55.5%.This varying frequency is probably related to the criteria on which the patients are selected (Maurer et al.,2001).In this study we investigated Yq11 microdeletions with the use of PCR analysis in a group of infertile men with severe oligozoospermia.

MATERIALS AND METHODS

Blood samples were collected from 89 males with severe oligozoospermia with a sperm count of <5 x 10 /mL, had been referred to the Royan-2 Infertility Center in Qom, Iran. Genomic DNA was extracted from whole blood by a nonorganic method involving lysis, proteinase K digestion and salting out of DNA with isopropanol precipitation(Miller et al.,1988, Grimberg et al.,1989).

An informed consent form was signed by all of the participants. Microdeletion analysis was made of the subregions AZFa, AZFb and AZFc STS robes (table 1).

Table 1. STS used in detect of microdeletions in the AZF region of the Y chromosome.

STS	Locus	Region	Sequence 5´ to 3´	pb
sY14	SRY	Yp11.3	F5'- GAATATTCCCGCTCTCCGGA R5'- GCTGGTGCTCCATTCTTGAG	472
ZFX/ZFY	ZFX/ZFY	Xq34 Yp22.3	F5'- ACCRCTGTACTGACTGTGATTACAC R5'- GCACYTCTTTGGTATCYGAGAAAGT	495
sY84	DYS273	AZFa	F5'- AGAAGGGTCTGAAAGCAGGT R5'- GCCTACTACCTGGAGGCTTC	326
sY86	DYS148		F5'- GTGACACACAGACTATGCTTC R5'- ACACACAGAGGGACAACCCT	320
sY127	DYS218	AZFb	F5'- GGCTCACAAACGAAAAGAAA R5'- CTGCAGGCAGTAATAAGGGA	274
sY134	DYS224		F5'- GTCTGCCTCACCATAAAACG R5'- ACCACTGCCAAAACTTTCAA	301
sY254	DAZ	AZFc	F5'- GGGTGTTACCAGAAGGCAAA R5'- GAACCGTATCTACCAAAGCAGC	400
sY255	DAZ		F5'- GTTACAGGATTCGGCGTGAT R5'- CTCGTCATGTGCAGCCAC	126

The STS probes used were sY84 and sY86 (AZFa), sY127, sY134 (AZFb), sY254 and sY255 (AZFc), and SRY and ZFX/ZFY (controls). In addition, a water sample that contains all reaction components but water instead of DNA was used for reagent contamination. PCR conditions used for STS probes were as follows: initial denaturation at 94°C for 5 min and subsequent denaturations at 94°C for 30 s were the same for all samples. Annealing was 56°C for 40 s, extension was 65°C for 4 min. Subsequent series were 35 cycles. Final extension was carried out at 72°C for 5 min. The PCR products were separated on a 1.5% agarose gel.

RESULTS

We selected 89 of the patients who had severe oligozoospermia with a sperm count of <5 x 10 /mL .Y chromosome microdeletion was determined in 26 patients in the patients. Microdeletions in the AZFa subregion (61%) were encountered more often than in AZFb (21%) and in AZFc (18%) in the patients.Also,

microdeletions of AZFa+b (50%),AZFa+c(50%)and AZFb+c(0%) were determined in the patients . Figure 1 shows a patient with microdeletion of AZFa (sY84, sY86)subregion. Patients ranged from 25 to 49 years, with a mean of 37 years.

Among the 89 severe oligozoospermic patients, 20 had deletions only in the AZFa region, 7 had microdeletions in the AZFb region and 6 in the AZFc region (Table 2).

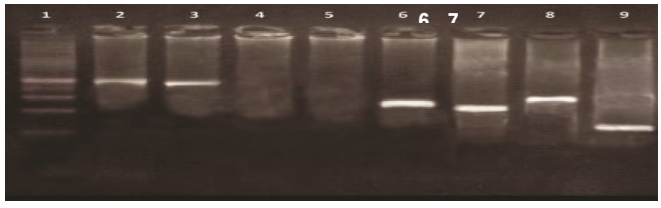

Figure 1.The image of severe oligozoospermia patient with a deletion of AZFa region
(sY84, sY86: there is no band).
Lane 1 =DNA ladder for 100 bp; lane 2 = ZFY/ZFX; lane 3 = SRY; lane 4 = sY84; lane 5 = sY86;
lane 6 = sY127; lane 7= sY134; lane 8 = sY254; lane 9 = sY255

Amplification of the genes SRY and ZFX/ZFY was detected in all the patients and in the positive controls, while only the ZFX/ZFY amplified in the negative control (Figure 2).

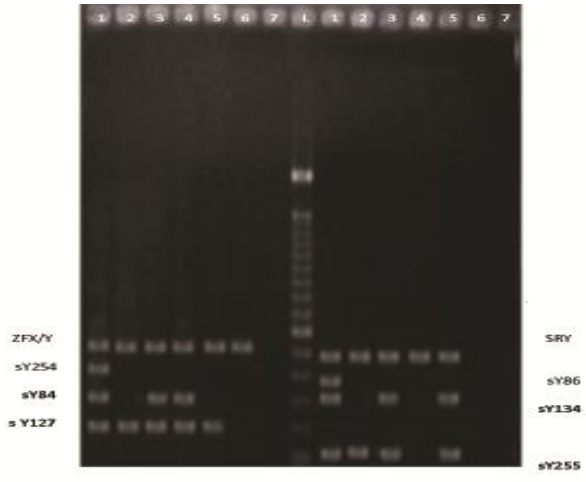

Figure 2. Electrophoresis on a 1.5% agarose gel showing deletion in the AZF region of the Y chromosome. Lane L, molecular weight 100 bp; ZFX/Y, 495 bp; SRY, 472 bp; sY255, 126 bp; sY254, 400 bp; sY134, 301 bp; sY127, 274 bp; sY86, 320 bp; sY84, 326 bp. Lane 1, positive controls; Lane 6, negative control ; Lane 7, water sample that contains all reaction components but water instead of DNA for reagent contamination;Lanes 2 - 5, severe oligozoospermic patients.

Table 2. Clinical data of the patients and regions deleted in AZF. STS markers deleted= (-),Y=year

patients	Number of patients with microdeletion	Age range	Sperm count ($\times 10^{-6}$/ml)	AZFa sY84 sY86		AZFb sY127 sY134		AZFc sY254	sY255
89	26	25-49y	1-5	14(-)	9(-)	0(-)	7(-)	5(-)	1(-)

DISCUSSION

The prevalence of the microdeletions in the Y chromosome has been reported from 1 to 55.5% . Prior studies have proposed that 3-18% of men with nonobstructive azoospermia or severe oligozoospermia may have deletions of the Y chromosome (Girardi et al.,1997).

There is no consensus about the marker that should be used for Y chromosome microdeletion analysis (Foresta et al.,1998,Le Bourhis et al.,2000,Krausz et al.,2001,Loginova et al.,2003,SaoPedro et al.,2003,Carrara et al.,2004,Dada et al.,2004,Simoni et al.,1999,Simoni et al .,2004,Vogt et al.,2004,Hellani et

al.,2006,Pina-Neto et al.,2006).Deletions in the AZF region are commonly found in patients with azoospermia and , severe oligozoospermia which we also found in our study, but a genotype-phenotype correlation has not been objectively demonstrated. Deletions in the AZFb region have been found to be associated with azoospermia, oligozoospermia; deletions in the AZFc region have been found to be associated with azoospermia and severe to mild oligozoospermia (Thangaraj et al.,2003).Studies suggest that deletions in AZFa can be events of recombination between specific repetitive regions defined as hot spots(Kamp et al.,2001) .

These comparative observations led us to believe the hypothesis that some molecular mechanism operating on defined hot spots in Yq could be responsible for a similar recurrence of AZFa deletions. The loci STS sY84 and sY86 used in the for analysis of AZFa deletions are always deleted in patients with complete AZFa deletions (Simoni et al.,1999,Simoni et al .,2004); which occurred in 11% of our patients.Prior studies have proposed that 3-18% of men with nonobstructive azoospermia or severe oligozoospermia may have deletions of the Y chromosome (Girardi et al.,1997).

Most AZF deficiencies are de novo events due to deletions occurring in germ cells or in post-zygotic stages. Germ cell deletions generate a mosaic sperm population carrying normal and AZF-deleted Y chromosomes. PCR loci in the same or in adjacent AZF subintervals (Kent-First et al.,1999,De Palma et al.,2005). Vogt et al (1996) have suggested that the deletions in the AZFa and AZFb are associated with the impairment of spermatogenesis being worse when compared with that of the AZFc region. Ferlin et al (1999), reported a high frequency of Y deletions in complete absence or strong reduction of germ cells, while milder forms of testiculopathy were not associated with Y deletions. Silber et al. (Silber et al.,1998) have suggested that when AZFb and beyond were missing, there was no detectable completion of spermatogenesis. This correlation has been supported by many other studies,but some others have not supported this correlation. Microdeletions are rarely found in a large control group of men with proven fertility (Simoni et al .,2004).

Studies of microdeletions in Yq will help in the development of better methods of diagnosis and will be useful for increasing our understanding of spermatogenesis. There is an urgent necessity for implementing molecular methods in medical clinics. Diagnosis of genetic alterations and knowledge on vertical transmission of these abnormalities are essential for studies of infertile men who participate in assisted human reproduction. Our results proved that there is a specific correlation between Y chromosome AZF subregions microdeletion and severe oligozoospermia.

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