ISSN: 0067-2904
GIF: 0.851

Molecular Analysis of Y Chromosome Microdeletions in Oligozoospermic Iraqi Patients

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Abstract

This study was designed to determine the correlation between Y chromosome azoospermia factor (AZF) subregions microdeletions and oligozoospermia in infertile men. Subjects included 50 infertile men with oligozoospermia who had been referred to the Fertility Center and infertility treatment in Kamal Al-Samarrai Hospital\Baghdad health office-Iraq. DNA was extracted from blood samples. Polymerase chain reaction (PCR) amplification of 3 loci spanning the AZFa, AZFb and AZFc subregions of the Y chromosome using sY84, sY127 and sY254 and were performed. The frequency of deletions involving AZFa subregion of the Y-chromosome was found in twelve of the patients (24%) in oligozoospermic infertile Iraqi men. While the other subregion (AZFb and AZFc) of the Y-chromosome were not determined microdeletion in oligozoospermic samples. The study showed that men with oligozoospermia should be evaluated for Yq11 microdeletionsbefore deciding to operate varicoceles or else scheduling them for assisted reproductive techniques and there is a specific correlation between Y chromosome AZFa subregions microdeletions and oligozoospermia.

Keywords: Male Infertility, Oligozoospermia,Y-Chromosome Microdeletion, PCR, AZF region.

التحليل الجزيئي لمناطق الحذف Microdeletions للكروموسوم Y في مرضى العقم بقلة النطاف Oligozoospermic العراقيين

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الخلاصة

حددت هذه الدراسة العلاقة بين الحذف الجزئي microdeletions لمناطق عامل الحذف الفرعي AZF على كروموسوم Y و قلة النطاف oligozoospermia في عقم الرجال . تضمنت الدراسة 50 عينه لرجال يعانون من العقم بقلة النطاف oligozoospermia من مركز الخصوبة وعلاج العقم في مستشفى كمال السامرائي \ دائرة صحة بغداد – العراق .استخلص DNA من عينات الدم .وتم تضخيم 3 مواقع فرعية شملت AZFa ، AZFb و AZFc للكروموسوم Y بتقنية PCR باستعمال البوادىء: sY84 وsY127 و sY254. وكانت شدة الحذف في المنطقة الفرعية AZFa لكروموسوم Yفي اثني عشر من المرضى بنسبة (24٪) في الرجال العراقيين يعانون من العقم نوع قلة النطاف oligozoospermic في حين أن المناطق الاخرى AZFb و AZFc على كروموسوم Y لم يتم تحديد الحذف في عينات المرضى بقلة

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النطاف oligozoospermic. وبينت الدراسة أن الرجال الذين يعانون من قلة النطاف oligozoospermia ينبغي تحديد الحذف على كروموسوم Yq11 قبل وضع جدول لتقنيات المساعدة على الإنجاب وهناك علاقة محددة بين كروموسوم Y ومناطق الفرعية AZFa التي تعاني من الحذف بقلة النطاف oligozoospermi.

Introduction

Infertility is a reproductive health problem that affects many couples in the human population. It is a major health problem today, affecting10 to 15 % of couples seeking to have children [1]. Male infertility is particularly problematic since it is estimated that the cause of the infertility is unknown in up to 50 % of the cases [2]. Regardless of whether it is primary or secondary infertility, affected couples suffer from enormous emotional and psychological trauma and it can constitute a major life crisis in the social context. Male infertility problems may be contributory to 30 to 40 % of infertile couples, and in another 20 % of cases, both men and women are affected [3, 4**]**. The male factor is, therefore, responsible in about 50 % of infertile couples. The infertile male partner has qualitative or quantitative abnormalities of sperm production [5, 6]. Y chromosome microdeletions(YCM) are the most frequently observed structural abnormalities in the male-specific region of the Y chromosome [7,8], and of primary spermatogenesis failures, 15% are related to at least 6 known major YCM patterns [9]. Microdeletions are present in 5 to 10% of infertile men [10]. Specifically, they have been reported in 6 to 16% of azoospermic men, and 4 to 5.8% of those with severe oligzooospermia [11, 8].

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 [12-14]. 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 [15, 7].

Deletions in the Y chromosome are mostly de novo [16].However; several cases of natural transmission of the microdeletion have been reported to date [17- 22]. Since Tiepolo and Zuffardi [23] reported cytologically detectable deletions of the proximal Yq in azoospermicmen, a tremendous amount of research has been done to scrutinize the mechanism of developing and characteristics of these deletions .In 1996, Vogt and colleagues [24] identified 3 recurrently deleted subregions in Yq11. These were termed the AZF and the 3 subregions were named as AZFa, AZFb, and AZFc. 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 786 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 [25]. 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 [26, 27]. This study aimed to investigate Yq11 microdeletions in AZF subregion (AZFa, AZFb, and AZFc) in a group of infertile men with oligozoospermia.

Materials and Methods

Samples: fifty infertile men compared to 20 normal fertile men from the Fertility Center and infertility treatment in Kamal Al-Samarrai Hospital\Baghdad health office, Iraq. The age of infertile men ranged from 25 to 50 years. They were subjected to detailed clinical and biological investigations, including cytogenetic and endocrinology studies, physical examination. On the basis of spermiogram, individuals (n = 50) were oligozoospermic patients (less than 20 x 10^6 spz/ml) according to the criteria of the World Health Organization (World Health Organization 1999). 20 males with proven fertility and normal sperm concentration, (more than 20 x 10^6 spz/ml) were selected as control (25 to 50 years).

Sample Collection: two ml of venous blood was collected in a tube containing ethylenediamine tetraacetate (EDTA) as an anticoagulant for DNA extraction.

Molecular Investigations:

Genomic DNA was extracted utilizing the Geneaid Blood Genomic DNA Purification Kit. Polymerase chain reaction (PCR) was performed according to the standard protocol for analysis of the

AZF region of the Y-chromosome. Three sub-regions were analyzed: AZFa, AZFb and AZFc, where sequence tagged site (STS) primers were used as shown in Table-1.

The PCR amplification comprised a total volume of 25µl, which contained 100 to 200 ng of human genomic DNA as template, 2.5mM dNTP's (2.5 mM each of dTTP, dCTP, dGTP, dATP), oligonucleotide primers (0.1 to 2.0µmol/ l each of the forward and reverse primers),10X Taq DNA polymerase assay buffer (Tris with 15mM MgCl2) and 3U Taq DNA polymerase. Samples were subjected to Polymerase Chain Reaction amplification using 35 cycles at 94^{o}C for 30 sec, 53^{o}C for 45 sec and 72^{o}C for 60 sec. Initial denaturation was done at 94^{o}C for 5 min and final extension at 72oC for 10 min. The PCR amplified products were submitted to electrophoresis on 2% agarose gels and stained with 0.5µg/ml ethidium bromide.

Primers Used for PCR:

Table 1- Sequence-Tagged Sites (STS) primer used for Y chromosomal microdeletion analysis

STS	Region	Sequence 5´ to 3´	pb	References
SY 84	AZFa	Forward: 5'-AGA AGG GTC TGA AAG CAG GT-3' Reverse: 5'-GCC TAC CTG GAG GAG GCT TC-3'	326 bp.	[3]
SY 127	AZFb	Forward: 5'-GGC TCA CAA ACG AAA AGA AA-3' Reverse: 5'-CTG CAG GCA GTA ATA AGG GA-3'	274 bp.	[3]
3 SY 254	AZFc	Forward: 5'-GGG TGT TAC CAG AAG GCA AA-3' Reverse: 5'-GAC CGT ATC TAC CAA AGC TGC-3'	380 bp.	[3]

Results:

Out of 50 oligozoospermic patients, 12 had microdeletions in AZF a (sY-84), but not deletion of AZFb (SY 127) and AZF c (sY- 254) as show in Figure-1. The details of sperm concentration are presented in Table-2 and the semen analysis is presented in Table-3.

Table 2- Classification of infertile males with their sperm concentration

Type of case	Sperm concentration 10^6/ml
Oligozoospermia	>5-20

Table 3- Classification of infertile males with their specific deletion and semen analysis

Type of case	AZFa deletion	AZFb deletion	AZFc deletion	Semen analysis
	SY 84	SY 127	3 SY 254	Motility %
Oligozoospermia	+	-	-	36-57%

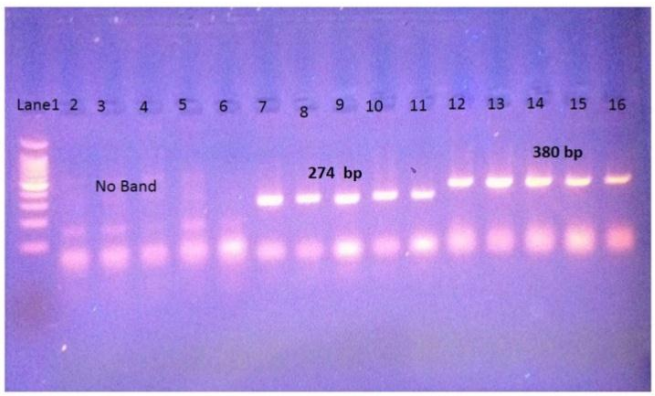

Figure 1-The image of Oligozoospermia patient with a microdeletion of AZFa region (sY84: there is no band). Lane 1 =DNA ladder for 100 bp; lane 2 = negative control; lane 3,4,5,6 = sY84; lane 7-11 = sY127; lane 12-16= sY254. Electrophoresis on 2% agarose gels and stained with 0.5µg/ml ethidium bromide.

Discussion:

Deletions in the AZFa region are commonly found in patients with 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 [28, 29], but in our samples study not found deletions.

Studies suggest that deletions in AZFa can be events of recombination between specific repetitive regions defined as hot spots [30, 3].

These comparative observations led 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 used analysis of AZFa deletions are always show deleted in patients with complete AZFa deletions [31, 30] which occurred in 24% of patients. Most AZF deficiencies are de novo events due to deletions occurring in germ cells or in post-zygoticstages. 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 [32, 33, 24] 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. It was reported a high frequency of Y deletions in complete absence or strong reduction of germ cells [34], while milder forms of testiculopathy were not associated with Y deletions. Other study [35] noticed that 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 [30]. Studies of microdeletions in Yq will help in the development of better methods of diagnosis and will be useful for increasing the 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 [36].

The results of this study revealed that there is a specific correlation between Y chromosome AZF subregions microdeletion and severe oligozoospermia.

Conclusion: The frequency of deletions involving AZFa region of the Y-chromosome is 24 % in oligozoospermic infertile men. The amplification of AZF locus is useful for the diagnosis of microdeletions in the Y-chromosome.

Acknowledgement

Financial support from the Institute of Genetic Engineering & Biotechnology for Post Graduate Studies, University of Baghdad.

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