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ORIGINAL ARTICLE

Frequency of Y Chromosome Microdeletions in Turkish Infertile Men: Single Center Experience

Türk İnfertil Erkeklerde Y kromozomu Mikrodelesyonlarının Sıklığı: Tek Merkez Deneyimi

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ABSTRACT

Objective: Infertility is defined as the failure to achieve a clinical pregnancy after twelve months of regular unprotected sexual intercourse. Both genetic and environmental factors affect infertility. The aim of the study is to establish the frequency of the Y chromosome microdeletions in Turkish infertile men who were referred to our center with severe oligozoospermia and azoospermia.
Methods: A retrospective chart review study on patients who referred to our center due to infertility were included in the study. We evaluated microdeletions of the Y-chromosome STS markers AZFa, AZFb and AZFc, ZFX/ZFY, and terminal sY160 regions. Y-chromosome STS markers were evaluated by DNA fragment analysis.
Results: The chart review indicated that a total of 319 men applied to our genetic diagnosis center between 2016 and 2020 due to infertility (mean age 32 ±7). Among the 319 infertile men, we determined 21 cases with Y chromosome microdeletions (6.89%), with the most common AZFc deletion (n=11, 52.3%).
Conclusion: Y-microdeletions are among the most common genetic causes of male infertility. In our study we most commonly detected AZFc deletions which is consistent with the literature. In azoospermic and oligospermic patients, cytogenetic tests, Y-chromosome microdeletions and NGS panel screening tests can give effective information before the use of assisted reproductive techniques.

Keywords: Azoospermia; Infertility; Microdeletion; Y-chromosome,

ÖZ

Amaç: İnfertilite, on iki aylık düzenli korunmasız cinsel ilişkiden sonra gebelik elde edilememesi olarak tanımlanmaktadır. Hem genetik hem de çevresel faktörler kısırlığı etkiler. Bu çalışmanın amacı, merkezimize 2016-2020 yılları arasında ağır oligozoospermi ve azospermi ile sevk edilen Türk infertil erkeklerinde Y kromozom mikrodelesyonlarının sıklığını belirlemektir.
Yöntemler: Çalışma için 2016-2020 yılları arasında merkezimize infertilite nedeniyle başvuran vakaların dosyaları retrospektif olarak incelendi. Y kromozomu STS belirteçleri AZFa, AZFb ve AZFc, ZFX/ZFY ve terminal sY160 bölgelerinin mikrodelesyonlarını değerlendirildi. Y kromozomu STS belirteçleri, DNA fragman analizi ile değerlendirildi. 101 hastaya sitogenetik analiz yapıldı.
Bulgular: Retrospektif çalışma 2016-2020 yılları arasında genetik tanı merkezimize infertilite nedeniyle (ortalama yaş 32 ±7) toplam 319 erkek başvurduğunu gösterdi. 319 infertil erkek arasında Y kromozom mikrodelesyonu (%6.89) olan 21 olgu belirlendi. Bu 21 olgu içinde en sık AZFc delesyonu (n=11, %52.3) saptandı.
Sonuç: Y-delesyonlar infertilitenin en sık genetik sebeplerinden biridir. Çalışmamızda litertürle uyumlu olarak en sık AZFc delesyonunu saptadık. Azospermik ve oligospermik hastalarda Y kromozom mikrodelesyonlarının tanımlanması, ayrıca karyotip ve moleküler analizler yardımcı üreme teknikleri öncesinde infertilite etiyolojini belirlemek için kullanılır. NGS panel testleri kullanılarak infertilite ile ilişkili genlerin taranması da infertilite etiyolojisi hakkında daha kesin bilgi verebilir.

Anahtar Kelimer: Azoospermia İnfertilite; Mikrodelesyon; Y-kromozom;

Introduction

Infertility is defined as the failure to achieve a clinical pregnancy after twelve months of regular unprotected sexual intercourse. It is a global health issue worldwide. It is estimated that 48 million couples have infertility (1). About 9% of men and about 11% of women of reproductive age in the United States have experienced fertility problems (2). The rate of infertility in the world is between 8-12% (3), while this rate varies between 10-20% in Turkey (4). About 20-30% of infertility cases are attributed to males (5). Both genetic and environmental factors affect infertility. Lifestyle factors such as smoking, excessive alcohol intake, obesity, and exposure to environmental pollutants have been associated with lower fertility rates in both men and women. Genetic causes are responsible for a small percentage of male infertility. Genetic factors are diagnosed in approximately 15-20% of severe male factor infertility (6). The genetic causes are Klinefelter syndrome, Y-chromosome microdeletions, and monogenic causes. Y-chromosomal microdeletions are the second most frequent genetic cause of male infertility after Klinefelter syndrome. The frequency of microdeletions is 1/4000 in the general population, and there is an increased frequency of microdeletions in infertile men (7). The aim of the study is to establish the frequency of the Y chromosome microdeletions in Turkish infertile men with severe oligozoospermia and azoospermia who were referred to the outpatient clinic of genetic diagnosis center (2016-2020).

Materials and Methods

A retrospective chart review study was performed in Istanbul University-Cerrahpaşa, Genetic Diagnosis Center (GETAM). Patients admitted to GETAM between 2016-2020 due to infertility were enrolled in the study. As per the Declaration of Helsinki, written informed consent was taken from all the subjects. The research study protocol was approved by the Institutional Review Board. We evaluated microdeletions of the Y-chromosome STS markers AZFa, AZFb and AZFc, ZFX/ZFY, and terminal sY160 regions by DNA Fragment analysis.

Cytogenetic Analysis

Peripheral venous blood was collected from patients in heparinized tubes, and lymphocyte culture was performed for 72 hours at 37 °C in phytohemagglutinin-induced media. Metaphase preparations obtained after culture were stained using the standard Giemsa-Trypsin G-banding technique and an analysis of 25 metaphase plates was performed. CBG and NOR banding techniques were used for heterochromatin region changes. At least 100 cells were analyzed in cases with suspected mosaicism.

Molecular Analysis

Peripheral blood samples (2 cc) were collected from patients for Y chromosome microdeletions, and genomic DNA was isolated from leukocytes using standard methods (Wizard Genomic DNA; Promega, Madison, WI, USA). Analysis of 17 STS regions was performed for Y chromosome microdeletions. Multiplex amplification of the STS containing the "AMXY marker" and a 5-dye fluorescence system by electrophoresis were used (Chr.X: 104bp, Chr.Y: 109bp; Xp22.1, Yp11.2) and evaluated on a genetic analyzer (Applied Biosystems 3500 Prism, Thermo Fisher Scientific, USA).

Fragment analysis was performed. The STS regions used for AZFa, AZFb, AZFc subregions and controls were: sY82, sY83, sY84, sY86, sY88, and sY1065 for the AZFa region; sY105, sY121, sY127, sY134, sY143 and sY153 for the AZFb region; sY1191, sY1291, sY254 and sY255 for the AZFc region respectively. ZFX/ZFY and terminalsY160 regions were amplified for internal control.

Statistical Analysis

All statistical analyzes were performed using the SPSS program (version 20.0 for Windows, SPSS Inc. Chicago, IL). The Kolmogorov-Smirnov test was used to analyze the normality of the data. Continuous variables with normal distribution were expressed as mean ± standard deviation and categorical variables were expressed as percentages when appropriate. A p-value of <0.05 was considered statistically significant.

Results

A retrospective review of charts was performed by the investigators. The cases were included if they were referred for infertility workup. The chart review indicated that a total of 319 men applied to our genetic diagnosis center between 2016 and 2020 due to infertility (mean age 32 ±7). The indication for referral/application were azoospermia (n=192, 60.4%), infertility (n=116, 37.1%), oligospermia (n=4, 1.3%) and primer infertility (n=2, 0.6%), anejaculation (n=1, 0.3%). All collected cases were included in the analysis. Among the 319 infertile men, we determined 21 cases with Y chromosome microdeletions (6.89%). Karyotype analyses were performed on 101 subjects. The distribution of karyotype results was demonstrated in Table 1. The distribution of Y chromosome microdeletions was demonstrated in Figure 1 with the most common AZFc deletion in 11 patients (52.3%) among 21 cases. The least common deletion was AZFb deletion detected in 1 patient (4.75). Three patients showed both chromosome abnormality and Y microdeletions. These were:

1. mos45,X[93]/46,XY(7) and Y chromosome deletions in AZFa, AZFb, AZFc, AZFd loci
2. mos 45,X[13]/46,XY[87] and Y chromosome deletions in AZFa, AZFb, AZFc, AZFd loci
3. mos45,X [15]/46,XY[25] and Y chromosome deletions in AZFb, AZFc loci

Table 1. Karyotype analysis results of 101 subjects. Frequency is calculated as percentage values

Karyotype	# of cases	Type of chromosome abnormalities	Frequency (%)
46, XY	83		82
Mosaicism	4	mos46,XX[20]/47,XXY[30] mos45,X[93]/46,XY[7] mos45,X[93]/46,XY[7] mos45,X0 [15]/46,XY[25]	4
Inversion	1	46,XY,inv(Y)(p11.2q11,23)	1
Polymorphism	2	46,XY,16qh+ 46,XY, 21ps+	2
Translocation	1	46,XY,t(8;20)(q24.2;p11.2)	1
47,XXY	10		10

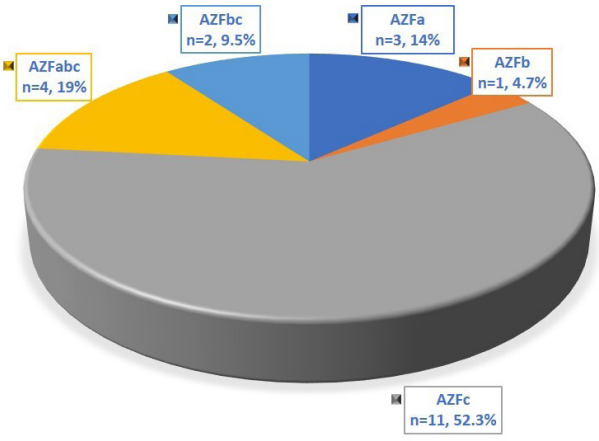

Figure 1. Distribution of Y-Chromosome deletions is demonstrated in pie graphics as the number of subjects (n) and the frequency (%) carrying the associated y microdeletion respectively

Discussion

Since the AZF region is associated with the differentiation and proliferation of male germ cells, microdeletion in AZF has a cause-effect association with spermatogenic impairment. The frequency of Y chromosome microdeletion in infertile men is 10-15% in non-obstructive azoospermia and 5-10% in severe oligozoospermia (8). The most common type of AZF deletions is AZFc region microdeletion (~80%) followed by AZFb (1%–5%), AZFbc (1%–3%), and AZFa (0.5%–4%) (9).

We screened 619 men admitted to the genetic center with infertility and determined Y chromosome microdeletions in 21 cases (6.89%). The most frequent deletion in AZFc occurred in 52.3% of the cases. The variations in the frequency of Y microdeletions in our study may be attributable to the small number of cases, genetic differences in and among populations, especially in Y chromosome-specific haplotypes, genetic background, environmental factors, and different types of primers for AZF-related microdeletions. Only gr/gr partial deletion of AZFc region has been described as a potential clinical interest (10). It removes half of the gene content with predominant expression in germ cells.Although there are numerous studies about Y microdeletions, the effect of AZFc deletions on germ cell functions in developmental steps is still a subject of research.

The other important genetic factors which can result in male infertility are numerical and structural chromosomal abnormalities. These abnormalities can be detected with cytogenetic analysis. If men have nonobstructive azoospermia and their total motile sperm count is <5 million, cytogenetic analysis is recommended (11).

Cytogenetic analysis is performed on 101 subjects. Small number of cases (n=4) had both Y-microdeletions and mosaic karyotypes. The remaining subjects with Y- microdeletion had normal karyotype results. Y- Chromosome microdeletions are common in men with mosaic karyotypes. The prevalence of Y microdeletions in mosaic karyotype has been found as 71.43% in recent literature (12). 45,X/46,XY chromosomal mosaicism covers a broad spectrum of clinical phenotypes with varying degrees of genital malformations, Turner syndrome, infertility, or normal male phenotype.

Conclusion

The etiology of infertility remains unknown and novel genes other than Y chromosome microdeletions should be identified with high throughput techniques. It is clear that we need more research on large independent study populations to understand this mechanism. In azoospermic and oligospermic patients, karyotype and molecular analysis of Y chromosome microdeletions can give more precise information before the use of assisted reproductive techniques. Also, genes associated with infertility should be screened by using next generation sequencing (NGS) panel tests.

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