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Original Article

The frequencies of Y chromosome microdeletions in infertile males

Y kromozomu mikrodelesyonlarının infertil erkeklerdeki sıklığı

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ABSTRACT

Objective: To determine the frequencies and the characteristics of Y chromosome microdeletions in infertile males.

Material and methods: The records of 1616 infertile males were included in the study. The cases were divided into groups according to the infertility etiology and semen analysis. The frequencies and the characteristics of Y chromosome microdeletions were investigated in groups.

Results: Y chromosome microdeletion was detected in 54 (3.3%) of 1616 cases. Microdeletions in the azoospermia factor (AZF) region were the most common (48.1%). When the cases were grouped according to causes of infertility that could be detected, no Y chromosome microdeletions were detected in some groups (cases with Klinefelter Syndrome, hypogonadotropic hypogonadism, congenital absence of vas deferens, and 47, XYY karyotype).

Conclusion: Y chromosome microdeletions were detected quite frequently in certain infertility subgroups. Therefore, detailed evaluation of an infertile man by physical examination, semen analysis, hormonal evaluations and when required, karyotype analysis may predict the patients for whom Y chromosome microdeletion analysis is necessary and also prevent cost increases.

Keywords: AZF; male infertility; Y chromosome microdeletion

ÖZ

Amaç: İnfertil erkeklerde Y kromozomu mikrodelesyonlarının sıklığını ve özelliklerini belirlemek.

Gereç ve yöntemler: Çalışmaya 1616 infertil erkek dahil edildi. Olgular infertilite etiyolojisi ve semen analizine göre gruplara ayrıldı. Gruplara göre Y kromozomu mikrodelesyonlarının sıklığı ve özellikleri incelendi.

Bulgular: Y kromozomu mikrodelesyonu 1616 olgunun 54'ünde (%3,3) saptandı. Mikrodelesyonlar en sık AZFc bölgesinde (%48,1) idi. Olgular infertilite nedenlerine göre gruplandırıldığında, bazı gruplarda (Klinefelter Sendromu, hipogonadotopik hipogonadizm vakaları, ductus deferens agenezisi olan vakalar ve 47, XYY karyotipi olan olgular) Y kromozomu mikrodelesyonları saptanmadı.

Sonuç: Y kromozomu mikrodelesyonları bazı infertilite alt gruplarında oldukça sık tespit edildi ancak bazılarında hiç tespit edilemedi. Bu nedenle, infertil bir erkeğin fizik muayene, semen analizi, hormonal değerlendirmeler ve gerektiğinde karyotip analizi ile detaylı olarak değerlendirilmesi, hangi hastada Y kromozomu mikrodelesyon analizinin gerekli olduğunu öngörebilir ve maliyet artışını önleyebilir.

Anahtar Kelimeler: AZF; erkek infertilitesi; Y kromozomu mikrodelesyonu

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Introduction

Infertility is defined as the inability to conceive after 1 year of regular and unprotected intercourse.[1] Approximately 15% of couples are infertile and male factors are responsible for infertility in over 50% of the cases.[2,3] Known genetic causes such as chromosomal abnormalities, Y chromosome microdeletions, x-linked and autosomal gene mutations contribute to

15-20% of the most severe forms of male infertility.[4] Y chromosome microdeletions are the most common causes of genetic abnormality in infertile men after Klinefelter Syndrome.[5]

Small deletions of the long arm of the Y chromosome (Yq) are not visible under the microscope and are called microdeletions.[6] The azoospermia factor (AZF) region of the Yq arm was genetically mapped in 1996. Deletions in this region were identified among men presenting with severe oligozoospermia or azoospermia.[7] The AZF locus harbors 14 protein coding genes critical for spermatogenesis.[8] These genes are organized in three different locations (AZFa, AZFb, and AZFc). Each of these regions may be deleted independently or in combination and are involved as the cause of defective spermatogenesis in 5% of men presenting with severe oligozoospermia, and in 10% of men with non-obstructive azoospermia (NOA).[7]

The tremendous development of assisted reproductive technologies (ART) such as in vitro fertilization, intracytoplasmic sperm injection and testicular sperm extraction have made reproduction possible for infertile males even if they are azoospermic. In addition, these techniques have increased the importance of Y chromosome microdeletions. Complete deletions of AZFa and AZFb portend an exceptionally poor prognosis for sperm retrieval[9]; however, if AZFc is impaired, successful surgical sperm retrieval is sometimes possible.[10] Thus, screening for AZF microdeletions before undergoing ART treatment is a critical diagnostic tool for prognosis.

The aim of the present study is to determine the frequencies and the characteristics of Y chromosome microdeletions in infertile males who attended to our clinic. We detected Y chromosome microdeletions in the AZFa, AZFb, and AZFc subregions, by using polymerase chain reaction (PCR).

Material and methods

The records of the patients who were admitted to our infertility clinic between 2009 and 2017 were investigated. From these, 1616 infertile males were included in the study.

All semen analyses were performed in the same laboratory. Semen samples were obtained after a 3–5 day-period of ejaculatory abstinence. Semen analysis was performed at least twice and pelleting was performed to confirm the condition of azoospermia.

The conventional method was used on the lymphocyte cultures for karyotype analysis. Generally, 20 metaphase fields were examined after staining by the G band technique and 550 level bands were obtained. The final results were written according to the International System for Chromosome Nomenclature guidelines. Analysis of the Y-chromosome microdeletions was performed by amplifying 14 markers (AZFa [Prox2], RBMY, AZFa [Sy84, Sy86], AZFb [Sy127, Sy133, Sy134], AZFd [Sy152, Sy153], AZFc [Sy157, Sy254, Sy255], control [SRY (Y14), ZFY]) using a commercially available kit (GML Y Chromosome Microdeletion Detection System Kit, GML AG, Altendorf, Switzerland). Polymerase Chain Reaction (PCR) was performed on a GeneAmp® PCR System 9,700 with a Silver 96-Well Block (Applied Biosystems, Foster City, CA, USA). Electrophoresis was performed using an Applied Biosystems 3130/3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

In the first instance, cases were divided into three groups according to the semen analysis results as cases with azoospermia, severe oligoastenozoospermia (sperm count less than 5 million/ml or pellet test was positive) and cases with a sperm count above 5 million/mL. Afterwards, cases were divided into eight groups according to the infertility etiology as revealed by physical examination, semen analysis, hormonal evaluation and cytogenetical analysis. Total number of 966 cases could be evaluated according to these characteristic features. The groups were formed as follows: 46, XY-karyotype cases with nonobstructive azoospermia (46, XY NOA) (n=547), cases with Klinefelter Syndrome (n=191), hypogonadotropic hypogonadism (n=61), congenital absence of the vas deferens (n=114), other karyotype anomalies (n=50) such as inversion/translocation, 45,X/46,XY mosaicism, 46, XX testicular disorder of sex development (DSD), and 47, XYY karyotype. The cases in each group were examined according to the detection rates of Y chromosome microdeletions.

Ethics committee approval was received for this study from the ethics committee of Erciyes University. Written informed consent was not obtained from the patients who participated in this study. The study was designed retrospectively and data were collected from the charts of the patients.

Results

Y chromosome microdeletion was detected in 54 (3.3%) of 1616 cases. Microdeletions in the AZFc region were the most common (48.1%), followed by AZFa+b+c (20.4%), AZFb+c (16.7%), AZFb (11.1%) and AZFa (3.7%). AZFd deletions were found in 4 patients who had also AZFc deletions. Since the importance of AZFd deletion is not known exactly, this deletion was not taken into account. The results are shown in Table 1.

Y chromosome microdeletions were more frequently detected (4.7%) in cases with azoospermia. Microdeletions were also detected (1.2%) in cases of severe oligozoospermia. No Y chromosome microdeletions were detected in cases with sperm counts above 5 million/mL (Table 2).

Table 1. AZF deletion patterns in cases with Y chromosome microdeletions

n, (%)	AZF microdeletion pattern					
	a	b	c	b+c	a+b+c	Total
	2 (3.7)	6 (11.1)	26 (48.1)	9 (16.7)	11 (20.4)	54

AZF: azoospermia factor

Table 2. Y chromosome microdeletion rates according to the results of semen analysis

Sperm count groups	Microdeletion n, (%)		
	Absent	Present	Total
Azoospermia	994 (95.3)	49 (4.7)	1043
0-5 million/mL	414 (98.8)	5 (1.2)	419
>5 million/mL	154 (100.0)	0 (0.0)	154
Total	1562	54	1616

Table 3. Y chromosome microdeletion rates according to infertility etiology

Groups	Microdeletion n, (%)		
	Absent	Present	Total
46, XY NOA	512 (93.6)	35 (6.4)	547
Klinefelter syndrome	191	0	191
Hypogonadotropic hypogonadism	61	0	61
CAVD	114	0	114
Other chromosomal abnormalities			
• Inversion/translocation	22 (91.7)	2 (8.3)	24
• 45X0/46XY mosaicism	5 (62.5)	3 (37.5)	8
• 46, XX testicular DSD	1 (9.1)	10 (90.9)	11
• 47, XYY	7	0	7
• Total	35 (70)	15 (30)	50
Total	913 (94.4)	54 (5.5)	967

NOA: nonobstructive azoospermia; CAVD: congenital absence of vas deferens; DSD: disorder of sexual development

When the cases were grouped according to the causes of infertility no Y chromosome microdeletions were detected in some groups (Klinefelter Syndrome, cases with hypogonadotropic hypogonadism, CAVD and 47, XYY karyotype). Y chromosome microdeletions were detected in the 46, XY NOA, inversion/translocation, 45, X0/46, XY mosaicism, and 46, XX testicular DSD groups. The frequency of AZF microdeletions were 6.1%, 8.3%, 37.5% and 90.9%, in these three groups, respectively (Table 3).

Discussion

In this study, the frequency of AZF microdeletion was 3.3% in 1616 infertile patients. This is lower than that reported in some previous Asian studies which had a similar number of cases to our study.[11,12] This difference between the results may be due to some factors such as ethnic differences, patient selection criteria, methodological aspects, and even the type and number of markers used in the studies. Moreover, the frequency of microdeletions detected in the present study was within the range reported by previous studies from Turkey (1.3-9.1%).[13-16] However, those studies consisted of limited number of cases and generally cases with azoospermia and severe oligozoospermia were evaluated.

In the present study, microdeletions in the AZFc region were the most common (48.1%), followed by those in the AZFa+b+c (20.4%), AZFb+c (16.7%), AZFb (11.1%) and AZFa (3.7%) regions. The frequent appearance of AZFc microdeletions was consistent with previous studies and the distribution rate of other microdeletions was similar.[17-19]

When the cases were analyzed according to semen analysis, microdeletion was most frequently (4.7%) found in the azoospermic group as expected. Furthermore, no microdeletions were detected in cases with sperm counts above 5 million/mL. Microdeletions occur in about one in 4000 men in the general population but their frequency is significantly increased among infertile men.[20] In a similar study in which the cases were assessed according to semen analysis, AZF microdeletions were detected in the moderate oligozoospermic group, even if its frequency was quite low.[21] In our study, we could not detect any AZF microdeletions in 154 cases with sperm counts above 5 million/mL. This discrepancy may be due to differences in genetic evaluation methods, patient selection, and ethnicity. Nevertheless, it may be appropriate, not to perform microdeletion assays in cases with sperm counts above 5 million/mL in terms of cost-effectiveness.

In the present study, no microdeletion was detected in some groups such as those with Klinefelter Syndrome, hypogonadotropic hypogonadism, CAVD and cases with 47, XYY karyotype. Although the number of cases in some groups is low, it may be significant that no microdeletions are detected in crowded groups such as Klinefelter Syndrome and CAVD. Three large studies in the literature have already supported this finding for

Klinefelter Syndrome.[22-24] The highest AZF microdeletion rates were found in non-obstructive azoospermic cases with 46, XY and some cases with other karyotype anomalies (except 47, XYY). In general clinical practice, karyotype analysis and Y chromosome microdeletion assays are often performed simultaneously. However, karyotype analysis before Y chromosome microdeletion analysis may favourably predict the necessity (if any) of microdeletion analysis.

Our study has some limitations. Our patient population was mostly made up of males from Central Anatolia. If the current study had been designed as a multicenter study from Turkey, our results would have approximately reflected the frequency of Y chromosome microdeletions among infertile men in the whole country. Also, the number of cases in some groups was smaller than in others. If the study had been conducted with a larger number of cases, our results would have been more conclusive.

In conclusion, analysis of Y chromosome microdeletion has a very important role in the management of infertile men and in the prediction of the success of ART. However, detailed evaluation of an infertile man by physical examination, semen analysis, hormonal evaluations and karyotype analysis may predict the patients for whom Y chromosome microdeletion analysis is necessary and prevent unnecessary health expenditure.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Erciyes University.

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