RESEARCH ARTICLE

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Evaluation of Y Chromosome Microdeletion and Chromosome Analysis Results in Infertile Male Patients

ABSTRACT

Objective: Genetic testing for male infertility is rarely performed in our country. Male infertility is caused by chromosome number or structural problems, Y chromosome deletions and gene alterations. Infertility is a problem seen in 15% of couples. Genetic causes are responsible for the etiology of 3-10% of those diagnosed with male infertility due to oligozoospermia and azoospermia. In this retrospective study, we aimed to determine both the chromosomal structure and the microdeletion of the azoospermic factor (AZF) region on the Y chromosome in infertile men admitted to our center before the application of assisted reproductive techniques.

Methods: We studied 327 patients who applied to our laboratory for routine analysis. Chromosome analysis was performed from peripheral blood by conventional cytogenetic method. DNA was isolated from peripheral blood and Y chromosome microdeletion was analyzed by fragment analysis method with Y chromosome microdeletion detection kit.

Results: Out of 327 patients, 32 had cytogenetic and 18 had molecular abnormalities and 4 had both cytogenetic and molecular abnormalities. Numerical and structural anomalies were detected in patients with anomalous karyotype. Among the patients with Y microdeletions, 1 patient had AZFa, 2 patient had AZFb, 6 patients had AZFc, 3 patients had AZFc+d, 2 patients had AZFb+c+d, 1 patient had AZFb+c+sY160, 1 patient had AZFa+b+d+c+sY90, and 2 patient had AZFb+d+c+sY90.

Conclusions: Our study shows that chromosomal abnormalities and Y chromosome microdeletions are important causes of male infertility and that chromosome analysis and Y chromosome microdeletion tests should be performed to explain these abnormalities. It also emphasizes the importance of genetic counseling in explaining male infertility.

Keywords: Male Infertility, Chromosomal Abnormality, Y Chromosome Microdeletion.

İnfertil Erkek Hastalarda Y Kromozomu Mikrodelesyon ve Kromozom Analizi Sonuçlarının Değerlendirilmesi

ÖZET

Amaç: Ülkemizde erkek infertilitesi için genetik testler nadiren yapılmaktadır. Erkek infertilitesine kromozom sayısı veya yapısal sorunlar, Y kromozomu delesyonları ve gen değişiklikleri neden olmaktadır. İnfertilite çiftlerin %15'inde görülen bir problemdir. Oligozoospermi ve azoospermi kaynaklı erkek infertilitesi tanısı alanların %3-10'unun etiyolojisinde genetik nedenler sorumludur. Bu retrospektif çalışmada, merkezimize başvuran infertil erkeklerde yardımcı üreme teknikleri uygulanmadan önce hem kromozomal yapının belirlenmesi hem de Y kromozomu üzerindeki azoospermik faktör (AZF) bölgesinin mikrodelesyonunun belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Laboratuvarımıza rutin analizler için başvuran 327 hasta çalışıldı. Bu hastalardan konvansiyonel sitogenetik yöntemle periferik kandan kromozom analizi yapıldı. Periferik kandan DNA izolasyonu yapılarak Y kromozom mikrodelesyon belirleme kiti ile fragman analizi yöntemi ile Y kromozomu mikrodelesyonu araştırıldı.

Bulgular: 327 hastanın 32'sinde sitogenetik ve 18'inde moleküler, 4'ünde hem sitogenetik hem moleküler düzeyde anomali belirlendi. Anomalili karyotipe sahip hastalarda sayısal ve yapısal anomaliler saptandı. Y mikrodelesyon belirlenen hastaların 1'inde AZFa, 2 hastada AZFb, 6 hastada AZFc, 3 hastada AZFc+d, 2 hastada AZFb+c+d, 1 hastada AZFb+c+sY160, 1 hastada AZFa+b+d+c+sY90, 2 hastada AZFb+d+c+sY90 bölgelerinde mikrodelesyon saptandı.

Sonuç: Çalışmamız kromozom anomalilerinin ve Y kromozomu mikrodelesyonunun erkek infertilitesinin önemli bir nedeni olduğunu ve açıklanmasında kromozom analizi ve Y kromozomu mikrodelesyon testlerinin yapılmasının gerekliliği gösterilmektedir. Ayrıca, erkek kaynaklı infertilitenin açıklanmasında genetik danışmanlık vermenin önemini vurgulamaktadır.

Anahtar Kelimeler: Erkek İnfertilitesi, Kromozomal Anomali, Y Kromozom Mikrodelesyonu.

INTRODUCTION

Changes in chromosome structure and number can cause various conditions ranging from dysmorphic appearance to recurrent fetal loss and even infertility (1-11). Today, DNA sequencing platforms are quite advanced with the developing technology and can detect structural and point mutations in DNA structure. Genetic studies have revealed that in addition to structural changes, point mutations and small indels are effective in phenotype (12-14). Y chromosome microdeletion (YCM) is a genetic disorder in which part of the Y chromosome is missing. It causes male infertility by affecting the ability to produce sperm. YCM is the second most common genetic cause of male infertility after Klinefelter syndrome. The prevalence of YCM ranges from 3-10% in infertile men and is rarely seen in the population. Generally, it occurs in 10% of non-obstructive azoospermia, 7% of severe oligozoospermia, and 1-2% of mild to moderate oligozoospermia (15,16). It also differs between populations, being higher in those of African and Asian descent (17). Deletions can occur in any of the four regions of the Y chromosome, including AZF (azoospermia factor) regions a, b, c, and d. The exact cause of YCM is unknown, but it is thought to be caused by mutations that occur during meiosis or mitosis (18). The absence of one or more of the genes responsible for spermatogenesis leads to reduced or absent sperm production, resulting in male infertility. The severity of the phenotype varies depending on the size and location of the deletion. The most common clinical manifestation of YCM is male infertility, which may be azoospermia or oligozoospermia. Other clinical manifestations may include testicular atrophy, hypospadias, and increased risk of testicular germ cell tumors (19). The diagnosis of YCM is made by genetic testing, particularly Y chromosome microdeletion analysis. This analysis can detect deletions in any of the three AZF regions. The test is usually performed on a blood or semen sample. Micro-deletions are seen in the four subtypes of AZF, AZFa, AZFb, AZFc, and AZFd (20,21). The diagnosis of YCM has implications for genetic counseling and assisted reproductive technologies. There is no specific treatment for YCM, but assisted reproductive technologies (ART) can be used to achieve pregnancy in couples affected by YCM. Intracytoplasmic sperm injection (ICSI) is the most commonly used ART technique in men with YCM. In cases with azoospermia, assistive treatments are applied in reproduction by performing testicular sperm extraction (TESE) (22,23).

MATERIAL AND METHODS

Between 2020-2023, 327 infertile men who applied to Çam and Sakura City Hospital Genetic Diseases Evaluation Center were analyzed retrospectively. All studies were approved by the ethics committee of Basaksehir Cam and Sakura City Hospital in accordance with the standards of the Declaration of Helsinki, and written informed consent for medical examinations, genomic analyses were obtained from the patients (KAEK-2023.07.290). After obtaining genomic DNA samples, the screening of AZF deletions was performed by multiplex polymerase chain reaction (PCR) method using Genetek Biopharma GT-AZF Screen PCR kit (Genetek Biopharma GmbH, Berlin, Germany) in accordance with company protocols.

Y Chromosome Microdeletion: sY190, AZFa (sY86, sY265, sY84), AZFb (sY127, sY130, sY131), AZFd (sY152, sY153) and AZFc (sY254, sY255) regions on the Y chromosome and control regions (SRY, ZFX/ZFY, Y/ , AMXY,) is based on PCR amplification of sequence-labeled regions. PCR products were analyzed using the ABI PRISM 3500 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Data analysis with GeneMapper v4.0 software (Applied Biosystems, Foster City, CA, USA).

Conventional Cytogenetics: For chromosome analysis, peripheral venous blood samples collected in heparinized tubes were inoculated into RPMI-1640 medium with phytohemagglutinin (PHA) and cultured at 37ºC for 72 hours. Colsemide was added 45 minutes before the study. Cultured blood cells were lysed with hypotonic solution and fixed with Carnoy's fixative. The cell suspension was spread on slides and aging was performed. The resulting chromosomes were analyzed after GTG banding in at least 20 metaphase plates.

RESULTS

Totaly 327 individuals who admitted to our Başakşehir Çam and Sakura Training and Research Hospital due to male infertility were included in the current study. The mean ages of individuals were 31.946±6.407 (min:16-max58). The distribution of the patients according to the indications is shown in Table 1. YCM was detected in 18 (5.5%) of 327 cases with primary male infertility and chromosomal abnormalities were detected in 32 (9.8%) cases (Table 2,3) (Figure 1).

Table 1. Indication distribution of patients.

Indication	Number	Percentage (%)
Azoospermia	204	62.4
Oligospermia	123	37.6
Total	327	100

Table 2. Y microdeletion results according to indication distribution of patients.

Indication	Azoospermia	Oligospermia
AZFa	1(0.5%)	0(0%)
AZFa + AZFb + AZFd + AZFc + sY90	1(0.5%)	0(0%)
AZFb + AZFc + AZFd	2(1%)	0(0%)
AZFb + AZFc + sY160	1(0.5%)	0(0%)
AZFb + AZFd + AZFc +sY90	2(1%)	0(0%)
AZFb	1(0.5%)	1(0.5%)
AZFc	3(1.5%)	3(2.4%)
AZFc + AZFd	1(0.5%)	2(1.6%)
NORMAL	192(94.1%)	117(95.5%)
Total	204(62.4%)	69(37.6%)

Table 3. Choromosomal abnormality results according to indication distribution of patients.

Karyotype	Azoospermia	Oligospermia
45,X[10]/46,XY[40]	1(0.5%)	0(0%)
45,X[48]/46,X,der(Y)[2]	1(0.5%)	0(0%)
45,XY,der(13;14)(q10;q10)	1(0.5%)	1(0.5%)
45,XY,der(15;22)(q10;q10)	1(0.5%)	0(0%)
46, XY, 22ps-	1(0.5%)	0(0%)
46,X,del(Y)(q11.23)	2(1%)	0(0%)
46,X,i(Y)(p10)	1(0.5%)	0(0%)
46,XY,inv(7)(p22q32)	1(0.5%)	0(0%)
46,XY,inv(9)(p11q13)	3(1.5%)	0(0%)
46,XYqh+	4(2%)	1(0.5%)
47,XXY	13(6.4%)	0(0%)
47,XYY	1(0.5%)	0(0%)
46,XY	174(85.3%)	121(99%)
Total	204(62.4%)	123(37.6%)

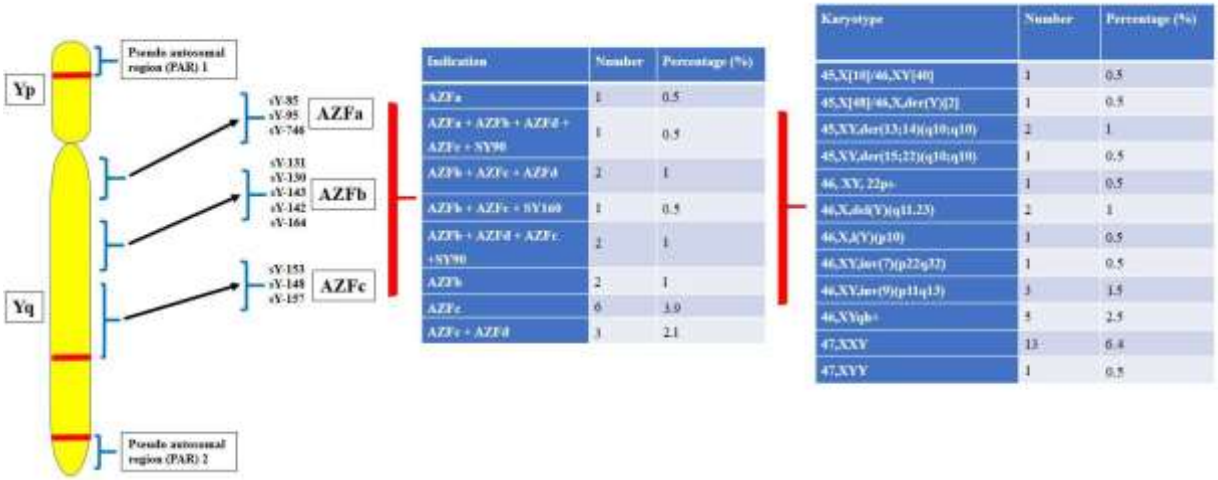

Figure 1. Y chromosome microdeletion test and distribution of chromosomal abnormalities in infertile patients.

The most common chromosomal abnormalities were found in the sex chromosomes (59.4%; 19/32). Others were autosomal translocations, additions or deletions of satellites (40.6%; 13/32). The most common chromosomal abnormality was Klinefelter syndrome (47,XXY) in 13 of 32 cases (Table 3) (Figure 1). Y microdeletion results according to indication distribution of patients were shown in the table 2. When the patients were divided as azoospermia and oligospermia, statistically significant differences were not found between two groups according to Chromosome Analysis results (χ2:17.321; p:0.138) (Table 3). When the patients were divided as azoospermia and oligospermia, statistically significant differences were not found between two groups according to Y microdeletion results (χ2:5.831; p:0.666) (Table 2).

In YCM, the AZFc region was found to be the most affected (33.3%), followed by AZF c+d (16.6%), AZFb+c+d (11.1%), AZFb+d+c+sY90 (11.1%), AZFb (11.1%), AZFa(5.5%),

AZFa+b+d+c+sY90 (5.5%), AZFb+c+sY160 (5.5%), respectively (Table 2). Abnormal karyotypes were found in 4 (22.2%) of 18 cases with YCM.

DISCUSSION

The male factor is responsible for 30-50% of infertility cases, and up to 20% of infertile men appear to be azoospermic (24). Azoospermia is generally observed with a frequency of 1% in the male population. Some conditions directly lead to azoospermia, while others occur after complex gene-environment interaction (24,25). A population-based study investigating the relationship between YCMs and male infertility showed that YCMs were significantly associated with infertility and azoospermia in this population (26-28). The prevalence of YCM varies between different populations, with some populations having a higher prevalence than others (29). Studies have shown that the prevalence of YCM is higher in infertile men, with rates ranging from 5% to 10% (30). The prevalence in the general population is estimated to be around 1 in 2000 men. The difference observed in these studies may be explained by the number of patients analyzed and the phenotype included in the studies. AZFc region deletions were most common in YCM and the Y chromosome contains several genes that play a role in male sex determination and fertility. YCM has been reported to cause loss of these genes, leading to various reproductive problems such as decreased sperm count, abnormal sperm morphology, and impaired sperm motility (31). However, some men with YCM may have normal sperm counts but still have difficulty conceiving due to other factors such as poor sperm motility or abnormal sperm morphology (32). In studies evaluating the efficacy of TESE and ICSI in men with FCM, it was found that TESE is a viable option for men with FCM and azoospermia, with an increased pregnancy rate. It was also emphasized that ICSI is effective in men with mild to moderate oligozoospermia (33,34). In a study, it was found that men with YCM who underwent IVF (in vitro fertilization) with ICSI had similar fertilization and pregnancy rates as men without YCM (35).

The most common type of YCM is the AZFc deletion, which occurs in the azoospermia factor c region of the Y chromosome (36) and the best prognosis is found in the AZFc deletion (37). MicroTESE is not recommended for complete AZFa, AZFb and AZFb+c deletions. The probability of finding sperm with micro-TESE in AZFc deletions is 50-60% (stahl2010). The transmission of AZFc region deletions to male babies is possible with the use of assisted reproduction method (38). However, some men with YCM have sperm production early in life and then have problems with sperm production. Therefore, it will be important steps for fertility of people with YCM or sex chromosomal abnormality to have their sons checked and, if necessary, to cryopreserve the sperm. However, pregnancy can also be achieved with the Preimplantation Genetic Diagnosis (PDG) method and options including the tran.

In our study, 327 infertile men were tested for YCM and CA. YCM was detected in 5.5% of patients, 9.8% of CA, and 1.2% of both YCM and CA (Table 2 and 3). While the frequency of chromosomal anomalies in the general population is approximately 0.6%-4%, it is reported as 2%-14% in male infertility cases (18). 5-15% of men with azoospermia or severe oligozoospermia have chromosomal abnormalities in an another study (7,39,40). In azoospermic men; The most common chromosomal abnormalities are Robertsonian translocations, inversions and Klinefelter syndrome (KS) (7,41,42). In our study, chromosomal variation was detected in 32 (9.8%) of 327 cases evaluated in terms of infertility. Except for the cases evaluated as chromosomal polymorphism, out of 23 (7%) patients, 14 numerical and 9 structural chromosomal variants were detected. 47,XXY (KS) was the most frequently detected chromosomal abnormality in our patient group (Table 3). CAs, whether numerical or structural, have serious adverse effects on fertility. CA was reported in 4% of patients who will undergo intracytoplasmic sperm injection (ICSI), 80% of which are related to the sex chromosomes (41,42) therefore, chromosomal analysis should be performed in patients evaluated for infertility.

KS is the most common genetic cause of male infertility and commonly affected individuals are taller than average and infertile. It is thought that the disease occurs as a result of the dosage effect of genes escaping from X inactivation in the extra X chromosome (43). In some cases, the symptoms are so mild that they are not diagnosed until puberty or adulthood. It is stated in the literature that 75% of the patients cannot be diagnosed in the early period (44). In our series, KS was found to be the most common chromosomal anomaly in infertility (13%), and we think that it is important to increase the awareness of clinicians in the early diagnosis of this disease. Patients with KS have small testicles that produce low amounts of testosterone. In some of the patients, sperm retrieval can be achieved by some specific methods called testicular sperm extraction; however because of the risk of gametes with chromosomal anomalies, these patients should be diagnosed with preimplantation genetics (45).

The relationship between YCM and testicular germ cell tumors has been investigated, and it has been reported that men with YCM have an increased risk of developing testicular germ cell tumors, especially those with partial or complete AZFc deletion (46). Although not fully established, it demonstrates that Y chromosome loss or ectopic expression of Y chromosome genes is closely

associated with a variety of male-based diseases, including selected somatic cancers (32,46).

Recent advances in genetic testing have allowed noninvasive detection of YCM using cell-free DNA in seminal plasma. This has the potential to reduce the need for invasive diagnostic procedures such as testicular biopsy (47,48). There is also ongoing research in the field of gene therapy for YCM, which may hold promise for future treatment options.

CONCLUSION

YCMs are a common genetic cause of male infertility. Diagnosis is made by genetic testing, and treatment options include assisted reproductive techniques such as ICSI and IVF. In our study, the genetic causes of male infertility who applied to our center were tried to be determinedThese genetic tests are the recommended test for all patients with azoospermia or severe oligospermia. Regenerative treatments such as stem cell therapy and gene therapy show promise in the treatment of male infertility caused by YCMs. These treatments aim to restore damaged or missing cells and genetic material to function properly to improve sperm production and function. Advances in genetic testing and regenerative therapies offer hope for the development of new treatments.

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