Iranian Journal of Reproductive Medicine Vol.7. No.2. pp: 79-84, Spring 2009

Results of ICSI in severe oligozoospermic and azoospermic patients with AZF microdeletions

Sevtap Kilic[1] M.D., Ph.D., Beril Yuksel[1] M.D., Nafiye Yilmaz[1] M.D., Erkan Ozdemir[2] M.D., Ufuk Ozturk[2] M.D., Serdar Ceylaner[3] M.D., Muammer Dogan[1] M.D.

1 Department of Reproductive Endocrinology, Dr. Zekai Tahir Burak Women's Health and Research Hospital, Ankara, Turkey.
2 Department of Urology, Dr. Zekai Tahir Burak Women's Health and Research Hospital, Ankara, Turkey.
3 Department of Genetics, Dr. Zekai Tahir Burak Women's Health and Research Hospital, Ankara, Turkey.

Received: 20 October 2008; accepted: 13 April 2009

Abstract

Background: The aim of this study was to determine the incidence of AZF (Azoospermia Factor) microdeletions of the Y chromosome in infertile Turkish male patients and intracytoplasmic sperm injection (ICSI) outcome of these patients.

Objective: This study was undertaken in order to evaluate the outcome of intracytoplasmic sperm injection (ICSI) in infertil man with AZF microdeletions

Materials and Methods: We evaluated 348 azoospermic and oligozoospermic patients retrospectively. Fourty of these patients had various types of AZF microdeletions. These patients had non-obstructive severe oligoasthenospermia or azoospermia with normal karyotype. Azoospermic patients underwent testicular sperm extraction and aspiration (TESE, TESA). Then ICSI was performed to patients who had testicular sperm or ejeculat.

Results: Fourty patients with AZF microdeletion were evaluated in this study. No spermium could be found in 27 patients. Three of these patients had only AZFa microdeletion, three had AZFb microdeletion, three had AZF (b+c), six had AZF (a+b+c) and 12 patients had AZFc microdeletion. Only two of all patients achieved a pregnancy and both had only AZFc microdeletion.

Conclusion: AZFc microdeletions have a better prognosis for achieving spermium in ejaculate or TESE, TESA materials.

Key words: *Y microdeletion, AZF, TESE, ICSI outcome.*

Introduction

It is clearly obvious that the Y chromosome is necessary for sexual development and spermatogenesis. In 1976, Tiepolo and Zuffardi (1) reported that the long arm of the Y chromosome carried genetic information that is essential for spermatogenesis. The deletions of the long arm of the Y chromosome could result breakdown in spermatogenesis, leading to infertility in human (2).

Corresponding Author:
Sevtap Kilic, Süleymanbey Sok. 29/10, Maltepe, Ankara, Turkey, PK: 06570
E-mail :sevtapkilic@gmail.com

The long arm of Y chromosome (Yq), particularly Yq11 .23 region, is considered to be critical for fertility. Macroscopic deletions of Yq11 are often observed in patients with azoospermia although many microdeletions have been implicated as significant causes of infertility. These microdeletions are often observed in the long arm, at the AZF (Azoospermia Factor) locus. Several subregions are distinguished in the AZF locus. In this locus, the structure of the Y-chromosome is rich in repeated palindromes and recombination between two flanking sequences and sharing a high degree of homology leads to various deletions: AZFa, AZFb, AZFc.

The frequency of these microdeletions differs among different ethnical populations. In a recent

study by Ristanovic *et al* deletions on Y chromosome were detected in 15.6% of cases, and of all cases 64.7% were detected in AZFc region, 17.6% in AZFa region and 17.6% in AZFb region (3). The most frequent deletion, AZFc, is usually associated with milder azoospermia and oligospermia (4).

Complete deletions of AZFa, AZFb+c and AZFb regions are associated with cases of more severe azoospermia. The testicular histology shows either total lack of germline cells (SCOS, Sertoli cell only syndrome) or arrested maturation of cells from the spermatogenetic cell lineage (5). The main purpose of this study was to determine AZF microdeletions of the Y chromosome among Turkish infertile patients with azoospermia or oligoasthenospermia and their relation with intracytoplasmic sperm injection (ICSI) outcome for these patients. The association between the types of Y chromosome genetic abnormality and various histopathological features were also evaluated.

Materials and methods

Patients

348 patients who applied to our ART (Assisted Reproductive Therapy) clinic between May 2006 and May 2007 and had the diagnosis of azoospermia or oligoasthenospermia were investigated retrospectively. An approval from local educational research committee was taken and written informed consents were obtained from all patients by post, prior to study. Twenty patients who had obstructive azoospermia and eighty patients with additional abnormal findings such as clinic varicocele, recurrent urogenital infections, recurrent urogenital surgery or monolateral cryptorchidism were excluded from the study. The remaining 248 patients were searched for Y microdeletions and forty men with normal karyotype presented AZF microdeletions of Y chromosome.

The patients did not have additional abnormal findings such as clinic varicocele, recurrent urogenital infections or monolateral cryptorchidism. All forty patients were primary infertile. Hormonal parameters and ultrasonographic testicular volume measurements were recorded. Hormonal parameters were studied with Electrochemiluminescence Immunoassay "ECLIA" (Roche, E170, ELECSYS, Mannheim, Germany) on Elecsys and cobas immunoassay analyzers.

The minimal detectable limit of the assay was 0.12 ng/ml and normal range of assay was 0.80-8.00 ng/ml for total testosterone. The sensitivity of the assay was 0.13 mIU/ml and range of assay was 0.13-200 mIU/ml for FSH (follicle stimulating hormone), inter-assay coefficients of variation sensitivity was %5 for this test.

The sensitivity of assay was 0.02mIU/ml, range of assay was 0.02-200 m IU/ml, inter-assay coefficients of variation sensitivity was 5% for LH (Luteinizing hormone). Testicular volume was calculated using the ellipsoid formula (volume = $4/3\pi$ a·b·c where a, b, and c are the semiaxes of the ellipsoid). We performed ICSI to 13 patients.

One surgeon performed all TESE procedures via micro dissection technique (6, 7) and one researcher reviewed the pathology of diagnostic biopsies.

The procedures were performed in conjunction with controlled ovarian stimulation cycles for ICSI via standard long protocol for female partners. Spermatozoa were not cryopreserved and fresh materials were used for ICSI.

Y chromosome analysis

Y chromosome analysis was carried out with a multiplex polymerase chain reaction (PCR) technique on DNA which was extracted from peripheral leukocytes.

Genomic DNA was extracted from peripheral blood. DNA was subjected to AZF-polymerase chain reaction (PCR) simplex. A multiplex protocol with primers from all the regions was also developed.

For patients who had failure of amplification of sequence-tagged site (STS) on Y chromosome, multiple primary pairs were used to confirm the absence of each site in a PCR reaction on multiple occasions. Method and primers described in the guideline orginezed by Simoni *et al* was used (8).

Statistical analysis

SPSS for windows version 11.0 was used for the frequency analysis and $p<0.05$ was accepted significant.

Results

Fourty patients with normal karyotype but AZF microdeletions of Y chromosome were identified. Mean FSH value for the patients was 20.91±4.01 mIU/ml, mean LH value was 11.71±3.06 and mean total testosterone value was 2.72±0.61.

Mean testicular volume was calculated as 1.49±0.58 cm^3. Twenty of these 40 patients had

AZFc microdeletions whereas 5 patients had AZFb, 3 patients had AZFa, 4 patients had AZFb+AZFc and 8 patients had total AZFa+AZFb+AZFc microdeletions (Table I).

Table I. Types of microdeletions, numbers and percentages of patients that had spermium in their ejaculate or TESE material and that achieved pregnancy.

Type of microdeletion	Nomber (percentage) of patients	Nomber (percentage) of patients that had spermium in the ejaculate or TESE material	Nomber (percentage) of patients that achieved pregnancy	Spermium positive patients for each type of AZF deletion
AZFa	3 (7.5%)	0 (0%)	0 (0%)	0/3 (0%)
AZFb	5 (12.5%)	2 (5%)	0 (0%)	2/5 (40%)
AZFc	20 (50%)	8 (20%)	2 (5%)	8/20 (40%)
AZFa + AZFb	4 (10%)	1 (2.5%)	0 (0%)	1/4 (25%)
AZFa+AZFb+AZFc	8 (20%)	2 (5%)	0 (0%)	2/8 (25%)
Total	40 (100%)	13/40 (32.5%)	2 /40 (5%)	13/40 (32.5%)

Table II. Sperm count, type of microdeletion, missing gene locus, pathology and treatment applied to patients. (AZF: azoospermia factor, SA: spermatocytic arrest, SC: sertoli cell only, hy: hyalinization, HS: hypospermatogenesis, GA: germ cell aplasia, OAS: oligoastenospermia, TESE: testicular sperm extraction).

Patient	Spermiogram	AZF	Sy missing	Pathology	Treatment
1	Azospermia	AZFc	Sy254-255	SC+SA	TESE
2	Azospermia	AZF c	Sy254-255	SA	TESE
3	Azospermia	AZF b	Sy127-134	HS	TESE
4	Azospermia	AZFb,c	Sy127-134 ,254,255	SA	TESE
5	Azospermia	AZF b	Sy127-134	SC+GA	TESE
6	OAS	AZF c	Sy254-255	-	-
7	Azospermia	AZFb,c	Sy127-134 ,254,255	SC+SA	TESE
8	Azospermia	AZFa,b,c	Sy86,84,127-134 ,254,255	SC+SA	TESE
9	Azospermia	AZF c	Sy254-255	SA	TESE
10	OAS	AZF c	Sy254-255	-	-
11	Azospermia	AZF c	Sy254-255	HS	TESE
12	Azospermia	AZFa	Sy 86,84	SC+HS	TESE
13	Azospermia	AZF c	Sy254-255	SA	TESE
14	Azospermia	AZFa,b,c	Sy86,84,127-134 ,254,255	SC+HS	TESE
15	Azospermia	AZFb,c	Sy127-134 ,254,255	HS	TESE
16	Azospermia	AZFa,b,c	Sy86,84,127-134 ,254,255	SA	TESE
17	OAS	AZF c	Sy254-255	HS	TESE
18	Azospermia	AZF b	Sy127-134	Hy	TESE
19	Azospermia	AZFa,b,c	Sy86,84,127-134 ,254,255	SA	TESE
20	Azospermia	AZFa	Sy 86,84	SC	TESE
21	Azospermia	AZF c	Sy254-255	SA	TESE
22	Azospermia	AZFa,b,c	Sy86,84,127-134,254,255	SC+Hy	TESE
23	Azospermia	AZF c	Sy254-255	SA	TESE
24	Azospermia	AZF c	Sy254-255	SC	TESE
25	Azospermia	AZFa,b,c	Sy86,84,127-134 ,254,255	SC	TESE
26	Azospermia	AZFa,b,c	Sy86,84,127-134 ,254,255	SC	TESE
27	OAS	AZF c	Sy254-255	-	-
28	Azospermia	AZF c	Sy254-255	SA	TESE
29	Azospermia	AZFa,b,c	Sy86,84,127-134 ,254,255	SC	TESE
30	OAS	AZF b	Sy127-134	-	-
31	OAS	AZF c	Sy254-255	-	-
32	Azospermia	AZFb,c	Sy127-134,254,255	SA	-
33	OAS	AZF c	Sy254-255	-	-
34	Azospermia	AZF c	Sy254-255	SA	TESE
35	Azospermia	AZF c	Sy254-255	SA	TESE
36	OAS	AZF c	Sy254-255	-	-
37	Azospermia	AZF c	Sy254-255	SA+SC	TESE
38	Azospermia	AZFb	Sy127-134	SC	TESE
39	Azospermia	AZFa	Sy 86,84	SC	TESE
40	Azospermia	AZF c	Sy254-255	SA	TESE

The patients were between ages of 24 and 36 years. From these 40 patients, 32 had azoospermia and 8 had oligoasthenospermia in their semen analysis. We achieved spermium from ejaculate or TESE material and applied ICSI to 13 patients. The incidence of Y microdeletion was 11.49 % in all infertile patients (40 of 348 patients). Types of microdeletions, numbers and percentages of patients that had spermium in their ejaculate or TESE material are stated in table I. The pathological results of testicular biopsies are summarized in table II. The patients with total deletions (AZFa+AZFb+AZFc) and only AZFa microdeletions had severe impaired spermatogenesis in pathologic findings and semen analysis.

Only two of all patients achieved a pregnancy and both had AZFc microdeletion. The patients were able to complete the pregnancy and gave birth to a healthy child. Spermia was obtained from ejaculate from one of these patients and from TESE material from the other patient. He had spermatocytic arrest in testicular pathology. We could not achieve pregnancy from patients with AZFa+b+c, AZFb+c, AZFa, AZFb microdeletions. From AZFa microdeletions, it was also impossible to achieve spermium.

Discussion

Microdeletions of the Y chromosome represent an important cause of male infertility and one of the most frequent genetic etiologies of severe testiculopathy. The development of Y-specific sequence-tagged sites (STS) - based mapping strategy has permitted the rapid screening of a large number of infertile patients for Y chromosome microdeletions (9).

The incidences of Y chromosome microdeletions in different ethnic populations reveal different results. Y chromosomal abnormality rate is reported to be 2.2% in non-selected group of males with subfertility in a study by Krausz *et al* (10). It was reported to be 7% in a study by Martinez *et al* (11) and the deletions were detected in 3.3% of Turkish male patients in a study by Sargin *et al* (12). In their study, Sargin *et al*. found that 47 patients had a diagnosis of idiopathic azoospermia (both obstructed and nonobstructed) and 13 had oligozoospermia of all 60 infertile patients with normal karyotype. In our study we determined the incidence of Y microdeletion as 11.49% in infertile male patients.
When we evaluated the pathological features of biopsies most of the biopsy specimens revealed to have sertoli only cell pattern in the cases of AZFa microdeletions, and this finding is consistent with Kamp *et al* (13). Moreover, no spermium could be found in any of the patients who had AZFa microdeletion which could lead to a conclusion that having AZFa microdeletion caused the worst prognosis for achieving spermium in the ejaculate or TESE material.

In our study the incidence of AZFb microdeletions was 12.5% among patients with azospermia. AZFb microdeletions of the Y chromosome are rare. Moreover, finding spermium is also rare from TESE material from the ones having AZFb microdeletion. In our study we could achieve spermium only from two patients with AZFb (5%). In addition, the histological findings revealed a severe impared semen analysis which concurs well with the literature. It is now well established by histological analysis that complete microdeletion of AZFb region usually results in severe spermatogenesis impairment, leading to azoospermia. Complete AZFb deletion is generally associated with a maturation arrest, frequently but not exclusively at the spermatocyte stage. It is characterized by the presence of spermatogonia and spermatocytes, but no postmeiotic germ cells are found in the majority of the tubules (14).

In general, testis histology of patients with complete deletions of the AZFa region and/or the AZFb region reveals a complete Sertoli cell-only syndrome (SCOS), while testis histology of patients with partial AZFa and AZFb deletions or with AZFc deletions can result in a broad array of testicular phenotypes ranging from complete SCOS or spermatogenetic arrest to hypospermatogenesis (15).

Consistent with the literature through our findings, AZFc microdeletion was the most frequent type of Y microdeletion and had the best prognosis for achieving spermium among the patients with azopermia (4, 9, 16). We determined a spectrum of histologies in the specimens of patients with AZFc deletions. AZFc deletions are known to be associated with a heterogeneous histological profile, varying from SCO-syndrome to spermatogenic arrest and hypospermatogenesis (17) and the seminal parameters range between azoospermia and oligozoospermia. Oates *et al* (18) could not even discern a correlation between AZFc deletions and testicular histology. This is not surprising, because most men with nonobstructive azoospermia have a heterogeneous pattern of spermatogenesis.

We determined mixed AZF microdeletions in twelve patients (four with AZFb+ AZFc and eight

with AZFa+ AZFb+ AZFc). The pathologic findings revealed SCOS predominantly but also spermatocytic arrest and hypospermatogenesis areas were also determined.

Based on these results we can conclude that AZFa microdeletions have the worst prognosis for the possibility of finding spermium in ejaculate or TESE material and consequently the poorest prognosis in ICSI outcome. In contrary, AZFc microdeletions provide the best results for achieving spermium and pregnancy. These results also concur well with the literature (19). Men with AZFc deletion are somatically healthy, will most likely have useable sperm and chance to experience biological paternity (18). Vertical transmission of AZF deletions from father to son by ICSI was demonstrated in several studies examining larger populations of AZF deleted male (19,20). Thus genetic inheritance should also be taken into consideration in the cases of ICSI conceived sons.
In our study, we could not find an absolute correlation between diagnostic testicular biopsy findings and type of microdeletion, nor even between biopsy findings and TESE success. TESE may not always be successful in patients with non-obstructive azoospermia, as targeting a focus of active spermatogenesis site is not always possible. Previous several studies have indicated that a single random biopsy cannot reliably predict sperm retrieval with TESE (20).

The pathologic scan of the TESE material does not give exact information about all testicular tissue. Testis histology could not predict oligospermia or azoospermia, nor could they predict whether sperm could be found in TESE material (18). But in our study we used microTESE, therefore, the possibility of finding spermium and achieving more uniform pathologic findings increased. However, still the pathologic parameters did not correlate with the possibility of achieving spermium.

Through assisted reproductive technology, men with Y microdeletions and severe oligozoospermia or azoospermia became capable of reproducing. The age, clinic parameters, hormonal assessments and histological evaluations are not enough to describe testicular failure in patients with Y microdeletions. As the type AZF deletions provide an important estimation for ICSI outcome, we point out the importance of genetic testing and counselling regarding Y chromosome microdeletion for couples requesting ICSI. A combination of these parameters should be used to increase the possibility of finding spermium and the chance to undergo ICSI.

References

1. Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum Genet* 1976; 34:119-1124.
2. Matsuda T, Horii Y, Ogura K, Nonomura M, Okada K, Yoshida O. Chromosomal survey of 1001 subfertile males: incidence and clinical features of males with chromosomal anomalies. *Acta Urol Jpn* 1992; 38: 803–809.
3. Ristanovic M, Bunjevacki V, Tulic C, Novakovic I, Nikolic A. Molecular analysis of Y chromosome microdeletions in idiopathic cases of male infertility in Serbia. *Genetika* 2007; 43: 850-854.
4. Choi JM, Chung P, Veeck L, Mielnik A, Palermo GD, Schlegel PN. AZF microdeletions of the Y chromosome and in vitro fertilization outcome. *Fertil Steril* 2004; 81: 337-341.
5. Ferlin A, Arredi B, Speltra E, Cazzadore C, Selice R, Garolla A, et al. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. *Clin Endocrinol Metab* 2007; 92: 762-770.
6. Tsujimura A, Matsumiya K, Miyagawa Y, Tohda A, Miura H, Nishimura K, et al. Conventional multiple or microdissection testicular sperm extraction: a comparative study. *Hum Reprod* 2002; 17: 2924-2929.
7. Schlegel PN. Testicular sperm extraction: Microdissection improves sperm yield with minimal tissue excision. *Hum Reprod* 1999;14: 131-135.
8. Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for moleculer diognosis of y-chromosomal microdeletions. *Int J Androl* 2004,27: 240-249.
9. Hadj-Kacem L, Hadj-Kacem H, Ayadi H, Ammar-Keskes L, Chakroun-Fki N, Rebai T, et al. Screening of Y chromosome microdeletions in Tunisian infertile men. *Arch Androl* 2006; 52: 169-174.
10. Krausz C, Bussani-Mastellone C, Granchi S, McElreavey K, Scarselli G, Forti G. Screening for microdeletions of Y chromosome genes in patients undergoing intracytoplasmic sperm injection. *Hum Reprod* 1999; 14: 1717-1721.
11. Martínez MC, Bernabé MJ, Gómez E, Ballesteros A, Landeras J, Glover G, et al. Screening for AZF deletion in a large series of severely impaired spermatogenesis patients. *J Androl* 2000; 21: 651-655.
12. Sargin CF, Berker-Karaüzüm S, Manguoğlu E, Erdoğru T, Karaveli S, Gülkesen KH, et al. AZF microdeletions on the Y chromosome of infertile men from Turkey. *Ann Genet* 2004 ;47: 61-68.
13. Kamp C, Hirschmann P, Voss H, Huellen K, Vogt PH. Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. *Hum Mol Genet* 2000; 9: 2563-2572.
14. Yogev L, Segal S, Zeharia E, Gamzu R, Maymon BB, Paz G, et al. Sex chromosome alignment at meiosis of azoospermic men with azoospermia factor microdeletion. *J Androl* 2004; 25: 110-116.
15. Luetjens CM, Gromoll J, Engelhardt M, Von Eckardstein S, Bergmann M, Nieschlag E, et al. Manifestation of Y-chromosomal deletions in the human testis: a morphometrical and immunohistochemical evaluation. *Hum Reprod* 2002; 17: 2258-2266.
16. Plaseski T, Novevski P, Kocevska B, Dimitrovski C, Efremov GD, Plaseska-Karanfilska D. AZF deletions in

infertile men from the Republic of Macedonia *Prilozi* 2006; 27: 5-16.
17. Luetjens CM, Gromoll J, Engelhardt M, Von Eckardstein S, Bergmann M, Nieschlag E, et al. Manifestation of Y-chromosomal deletions in the human testis: a morphometrical and immunohistochemical evaluation. *Hum Reprod* 2002; 17: 2258-2266.
18. Oates RD, Silber S, Brown LG, Page DC. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. *Hum Reprod* 2002; 17: 2813-2824.
19. Lynch M, Cram DS, Reilly A, O'Bryan MK, Baker HW, de Kretser DM, et al. The Y chromosome gr/gr subdeletion is associated with male infertility. *Mol Hum Reprod* 2005; 11: 507-512.
20. Amer M, Haggar SE, Moustafa T, Abd El-Naser T, Zohdy W. Testicular sperm extraction: impact of testicular histology on outcome, number of biopsies to be performed and optimal time for repetition. *Hum Reprod* 1999; 14: 3030-3034.