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Evaluation of the Genetic Analysis Results in Infertile Patients with Non-Obstructive Azoospermia

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What's known on the subject? and What does the study add?

Genetic factors are important among the causes of non-obstructive azoospermia (NOA). Genetic tests can provide information about the possibility of sperm retrieval before microscopic testicular sperm extraction (micro-TESE) is applied to NOA patient. Age, testis volume, serum FSH and testosterone levels, and the presence of Klinefelter syndrome (KS) were found to affect the sperm retrieval rates (SRR) in micro-TESE. Moreover, sperm retrieval rate (SRR) of patients with KS and Y chromosome microdeletion was lower than the literature.

Abstract

Objective: To evaluate the genetic analysis results of patients who referred to our clinic infertility and whom semen analysis revealed non-obstructive azoospermia (NOA).

Materials and Methods: Among 994 patients who underwent a microscopic testicular sperm extraction (micro-TESE) operation for NOA, 497 patients who were tested for karyotype analysis and 450 patients who were tested for chromosome Y microdeletion were included in our study. The rates of Klinefelter syndrome (KS) and Y chromosome microdeletion, sperm retrieval rates (SRR) in these genetic anomalies and the factors affecting them were investigated. Additionally, the association between the age, duration of infertility, testicular size, serum follicle stimulant hormone (FSH) and testosterone levels of patients and sperm extraction rates of micro-TESE operations were also evaluated.

Results: The overall SRR of NOA patients who underwent micro-TESE was 47.5%. Among 104 patients with KS, sperm was successfully found after micro-TESE in 22 (21.2%). Fourteen patients were diagnosed with the Y chromosome microdeletion and sperm was successfully found in 4 (28.6%) of them; while the duration of infertility did not affect the SRR after micro-TESE ($p=0.712$); age, testicular volume serum FSH and testosterone levels had a significant effect on the SRR ($p<0.005$).

Conclusion: In this study, the SRR of patients who have chromosome Y microdeletion or KS, was found to be lower than other studies in the literature. This difference could be derived from the genetically tested population's structure, variance in the gene areas used for scanning and different demographic characteristics of different regions.

Keywords: Genetic analysis, Klinefelter syndrome, micro-TESE, non-obstructive azoospermia, Y microdeletion

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Introduction

Infertility is defined as the failure to achieve pregnancy after a year or more of regular and unprotected sexual intercourse (1). When all married couples are considered, the worldwide prevalence of infertility is thought to be 15%. In studies consisting of normal healthy couples having unprotected sex, it has been shown that pregnancy can be achieved within 6 months in 60-75%, and within 1 year in 90% of them (2).

Azoospermia is defined as the absence of sperm in the ejaculate, and has been determined in 1% of all males and 10-15% of infertile males (3). Non-obstructive azoospermia (NOA) is defined as the absence of spermatozoa in the ejaculate due to minimal or inability to produce mature sperm in the testicles. Genetic factors are important among the causes of NOA. Genetic tests can provide information about the possibility of sperm retrieval before microscopic testicular sperm extraction (micro-TESE) is applied to patients with NOA.

Micro-TESE is a surgical method in which spermatogenesis is shown to continue in the testes in small foci under a microscope and mature sperm cells are obtained from those foci. In patients who present because of infertility, Y-chromosome microdeletion genetic analysis should be applied to patients with a sperm count of <5 million, and karyotype analysis to patients with sperm count of <10 million, as well as to those with azoospermia (4,5). While chromosome anomalies are seen in 0.5% of the normal population, this rate increases up to 5.8% in infertile males (6). Therefore, it is recommended screening for genetic anomalies in infertile males before intracytoplasmic sperm injection (ICSI). These genetic analyses should include genetic tests related to sex chromosome anomalies [Klinefelter syndrome (KS), XYY syndrome, XX male syndrome, mixed gonadal dysgenesis, Y chromosome microdeletion, other Y chromosome structural anomalies, reciprocal translocation between sex chromosomes], anomalies in autosomal chromosomes (reciprocal translocations, Robertsonian translocations, chromosomal segmental inversion, other autosomal chromosome anomalies) and genetic abnormalities in reproductive cells.

In daily practice, patients with KS and Y chromosome microdeletion are the most frequently encountered of the infertile patients. This study aimed to evaluate the genetic analysis results of patients who presented because of infertility, were diagnosed with NOA and underwent a micro-TESE operation.

Materials and Methods

Data of 1076 patients who presented to our clinic with the inability to achieve pregnancy and were found to have NOA were retrospectively analyzed. Males older than 18 years who had not received any prior treatment for infertility, undergone any assisted reproductive technique and undergone a urological operation; NOA patients who tested for genetic analysis and underwent micro-TESE operation in our clinic were included in the study. The exclusion criteria were as follows: Patients who were lost to follow-up or whose retrospective information was not available from the hospital database, obstructive azoospermia patients and patients who had chemotherapy and radiotherapy.

A clinical examination included secondary sexual characteristics, testicular size and consistency, epididymal distension, the presence of the vas deferens and varicocele. Patient's age, the duration of infertility, the volume of the testes, serum follicle stimulant hormone (FSH) and testosterone levels were recorded. Patients with KS and Y chromosome microdeletion according to the results of genetic analysis and those without genetic anomaly were compared separately in terms of the probability of finding sperm in the micro-TESE operation. No medical treatment was administered to the patients before micro-TESE. Approval for the study was granted by the Local Ethics Committee (project no: KA14/277, date: 24.09.2014 - Baskent University Ethics Committee for Non-Interventional Clinical Trials).

Statistical Analysis

Data were analyzed statistically using SPSS v.22 Software (Statistical Package for Social Sciences). Independent Samples t-test was used for evaluation of the parameters. The chi-square test was applied to determine the relationship between micro-TESE result and KS or Y chromosome microdeletion. Logistic regression analysis was performed by forming a model with variables determined in One-Way analysis to affect the micro-TESE result, and the factors affecting the micro-TESE result were determined. A value of $p<0.05$ was set as statistically significant in all analyses.

Results

The total number of patients included in the study with NOA who underwent micro-TESE operation in our clinic was 994. While sperm were detected in 472 (47.5%) of these patients, no sperm could be found in 522 (52.5%) of them.

The number of patients who underwent karyotype analysis and underwent micro-TESE was 497. KS was determined in 104 (20.9%) of these patients, of which non-mosaic KS (47, XXY) was present in 101 (97.1%). In micro-TESE, while sperm was detected in 22 (21.2%) of the 104 patients determined by KS, sperm was found in 176 (44.7%) of 393 patients with normal chromosome analysis. The sperm detection rate in micro-TESE was found to be significantly lower in patients with KS compared to patients with normal karyotype analysis. The rates of sperm detection

and the distribution of age, testis volume, FSH and testosterone levels of the 497 patients subjected to karyotype analysis are shown in Table 1.

In the 450 patients examined for Y chromosome microdeletion and subjected to micro-TESE operation, Y chromosome microdeletion was present in only 14 (3.1%). In 12 (85%) patients there was *AZFc* deletion, and in 2 (15%) *AZFb* deletion. Sperm was found in micro-TESE in 4 (28.6%) of the 14 patients. Sperm was found only in *AZFc* deletion patients. The rates of sperm detection and the distribution of age, testis volume, FSH and testosterone levels of these two groups are shown in Table 2.

Age, testis volume, serum FSH and testosterone levels, and the presence of KS were determined to affect the sperm retrieval rate (SRR) in micro-TESE (p<0.05). The duration of infertility and the presence of Y chromosome microdeletion were not determined to affect the rate of SRR in micro-TESE (p>0.05). The factors affecting sperm detection in micro-TESE in patients NOA are shown in Table 3.

Discussion

Genetic analysis results of patients diagnosed with NOA who underwent micro-TESE were evaluated and some important findings emerged in this study. To the best of our knowledge, this study is a study that included the largest patient series examining both KS and Y chromosome microdeletion with SRR. The overall SRR of NOA patients was similar to the literature. However, SRR of patients with KS and Y chromosome microdeletion was lower. Age, testis volume, serum FSH and

Table 1. Descriptive information of patients examined karyotype analysis

	KS (-) (average ± SD)	KS (+) (average ± SD)	p-value
Age (year)	33.78±6.06	32.74±5.55	0.115*
Testis volume (mL)	12.24±6.59	5.19±3.37	<0.001*
FSH (mIU/mL)	15.55±11.57	30.99±13.57	<0.001*
Testosterone (ng/mL)	4.31±1.96	3.29±2.61	<0.001*
Sperm retrieval rate (n, %)	176/393 (45%)	22/104 (21.2%)	<0.001**

FSH: Follicle stimulant hormone, KS: Klinefelter syndrome, *Independent groups t-test, **chi-square test

Table 2. Descriptive information of patients examined Y-microdeletion test

	Y-microdeletion (-) (average ± SD)	Y-microdeletion (+) (average ± SD)	p-value
Age (year)	33.36±5.64	31.86±5.80	0.329*
Testis volume (mL)	11.56±6.77	15.93±5.56	0.012*
FSH (mIU/mL)	18.27±13.63	14.26±8.69	0.276*
Testosterone (ng/mL)	4.13±1.82	4.13±1.82	0.284*
Sperm retrieval rate (n, %)	170/436 (39%)	4/14 (28.6%)	0.580**

FSH: Follicle stimulant hormone, *Independent groups t-test, **chi-square test

Table 3. Factors affecting the presence of sperm in micro-TESE in nonobstructive azoospermic patients

		TESE results		p-value
		Sperm (-)	Sperm (+)	
Klinefelter syndrome	Yes (n=104)	78.8%	21.2%	<0.001*
	No (n=393)	55%	45%	
Y-microdeletion	Yes (n=14)	71.4%	28.6%	0.580*
	No (n=436)	61%	39%	
Age (year)		33.82±6.01	34.99±6.55	0.004**
Infertility duration (year)		6.92±5.12	6.80±4.97	0.712**
Testis volume (mL)		11.21±6.55	14.18±6.32	<0.001**
FSH (mIU/mL)		19.67±13.60	14.75±12.50	<0.001**
Testosterone (ng/mL)		4.13±2.08	4.53±1.98	<0.001**

FSH: Follicle stimulant hormone, *chi-square test **Independent groups t-test

testosterone levels, and the presence of KS were found to affect the SRR in micro-TESE.

The fertility management of patients with NOA relies on surgical sperm retrieval techniques. Micro-TESE is one of the most frequent used techniques in this regard. In a review including 116 studies and 4.895 patients, similar to this study, SRR of patients with NOA was found to be 46.6% (7). Unlike these studies, a recent study, which included 85 patients with clinical NOA from Australia, showed an overall SRR of 61.2%, which was found to be higher than that literature (8). The difference in surgical experience, histological pattern, etiology, geographical and genetic differences may cause these variable results.

KS is the most common genetic abnormality causing infertility and approximately 90% of patients with KS have NOA. The most frequently seen karyotype of KS is non-mosaic 47, XXY (80-85%). The mosaic form (46, XY/47, XXY) is seen less often (9). In this study, similar to other studies in the literature, the non-mosaic form was seen more common. However, the rate of KS non-mosaic form was higher than that in other studies, with 97.1%. In a multicenter study from Turkiye, non-mosaic karyotype of KS was found to be 84.4%, and mosaic karyotype was 15.6% (10). There are many studies in the literature that have investigated the sperm detection rates of in patients with KS. In a study by Sabbaghian et al. (11) comprising of 134 patients, SRR was found to be 28.4% in micro-TESE operations in patients with KS. In a recent study, which included 142 patients with KS, SRR was found to be 57.7% (10). Schiff et al. (12) found the highest rate of SRR in micro-TESE operations in patients with KS (70.4%). In this study, SRR was found to be 21.2% of the patients with KS. This rate is lower than in other studies in literature, which could be due to different demographic characteristics in different geographical regions, the inexperience of the surgeon, differences in rates of mosaic and non-mosaic forms of KS, or lack of technical facilities.

Y chromosome microdeletion is seen in 10-15% of azoospermic males and 7-10% of severe oligospermic males. The most frequent deletion type in the literature is the AZFc deletion. While sperm can be obtained from 50% of patients with *AZFc* deletions and partial *AZFb* deletion, there is almost no chance of detecting mature spermatozoa in complete *AZFb* and *AZFa* deletions. In a study that included 374 patients with primary infertility, the incidence of Y chromosome microdeletion was shown as 1.07% (13). The frequency of Y chromosome microdeletion was reported as 1.3% in a Turkish population by Balkan et al. (14), 3.3% by Sargın et al. (15), 3.93% by Akin et al. (16), and 22.64% by Müslümanoglu et al. (17). In this study, Y chromosome microdeletion frequency was found to be 3.1%.

In a study by Oates et al. (18) on 42 infertile males with *AZFc* microdeletions, the SRR was reported as 42%. Of the 42 males examined in that study, 38% were severely oligospermic and 62% were azoospermic. In a study by Simoni et al. (19), a rate of 60% sperm detection with micro-TESE was reported in azoospermic infertile males determined by *AZFc* microdeletions. Mulhall et al. (20) reported 50% sperm detection in cases with deletions affecting the *AZFc* region. In this study, the SRR was found to be 28.6% in patients with Y chromosome microdeletion, which is a lower than that reported in the literature. The reason for this low rate was 2 patients with *AZFb* deletion, for which there is almost no possibility of sperm detection, and sperm was not detected in those patients in micro-TESE. Of the 12 patients in this study with *AZFc* deletion, sperm was detected in 4 (33%) of them and this rate was also lower than findings in the literature. The small number of patients with Y chromosome microdeletion who underwent micro TESE could be the reason for the low rate of sperm detection.

There are very few studies in literature that include both KS and Y chromosome microdeletions. In a study by Altintas et al. (21) consist of 165 patients, sperm was found in micro-TESE in 37.5% of all patients, in 27.2% of 10 patients with *AZFc* deletion, and no sperm was found in 7 patients with KS. Balkan et al. (14) studied 80 infertile males in Turkiye, 71 of them were found to have a normal karyotype, KS was determined in 7 patients and autosomal chromosomal disorder in 2. Apart from these studies, although there are insignificant series of studies evaluating both genetic disorders together in the literature, there is no study evaluating sperm detection rates together in micro-TESE operations in NOA patients, as in this study.

Some variables affecting SRR in micro-TESE operations and some different results are shown. In this context, in the Australian study mentioned above, no correlation was found between SRR and age, serum LH, FSH and testosterone levels (8). However, in another study examining only patients with KS, SRR was associated with patient age and serum testosterone level, but not with serum FSH and LH (10). Also, there are conflicting results on the relationship between testicular volume and SRR (22,23). In this study; age, testis volume, serum FSH and testosterone levels, and the presence of KS were found to affect the SRR in micro-TESE and the duration of infertility and the presence of Y chromosome microdeletion were not determined to affect the SRR in micro-TESE.

Study Limitations

Although karyotype analysis and Y chromosome deletion analysis are recommended for azoospermic patients in the latest guidelines, the lack of genetic analysis of approximately half of the patients undergoing micro-TESE can be considered a limitation of this study. Reasons for this may include increased costs, the long waiting time for genetic test results and that surgery was not preferred or recommended. Another limitation

of this study is its retrospective design. Moreover, the outcome measure utilized, SRR, does not encompass the final goal, which is live birth. The pregnancy rates of the couples were not evaluated as well. Furthermore, some variables, which have relevant albeit controversial predictive values, such as inhibin B, Johnsen score, smoking, and lifestyle, were not recorded.

Conclusion

In this study, the SRR of patients who have chromosome Y microdeletion or KS, was found to be lower than other studies in the literature. This difference could be derived from the genetically tested population's structure, variance in the gene areas used for scanning and different demographic characteristics of different regions.

Ethics

Ethics Committee Approval: Approval for the study was granted by the Local Ethics Committee (project no: KA14/277, date: 24.09.2014 - Baskent University Ethics Committee for Non-Interventional Clinical Trials).

Informed Consent: Retrospective study.

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Authorship Contributions

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