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Correlation between high Choline metabolite signal in spectroscopy and sperm retrieval chance at micro-TESE

C. KARAKUS[1], R. OZYURT[2]

[1]Vocational School of Health Services, Beykent University, Istanbul, Turkey
[2]Istanbul IVF-Center, Istanbul, Turkey

Abstract. – OBJECTIVE: To determine the relationship between choline (Cho) signal intensity measured before micro-dissection testicular sperm extraction (micro-TESE) and sperm retrieval rates in mTESE in non-obstructive azoospermia (NOA) patients.

PATIENTS AND METHODS: A total of 20 patients who underwent testicular MR spectroscopy were included in the study. Participants consisted of 10 patients diagnosed with NOA and 10 fertile cases with normal sperm counts. Both groups of participants underwent bilateral testis MR spectroscopy. Ten patients in the NOA group underwent mTESE after spectroscopy. The signal intensities of Cho, creatine (Cr), lactate, and lipids were analyzed and compared with the results of fertile control. Cho signal intensity detected before mTESE in the NOA group and sperm retrieval rates were compared.

RESULTS: Sperm was found in 5 of 10 patients who underwent mTESE. No sperm was found in five NOA cases. The main metabolites detected in NOA cases with sperm in mTESE were Cho and Cr. Cho and Cr signals were found to be significantly lower in NOA cases where no sperm could be found in mTESE. Cho and Cr signal intensities of the fertile group were similar to NOA patients with sperm in mTESE but were significantly higher than those with NOA without sperm. While the cut-off value of Cho was 1.24 ppm (AUC 0.665, $p = 0.01$ [95% CI: 0.722–1.00]) the cut-off value of Cr was 1.18 ppm (AUC 0.887, $p = 0.02$ [95% CI]): 0.620–1.00]) for positive sperm retrieval.

CONCLUSIONS: Detection of high Cho metabolite in the spectra before TESE in NOA patients increases sperm retrieval rates in mTESE.

Key Words:
Non-obstructive azoospermia, mTESE, MR spectroscopy, Cho signal, Sperm retrieval rate.

Introduction

Azoospermia is characterized by the absence of sperm in the ejaculate. It is seen in approximately 8-10% of infertile men[1-3]. More than half of the men diagnosed with azoospermia constitute the non-obstructive azoospermia (NOA) subgroup. In men with NOA, there is no obstruction that would prevent the sperm transport from the testicular tubules to ejaculte[4]. The main reason for the absence of sperm in the ejaculate is due to sperm production defects in the testicles. Sperm is either not produced at all or maturation defect occurs at certain stages of development. In case of complete absence of sperm, couples can only get pregnant with sperm donation.

In NOA patients who do not pass sperm to the ejaculate, although sperm production continues, albeit limited, in their testicles couples have a chance to have a child by collecting sperm with some sperm retrieval procedures and using them in intra-cytoplasmic sperm injection[1,4]. Micro-dissection testicular sperm extraction (Micro-TESE) is the most reliable and successful way to obtain sperm in NOA cases. With this technique, sperm can be obtained in more than half of men with NOA[5]. However, sperm cannot be found in half of men with NOA undergoing mTESE and repeated surgery may be required. Unfortunately, repeated mTESE may cause infection, adhesion, defect in steroid synthesis, and decrease in testicular volume. In order to reduce the number of repeated mTESE, some predictive methods have been developed to estimate the chance of finding sperm before the procedure. Evaluation of serum FSH, inhibin B, testicular volume, histology, genetic analysis, age, and varicocele have been used to predict the chance of finding sperm in mTESE[6-8]. However, none of these methods could fully and definitively predict whether sperm could be found in mTESE.

In 2020, Celik et al[1] published a study reporting that testicular MR spectroscopy performed before mTESE in NOA patients is a highly ef-

Corresponding Author: Cemil Karakus, MD; e-mail: drckarakus@gmail.com

fective, noninvasive, reliable and inexpensive method to predict whether sperm can be found in mTESE. This study was followed by a study conducted by Ntorkou et al[9] that investigated the predictive value of spectroscopy before mTESE in men with NOA. However, testicular spectroscopy technique was not widely used in clinical practice, since no further studies were conducted after these two studies. MR spectroscopy was developed as a method that provides information about the nature of the brain lesion without biopsy or other lesions where making biopsy is difficult. The metabolite densities determined by spectroscopy in the living tissue provide information about the physiological or pathological functions of the cells[10]. The main metabolites detected in spectroscopy are choline (Cho), creatine (Cr), lactate and lipid. Cr is an essential energy reservoir of living cell. Lactate is the end product metabolite of anaerobic glycolysis. It indicates necrosis or ischemia within relevant tissue. Lipids have been recognized as potential indicator of necrosis and malignancy. Cho signal is main component of the cell membrane, which shows the degree of membrane turnover and mitosis[10]. The high Cho intensity found in the testis spectroscopy of fertile male suggested that Cho could be used as a predictive spermatogenesis marker[1,11]. This study was, therefore, designed to determine relationship between the signal intensity of Cho, Cr, lactate and lipid metabolites to be measured with spectroscopy before mTESE and sperm retrieval rates in patients with NOA.

Patients and Methods

This case-controlled study was conducted on infertile men who applied to Istanbul IVF-Center between 2019-2020 with the diagnosis of azoospermia. Azoospermia was defined as the absence of sperm cells in the seminal fluid after centrifuge. Men in the NOA group were confirmed to be azoospermia through at least two semen analyses after at least three days of abstinence. A total of 20 men who underwent testicular MR spectroscopy were included in the study. Participants consisted of 10 patients diagnosed with NOA and 10 fertile men with normal sperm counts. Men in the NOA group and the fertile group were matched in terms of age. Men with NOA undergoing mTESE for the first time were included in the study. Serum levels of FSH, LH, PRL, and testosterone were recorded in both groups. The NOA men with unilateral or undescended testes, benign or malignant testicular lesions or torsion, and abnormal karyotypes were excluded. Due to possible change in metabolite signals patients with a history of mTESE were not included the study.

Both groups of participants underwent bilateral testis MR spectroscopy by using a 3-T MRI. Each testis was visualized using magnetic resonance imaging before the voxels were located. T1-weighted (time repetition/time echo, 500/20) and T2-WI images were obtained in the axial and coronal planes. Both single and multi-voxel spectroscopy sequences with short (35 ms) and long (140 ms) TEs were used. The volume of interest was centered of the testicular parenchyma. The metabolites detected in the spectrum were denominated parts per million (ppm). Metabolite signals were quantitatively analyzed from spectroscopic images and recorded within a range of 0–4.1 ppm. These peaks included Cho located at 3.2 ppm, Cr located at 3–3.1 ppm, lactate located at 1.4 ppm, and compound peak containing lipids and lactate located at 0.8–1.4 ppm. The signal intensities of Cho, Cr, lactate, and lipids were analyzed and compared with the results of fertile control. The signal intensities of Cho and other metabolites detected before mTESE in the NOA group and sperm retrieval rates were compared. Ten patients in the NOA group underwent mTESE after spectroscopy. Regardless of the metabolite signals obtained before surgery mTESE was performed in all men with NOA. mTESE was performed with 8x magnification and when necessary, increased up to 20x magnification and seminiferous tubules were made visible. The tubules were cut into small pieces and sent to the embryology laboratory, and the samples were searched for sperm[11]. Detailed information about the micro-TESE can be found elsewhere[12]. The Cho, Cr, lipid, and lactate values obtained from spectroscopy of the men with NOA were analyzed quantitative manner and then correlated with the outcome of subsequent mTESE. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and informed consent was obtained from participants.

Statistical Analysis

Analyses of all data were performed on SPSS 21 (SPSS Inc., IBM, Armonk, NY, USA). Q-Q and histogram plots were used to determine whether variables were normally distributed.

Table I. Demographic and laboratory characteristics of NOA and fertile groups.

	Fertile group (n = 10)	NOA group (n = 10)	*p*-values
Age (year)	33.4 ± 1.22	31.9 ± 3.08	0.06
BMI (kg/m^2)	26.8 ± 1.25	25.9 ± 2.09	0.08
Number of IVF attempt	0	0	NA
FSH (IU/L)	8.9 ± 3.22	10.6 ± 3.03	0.07
LH (IU/L)	6.4 ± 0.44	7.13 ± 2.20	0.06
Prolaktin (ng/mL)	11.2 ± 1.30	12.4 ± 5.21	0.057
Testosterone (ng/dL)	390.2 ± 12.1	366.4 ± 11.2	0.07

Quantitative data were expressed as mean ± standard deviation and median and range (minimum-maximum). Normally distributed variables were analyzed with the independent samples *t*-test. Non-normally distributed variables were analyzed with the ANOVA test. The correlations between demographic, hormonal parameters and metabolite values were evaluated using Pearson correlation coefficients. The receiver operating characteristic (ROC) curve analysis was used to determine the cut-off values for the Cho, Cr, lactate, and lipid metabolites for the comparison of the success rates of sperm retrieval at mTESE. A value of $p < 0.05$ was accepted as statistically significant.

Results

Clinical and laboratory findings of NOA and fertile group were shown in Table I. Spectroscopy technique was successfully applied to all patients in both NOA and control groups. Each patient had two testicles, and metabolite levels were recorded by spectroscopy in each testicle. We were able to image 4 of the 5 metabolites we previously aimed to find in spectroscopy. These metabolites were visualized as Cho, Cr, lactate and lipid. Since we could not detect the myoinositol signal in amounts that could be statistically evaluated, we excluded it from the evaluation. Sperm was found in 5 out of 10 patients who underwent mTESE. No sperm was found in the remaining five men with NOA.

Both in the fertile group and men with NOA with sperm in their mTESE, the two most prominent metabolite peaks were Cho and Cr (Table II). The main metabolites detected in men with NOA with sperm in mTESE were Cho and Cr. Cho and Cr metabolite levels were found to be significantly lower in the other five NOA patients whose sperm were not found in mTESE. Cho and Cr signal intensities of the fertile group were similar to NOA patients with sperm in mTESE, but they were significantly higher than those with NOA without sperm. Cho and Cr values of patients with NOA who did not have sperm in mTESE were significantly lower than those with sperm in mTESE. The lactate and lipid signal intensities of the fertile group were significantly lower than the men with NOA. Lactate and lipid signal intensities were similar between NOA patients with sperm and NOA patients without sperm.

A positive and significant correlation was found between Cho, Cr and serum androgen levels (r=0.678, p<.002, r=0.456, p<0.001 respectively). Similarly, a negative and significant correlation was found between age and Cho metabolite (r=-

Table II. Comparison of Cho, Cr, lactate, and lipid metabolites levels in the testes of fertile and NOA groups (Results are presented as median).

	Cho	Cr	Lactate	Lipid
1. Fertile group (n = 10)	2.653	2.123	0.511	0.632
2. NOA patient with sperm on mTESE (n = 5)	2.201	1.91 ± 0.20	1.502	1.122
3. NOA patient without sperm on mTESE (n = 5)	1.344	0.870	1.334	1.303
		p-value		
1 *vs.* 2	0.40	0.23	0.02	0.02
1 *vs.* 3	0.03	0.02	0.02	0.01
2 *vs.* 3	0.01	0.01	0.56	0.07

0.655, $p<0.03$). No correlation was found between metabolites and other laboratory and demographic parameters. While the cut-off value of Cho was 1.24 ppm (AUC 0.665, $p = 0.01$ [95% CI: 0.722–1.00]) the cut-off value of Cr was 1.18 ppm (AUC 0.887, $p = 0.02$ [95% CI]): 0.620–1.00]) for positive sperm retrieval. Cho and Cr cut-off values of NOA patients whose sperm were obtained in mTESE were found to be >1.24 and >1.18, respectively. The Cho and Cr signals were under the cut-off values in five NOA patients with negative sperm retrieval in mTESE. The sperm retrieval rate in the men with NOA was 50%. Decreased Cho and Cr metabolite signals were detected in 100% of the NOA men with negative sperm retrieval in micro-TESE (Figure 1). Increased Cho and Cr metabolite peaks were detected in 100% of the NOA men with positive sperm retrieval in micro-TESE. Although testicular lengths were higher in the fertile group than in the NOA group, the difference was not significant. No significant correlation was detected between metabolites, serum androgen, other hormonal parameters and testicular dimensions.

Discussion

mTESE is the gold standard approach in obtaining sperm from the testicles, and with the use of collected sperms in ICSI, infertile couple with azoospermia can have a baby from their own genetic material. However, since every mTESE procedure performed in men with azoospermia does not result in sperm retrieval, patients cannot fully comply with their treatment due to both the psychological pressure caused by the absence of sperm and the stress of the surgical procedure. Since one out of every two NOA patients undergoing mTESE is exposed to this stress, the search for a test or method that predicts whether sperm can be found in mTESE continues in these patients. Although many predictive tests are used, mTESE results cannot be predicted exactly. In a recent study conducted on NOA patients, it was reported that performing testicular spectroscopy before mTESE highly predicted whether or not sperm could be obtained. In that study by Celik et al[1], a total of 18 NOA patients underwent spectroscopy before mTESE and metabolite peaks were analyzed. According to the results of that

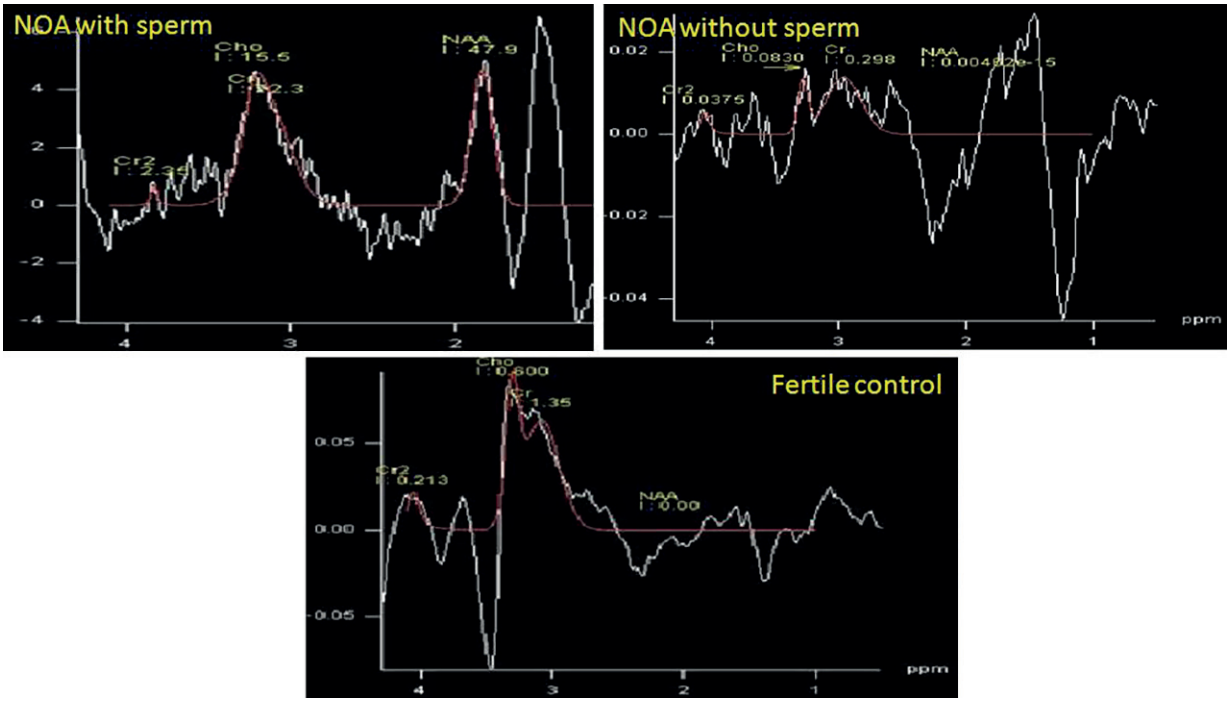

Figure 1. Despite high Cho and Cr peaks in the NOA group with sperm in mTESE, weak Cho and Cr peaks were detected in NOA patients without sperm. High Cho and Cr peaks are noteworthy in the fertile group.

study, it has been reported that the detection of a high Cho peak in the spectroscopy predicts the presence of sperm with 87.5% sensitivity, 100% specificity and 100% positive predictive value. In the same study, it was noted that high Cr peak strongly predicted sperm retrieval rates in mTESE[1]. Similarly, Ntorkou et al[9] reported decreased Cho, myo-inositol, total lipids and macromolecules levels in men with NOA. In addition, they showed that glutamate levels were markedly increased in NOA patients who could not find sperm in mTESE.

In the present study, we evaluated four main testicular metabolites in the spectroscopy performed before mTESE in ten men with NOA. We found sperm in five of the ten patients in mTESE, and we could not find any sperm in the remaining five. Our sperm retrieval rates in mTESE were consistent with the literature[1-3]. Cho and Cr were the most prominent metabolite peaks obtained from the spectroscopic examination of five men with NOA with sperm obtained at mTESE. The cut-off value of the Cho peak was found to be >1.2 in five patients, and sperm were obtained in all of them. Similarly, the cut-off value of the Cr peak was found to be >1.1 in five patients and sperm were obtained from all of the patients. In five NOA patients without sperm in mTESE, the decrease in Cho and Cr peak intensities was accompanied by a decrease in cut-off values. As a result, we noted the significant increase in Cho and Cr peaks and high cut-off values for Cho and Cr, as the finding that most strengthens the possibility of sperm retrieval in mTESE. Similarly, in cases of decreased Cho and Cr metabolite, the chance of obtaining sperm in mTESE was significantly reduced. If the cut–off values of Cho and Cr were smaller than 1.2 and 1.1, respectively, the chances of sperm retrieval in micro-TESE are very low. Indeed, no evidence of active spermatogenesis was found in the mTESE of patients with low Cho or Cr peak.

What is the scientific basis for the significant correlation between Cho and Cr metabolites and active spermatogenesis? Detection of these two metabolites in spectroscopy shows the viability of the cell and the continuation of its energy production capacity. It is not possible to detect the Cho signal in dead cells or completely necrotic tissues[11]. Cho is the main indicator of cell membrane integrity and regeneration. It is detected more prominently in healthy tissues and regions with active mitosis[10,11]. In addition to physiologically healthy cells, there may be an increase in Cho peak in premalignant and malignant cell with increased mitotic activity. Since spermatogenesis is characterized by increased mitotic activity and new cell membrane formation, an increase in the Cho metabolite suggests that spermatogenesis is healthy. The fact that the Cho peak is more pronounced in the fertile group than in men with NOA supports this view. Similarly, the detection of a lower Cho peak in NOA patients with no sperm found in mTESE can be considered as evidence of inactivated spermatogenesis[1,10,11]. The Cr metabolite is an indicator of the energy capacity of the cell and is significantly higher in the fertile group with healthy spermatogenesis[1,11]. Similarly, the Cr peak increased in patients whose sperm were obtained in mTESE. This finding confirms our assumption that sufficient energy is produced to support active spermatogenesis in NOA patients whose sperm are retrieved on mTESE[1]. On the other hand, the low Cr peak in the NOA group in which no sperm was obtained in mTESE suggesting inactive spermatogenesis. Although Cho signal intensity decreases with age, it decreases earlier in NOA patients[12]. In our study, a negative correlation was found between advancing age and Cho peak in the NOA group. Widespread collagen deposition and Leydig cell scarcity in the seminiferous tubules may lead to a decrease in both Cho and Cr signals.

Despite the positive and significant correlation between Cho and Cr signal intensity and sperm retrieval rates, no significant correlation was found between lactate and lipid signals and sperm retrieval rates. The positive correlation between androgen levels and Cho and Cr metabolites is a finding that supports the role of androgens in spermatogenesis. Although both lactate and lipid levels were higher in the NOA group compared to the fertile group, the levels of these two metabolites were found to be similar in the NOA patients with and without sperm. In the light of our results, we thought that the increased lactate in NOA patients does not mean that aerobic glycolysis is completely disrupted. Similarly, the presence of sperm in mTESE despite increased lipid metabolite is evidence that membrane integrity is preserved in testicular cells.

Despite the small number of participants and the lack of histological staging, this study is the third study investigating the predictive value of spectroscopy before mTESE in NOA patients. Our number of participants is higher than Celik

et al[1] and less than Ntorkou et al[9]. Although the investigated metabolites were similar in three studies, Ntorkou et al[9] evaluated total lipids and macromolecules levels differently from us. In addition, authors divided NOA patients into histological subtypes and compared metabolite levels and subtypes. The common point of the two previous studies and our study is that there is a defect in the production of some testicular metabolites in men with NOA. This defect becomes more evident in patients with NOA without sperm in mTESE.

Conclusions

If the predictive value of testis spectroscopy is clearly proven with more comprehensive studies, infertility physicians and urologists will have a cheap, easily applicable and noninvasive screening test. Thanks to this screening test, unnecessary and repetitive mTESE will be avoided. Men with NOA with weak Cho and Cr signals before mTESE have a low chance of finding sperm in the surgical procedure. Thus, men with NOA can turn to different options, especially donation. Finally, *in vivo* testis MR spectroscopy may provide complementary information on spermatogenesis, which may assist enable better understanding of biochemical pathways within the testis of men with NOA.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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