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The timing of micro-TESE: what is the ideal age for male and female partner to bring a child to home?

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SUMMARY

OBJECTIVE: The aim of this study was to analyze the results of microsurgical testicular sperm extraction (micro-TESE) and investigate the potential factors that may affect the successful sperm retrieval and timing of micro-TESE.

METHODS: A total of 56 patients with nonobstructive azoospermia (NOA) who underwent micro-TESE procedure between January 2017 and December 2019 were retrospectively analyzed. The patient age, marriage duration, infertility duration, smoking, chronic illness, varicocele status, previous scrotal surgeries, and the presence of genetic disease were noted by an urologist for all patients.

RESULTS: The mean age of patients was 33.28±4.4 (22–44) years. Our total sperm-retrieval rate was 55.4% (n:31). Sixteen (28.6%) pregnancies were achieved and 15 (26.8%) healthy live births could be managed. Only the marriage duration (p=0.016) and infertility duration (p=0.015) were detected to be the significant factors to manage successful sperm retrieval. Men with NOA younger than 35.2 years and having a female partner younger than 36.9 years seemed to have the best chance to have a living healthy baby.

CONCLUSIONS: The fertility decreased by both male and female age and for men with NOA. The early visit to doctor seemed to have positive effect.

KEYWORDS: Infertility. Live birth. Testicular sperm retrieval. Nonobstructive azoospermia. Pregnancy.

INTRODUCTION

Infertility is the inability of a sexually active couple to get pregnancy despite unprotected regular sexual intercourse for at least 1 year[1]. Approximately 15% of the couples cannot get pregnancy in the first year and as a result they seek help from reproductive health care centers for infertility treatment[2,3]. Male factor is responsible for approximately half of the infertility cases[4]. Azoospermia is the absence of spermatozoa in the ejaculate. It is found in approximately 10% of the males undergoing treatment for infertility[3,5]. The majority of the cases are nonobstructive azoospermia (NOA), a condition that is essentially a spermatogenic impairment[6]. Impaired spermatogenesis may be caused by idiopathic factors, cryptorchidism, orchid, radiation, gonadotoxic agents, systemic diseases, testicular trauma, and testicular torsion[7]).

Micro-TESE (microsurgical testicular sperm extraction) has been first described by Schlegel et al.[8] and used as the most important method in retrieving sperm in the patients with NOA. Despite many studies on this field, unfortunately, sperm-retrieval rate (SRR) by micro-TESE reached approximately 50–60%[5,9,10]. Even though sperm is retrieved with this method, clinical data related to pregnancy and live birth rates are limited since a follow-up process is necessary after sperm retrieval[5,10].

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There is no clinical parameter that definitely indicates whether spermatozoa are present in the testicles prior to micro-TESE in patients with NOA; studies on this subject are currently ongoing. The evaluated parameters included the result of the testicular biopsy, testicular size, serum hormone level, hormonal therapy (HT), genetic analysis result, and TESE method[3,5,7,11]. In the light of these data, the chance of live birth for the couples increases with an increase in successful sperm retrieval by micro-TESE.

Advanced ages of both male and female spouses affect reproductive functions negatively. Sperm count and quality are reduced in males with advanced age[12]. In the literature, there is no study on the relationship between the timing of micro-TESE procedure and the ideal age required for couples to bring a child to home. The present study aims to analyze the results of micro-TESE procedure as a minimally invasive, safe, and effective sperm-retrieval method and investigate the potential factors that may affect the successful sperm retrieval and timing of micro-TESE procedure.

METHODS

Participants and study design

A total of 56 patients with NOA who underwent micro-TESE procedure between January 2017 and December 2019 in our clinic were retrospectively analyzed. The diagnosis of azoospermia was confirmed by performing sperm analysis in accordance with the World Health Organization guideline from the ejaculates obtained by masturbations in two different times from all the patients[12]. The patient selection criteria for micro-TESE were:

a) infertility because of NOA;
b) female partner with healthy reproductive systems;
c) age at least 18 years;
d) the absence of a mental illness or a psychiatric disorder; and
e) the ability to read and write in Turkish language.

After obtaining medical history, the patients underwent a detailed physical assessment of urogenital system including secondary sexual characteristics, testicular consistency and size, the presence of vas deferens, and varicocele examination. The sociodemographic data such as patient and spouse age, duration of marriage and childbirth expectation, presence of child, cigarette smoking status, educational status, history of chronic disease, and surgery were enquired. The presence and type of previous treatment for azoospermia were questioned.

Hormonal assessment included serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone (TT). The patients were divided into etiological groups as hypergonadotropic hypogonadism (hyper–hypo: high FSH and LH, low TT), hypogonadotropic hypogonadism (hypo-hypo: low FSH, LH, and TT), and idiopathic (normal FSH, LH, and TT). All the hormone tests mentioned above were evaluated 1 month before micro-TESE.

Testicular size (mL), the presence of reflux flow in the venous system, and maximum venous diameter (mm) were assessed using scrotal color Doppler ultrasonography. The presence of varicocele was investigated radiologically.

The genetic analysis results of the patients including karyotype analysis and Y-chromosome microdeletion (AZFa, AZFb, and AZFc) were evaluated.

Data including the side of micro-TESE procedure, SRR, and the side of the testicle from which sperm was retrieved were recorded. At the end of the procedure, histopathological examination of the testicular tissue (tubular atrophy, Sertoli cell only, maturation arrest, hypospermatogenesis, normospermatogenesis, and granulomatous inflammation) was performed.

Micro-TESE procedure

A complete micro-TESE procedure was performed by two urologists experienced in microsurgery in the supine position under spinal anesthesia. Scrotal raphe was entered with midline longitudinal incision, and testicle was unilaterally exposed. Then, an incision was made in the tunica albuginea and testicular parenchyma was exposed in its equatorial plane. The dilated seminiferous tubules were detected under microscope (at 20x magnification) and adequate samples were obtained from the tissue that may contain spermatozoa. When no spermatozoa could be detected in one testicle, the other testicle was incised using same approach and spermatozoa were searched. These tubules were examined by the embryologist, and live spermatozoa were prepared to be used in intracytoplasmic sperm injection (ICSI) procedure or frozen if detected. In the same session, a biopsy was obtained from the testicular parenchyma and put into Bouin's solution and sent for histopathological examination. After obtaining the samples, the tunica albuginea was closed using 4/0 absorbable suture. Antibiotherapy, analgesic agent, and bed rest were recommended postoperatively for 5 days after the procedure.

Ovarian stimulation, fertilization, and embryo transfer

All recruited women had regular menstruation periods ranging from 24 to 35 days. Women who were treated with gonadotropin-releasing hormone (GnRH) antagonist-based protocols without pretreatment by hormonal suppression were also included. Controlled ovarian hyperstimulation was

performed with the administration of gonadotropin using recombinant FSH: either Gonal-F (Serono Laboratories, Germany) or human menopausal gonadotropin Menogon (Ferring Pharmaceuticals, Switzerland), individually or combined, starting on day 2 of the menstrual cycle. The GnRH antagonist Cetrotide (Serono) was given on day 6 of the stimulation at 0.25 mg/day subcutaneously and continued until human chorionic gonadotropin (hCG) was administered. Initial dosage of the gonadotropins was determined on the basis of patient's age, antral follicle count, day 3 FSH level, and body mass index. The stimulation was generally achieved with daily dosing of a total of 150–300 IU/day, with the gonadotropin dose being adjusted according to the ovarian response assessed by E2 levels and a transvaginal sonography. Oocyte maturation was triggered by 10,000 IU of hCG (Choriomon 5000 IU; IBSA) or recombinant hCG (Ovitrelle 250 mg; Serono) or 0.2 mg triptorelin, GnRH agonist (Gonapetyl 0.1 mg; Ferring), which was administered after at least two follicles had a mean diameter of 17 mm. Transvaginal oocyte retrieval was performed 36 h after hCG administration in the standard manner, with the patient under general anesthesia. All oocytes were inseminated with an ICSI. Luteal progesterone (P) supplementation was started 1 day after retrieval, using either 8% P vaginal gel (Crinone; Serono) daily or vaginal P suppositories (200 mg) three times a day or an additional bolus of 1.500 IU hCG on the day of oocyte retrieval in GnRH agonist trigger cycles. Embryo transfer was performed by a soft catheter (Gynetics, Belgium) with the guidance of transabdominal ultrasonography on day 3 or 5.

Serum β-hCG levels were determined 2 weeks after embryo transfer. A biochemical pregnancy was defined as a positive serum β-hCG test with no sonographically detectable gestational sac, whereas miscarriage was defined as pregnancy loss following ultrasound confirmation of an intrauterine gestation sac. The live birth was defined as delivery of a viable infant at ≥28 weeks of gestation after embryo transfer.

Ethical approval

This study was approved by the Ethics Committee of Gülhane Training and Research Hospital, Ankara, Turkey (approval number: 2020-44).

Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences 20.0 software for MAC (Chicago, USA) by an expert biomedical statistician. Kolmogorov-Smirnov test was used to assess normalization of the variables. Descriptive statistics were noted as mean±standard deviation (minimum-maximum) if the variables are in normal distribution, and median (minimum-maximum) if the variables are in non-normal distribution, or as numbers or percentiles. Mann-Whitney *U* test was used for comparing groups. A $p<0.05$ was accepted as statistically significant.

RESULTS

There were 56 male patients in our study. The mean age of patients and their spouses at the time of micro-TESE was 33.28±4.40 (22–44) and 30.42±4.62 (20–41), respectively. Only 3 (5.4%) patients had a child before. Sociodemographic characteristics and descriptive data of the patients are presented in Table 1.

Sixteen (28.6%) patients had a treatment for infertility before: 11 had TESE without any additional treatment, 2 had HT, and 3 had HT+TESE. Of the 11 (19.6%) patients who had TESE without any additional treatment before, 6 (54.7%) had successful sperm retrieval at the first micro-TESE and 2 (33.3 of 54.7 of 19.6%) had a child. Two (100 of 3.6%) patients who had HT before had successful sperm retrieval at masturbation and one (50 of 100 of 3.6%) had

Table 1. Sociodemographic characteristics, sonographic evaluation results, and micro-TESE results of patients with nonobstructive azoospermia.

Factors	Mean±SD	Min–Max
Marriage duration (years)	5.14±2.53	2–11
Infertility duration (years)	3.42±2.15	1–10
Factors	**n**	**%**
Smoking	21	37.5
Chronic illness	8	14.3
Education degree		
Elementary and secondary school	6	10.7
High school	31	55.4
University	19	33.9
Previous inguinoscrotal surgery	10	17.9
Orchiopexy	4	7.14
Orchiectomy	4	7.14
Varicocelectomy	1	1.78
Herniorrhaphy	1	1.78
Etiological classification		
Hyper-hypo	27	48.2
Idiopathic	29	51.8

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Table 1. Continuation.

Factors	n	%
Genetic diseases		
Klinefelter syndrome	3	5.4
Microdeletion of Y chromosome	2	3.6
Physical examination		
Right small testes	17	30
Left small testes	21	37.5
Palpable vas deferens	56	100
Varicocele	18	32.1
Right	1	1.78
Left	17	30.3
Factors	**Mean±SD**	**Min–Max**
Maximal vein diameter (mm)		
Right	0.11±0.5	0.2–2.3
Left	0.9±0.3	0.4–5.5
Testicular volume (mL)		
Right	9.56±4.39	0–14.2
Left	9.57±4.42	0–14.8
Factors	**n**	**%**
Side of micro-TESE		
Right	19	34
Left	10	18
Bilateral	27	48
Sperm retrieval after micro-TESE		
Sperm positive (presence)	31	55.4
Sperm negative (absence)	25	44.6
Sperm positive testicles		
Right	18	32.1
Left	10	17.9
Bilateral	3	5.40
Pathologic examination		
Tubular atrophy	3	5.35
Sertoli cell only	15	26.78
Maturation arrest	8	14.28
Hypospermatogenesis	7	12.50
Normospermatogenesis	10	17.85
Granulomatous inflammation	1	1.78
Missing	12	21.42

a child. Three (100 of 5.4%) patients who had HT+TESE before had successful sperm retrieval at the first TESE, but none of them (0 of 100 of 5.4%) had a child.

The physical examination and sonographic evaluation results of the patients are presented in Table 2.

Our total SRR was 55.4% in 56 patients. Micro-TESE results of the patients are summarized in Table 1. Sixteen (28.6%) biochemical pregnancies were achieved, and 15 (93 of 28.6%) (26.8% of total) healthy live births could be managed. Miscarriage was noted in one case.

Only the marriage duration and infertility duration were detected to be the significant factors to manage successful sperm retrieval. The factors such as patient age, smoking status, having a chronic illness or not, education degree, previous scrotal surgeries, varicocele status, karyotype analyze, and Y-chromosome microdeletion were not found to affect SRR (Table 2). The marriage duration was 4.41±3.91 and 6.04±2.93 years in successful sperm-retrieval group and failure group, respectively (p=0.016). The infertility duration was 2.8±1.7 and 4.2±2.43 years in successful sperm-retrieval group and failure group, respectively (p=0.015).

For having a healthy live birth, concomitant use of patient and female partner age were found to be important factors. The receiver operating characteristic (ROC) curve of both these factors gives 0.611 as an area under curve value, implying the moderate strength for use. The SRR decreased significantly when age exceeded 35.2 years for men, and healthy live birth rate decreased significantly when the female partner age exceeded 36.9 years (Figure 1).

Table 2. Factors affecting the sperm retrieval in micro-TESE operations.

Factors	p-value*	Odds ratio
Patient age	0.197	0.920
Marriage duration	0.016	0.894
Infertility duration	0.015	0.831
Smoking	0.790	0.835
Chronic illness	0.459	2.270
Education degree	0.072	2.726
Previous inguinoscrotal surgery	0.205	3.057
Varicocele	0.841	0.872
Karyotype analyze	0.828	1.572
Y-chromosome microdeletion	0.999	1.000

*Logistic regression.

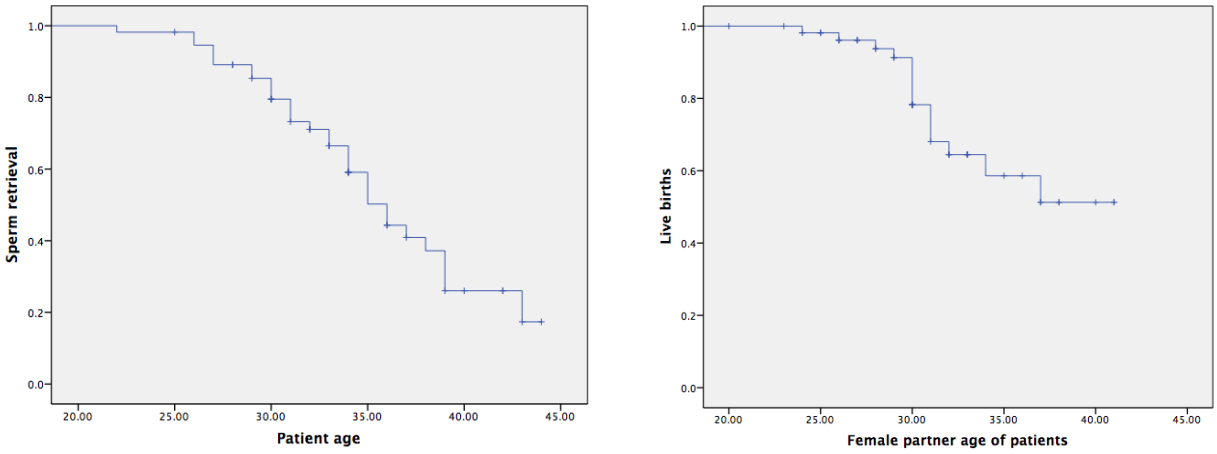

Figure 1. Sperm-retrieval rate according to patients' age and live birth rate according to the female partner age of patients who had micro-TESE.

DISCUSSION

NOA was historically accepted as an incurable condition which requires donor sperm for fertilization. Substantial improvements regarding fertilization have been achieved in patients with NOA after the introduction of micro-TESE procedure[13]. In the present time, the most beneficial and common treatment method for the males with NOA is combined micro-TESE and ICSI.

Mean age of the patients in our study (33.28±4.4) was similar to the previous studies in the literature[3,5,11,14,15]. In the literature, we found that even a 65-year-old patient had micro-TESE[3]. The oldest patient in our study was 44 years old. The relationship between patient age and sperm retrieval by micro-TESE has been investigated in many studies. Although assisted reproductive techniques have been accepted to be more effective in the young patients than in elderly ones, data on this subject vary[16]. In our study, we have found no statistically significant difference between SRR regarding patient age (p=0.197). Eken and Gulec[5] conducted a study on sperm retrieval by micro-TESE procedure in patients with NOA and found no significant relationship between patient age and SRR (p=0.14). Spahovic et al.[15] applied correlation test between the patients with positive and negative micro-TESE results and determined no statistically significant correlation in terms of age (p>0.05). Enatsu et al.[11] in a case series of Japanese males found interestingly a statistically significant correlation between advanced age and successful SRR (p=0.01). In contrast, Ramassamy et al.[17] stated that patient age does not affect sperm retrieval negatively in the males who underwent micro-TESE and that elderly males predispose to acquired NOA.

Smoking is a well-documented risk factor that has many negative effects, particularly on sperm count and sperm morphology[16,18,19]. We have detected in our patients that smoking is not predictive factor on successful sperm retrieval by micro-TESE (p=0.79). The patients were divided into two groups as smokers and nonsmokers in a study in which the results of TESE/ICSI procedures were performed on the azoospermic patients[19]. A higher sperm motility was detected in the nonsmokers compared with smokers after TESE and before sperm freezing process (45.5 and 14.8%, respectively; p<001). No significant correlation was determined in another study that evaluated the relationship between successful sperm retrieval by micro-TESE and cigarette smoking (p=0.535)[3]. Pavan-Jukic et al.[20] have investigated the independent variables that may predict sperm retrieval prior to TESE. Cigarette smoking was found only to be a prominent statistically negative risk factor for sperm retrieval (p=0.045).

An analysis of patients with NOA may reveal surgery history such as orchiopexy, orchiectomy, varicocelectomy, and herniorrhaphy. It was determined that 10 (17.9%) of 56 patients had previous inguinoscrotal surgery. The most common previous surgeries were orchiopexy (n:4) and orchiectomy (n:4). In our study, these previous surgery histories were evaluated together and found to have no impact on sperm retrieval prior to micro-TESE (p=0.205). In contrast, another study has analyzed the inguinoscrotal surgeries separately and found only varicocelectomy to predict spermatozoa retrieval (p=0.009)[3]. Only 1 (10%) of 10 patients had previous varicocelectomy in the history of inguinoscrotal surgery (1.78% of 56 patients). A history of previous varicocelectomy may

be determined in a part of the patients with NOA since they have a long-term expectation of fertility. Although the rate of varicocelectomy history (n:1; 1.78%) was low in our study, data reported in other studies were limited (19.5, 1.51, 10, and 17.7%, respectively)[3,11,15,20].

The presence of varicocele has a progressive harmful effect on the testicles in the normal population. Even though treatment of varicocele in patients with NOA is controversial, the number of the motile spermatozoa increase after varicocelectomy; therefore, some studies recommended the treatment of varicocele before undergoing TESE/ICSI procedure[6,21]. In contrast, the adverse studies suggest that many patients with NOA need ICSI procedure even in repaired varicocele[6]. Hence, the couples should be informed comprehensively about the necessity of varicocelectomy in patients with NOA. We have detected varicoceles in 18 (32.1%) of 56 patients and most of them were left-sided varicoceles (n:17; 30.3%). Varicocele was found in varying frequencies (18.8, 9.42, and 29%, respectively) in this patient group[3,11,20]. We have demonstrated that the presence of varicocele has no impact on successful sperm retrieval before micro-TESE (p=0.841). Other studies showed that the presence of varicocele did not make a statistically significant difference between the TESE-positive and TESE-negative groups (p=0.828 and p=0.263, respectively)[3,20].

Klinefelter syndrome (47,XXY) is the most commonly found chromosome anomaly associated with gender chromosomes in men and is responsible for 10% of the azoospermic patients[2,6]. The patients with Klinefelter syndrome are usually azoospermic; however, spermatozoa have been encountered in 69% of patients by micro-TESE in the literature[6]. We have detected 3 (5.4%) patients with Klinefelter syndrome in our study. Of these three patients, spermatozoa were obtained by micro-TESE in two patients, and biochemical pregnancy in one patient with ICSI. However, this pregnancy did not result in live birth. AZF subregions (AZFa, AZFb, AZFc) related to sperm production are located in the q11 region on the long arm of Y chromosome. The microdeletions in this region may cause azoospermia[2,6]. AZFc is the most commonly encountered microdeletion (75%) and is associated with higher chance of sperm retrieval. AZFc and AZFa+AZFc were determined in one patient each. However, biochemical pregnancy could be obtained in these two patients. SRR was not found to be significantly correlated with karyotype analysis and Y-chromosome microdeletion (p=0.828 and p=0.999, respectively).

As stated above, we have analyzed the various factors that may affect sperm retrieval before micro-TESE procedure. The marriage duration and infertility duration were found to be statistically significantly effective on successful sperm retrieval (p=0.016 and p=0.015, respectively). Regarding marriage duration, the results of micro-TESE procedure were 4.41±3.91 and 6.04±2.93 years in the positive and negative groups, respectively. Boeri et al.[22] have conducted an observational study with a large population to analyze the effect of infertility duration on the semen parameters and concluded that sperm concentration was negatively correlated with prolonged infertility duration. Additionally, higher rates of azoospermia were associated with longer infertility duration (p=0.03). In our study, infertility durations were 2.8±1.7 and 4.2±2.43 years in the positive and negative groups, respectively. From this point of view, prolonged duration of marriage and infertility can be considered as potential risk factors for fertilization.

In our study, SRR by micro-TESE was 55.4% (n:31). Taking into consideration that varicocele was found more commonly in the left side, we started micro-TESE procedure usually at the right side (except atrophic testicle). SRR in the right testicle (n:18; 32.1%) was found to be higher than the left testicle (n:10; 17.9%). Sperm could be retrieved from both testicles in 3 (5.4%) patients. Biochemical pregnancy (positive β-hCG) was monitored in 16 (28.6%) of 31 patients. Live birth was achieved in 15 (26.8) of these 16 patients. SRR by micro-TESE in patients with NOA is approximately 50–60%[5,9,10,20]. Our testicular SRR was similar with the literature. Only a small number of studies have provided data on pregnancy and live birth following TESE procedure[5,10,14]. Eken and Gulec[5] have retrieved sperm in 95 (65.5%) of the 145 patients with NOA by micro-TESE. In their study, the numbers of clinical pregnancies and live births were 41 (28.2%) and 26 (17.9%), respectively. In a current meta-analysis, cumulative pregnancy and live birth rates per ICSI cycle were 29% and 24%, respectively[10]. SRR, pregnancy, and live birth rates in another study investigating the success of TESE/ICSI were 40.5, 18, and 13%, respectively[14]. In the light of the given data, our pregnancy rate was close to the literature data whereas our live birth rate was somewhat higher. Live birth did not occur in only 1 of the 16 patients in whom biochemical pregnancy was achieved. In other words, according to our data, live birth may occur with a great probability if biochemical pregnancy could be achieved by TESE/ICSI procedure in patients with NOA.

It is known that ages and reproductive reserves of the couples are substantial for a healthy live birth. The analysis of ROC curve with respect to the patient age and SRR in the males with NOA indicated that SRR significantly decreased if the patient age exceeds 35.2 years. Healthy live birth rate significantly decreased when age of the female partner exceeded 36.9 years. We have analyzed the relationship between the timing of

micro-TESE procedure and ages of the spouses, thereby providing additional information to the literature.

Two major limitations exist in our study. First is the retrospective design of the study and second is the lack of participants. Despite these limitations, our study has provided useful data on biochemical pregnancy rates, live birth rates, the effect of the patient age on SRR, and the effect of the female partner's age on the live birth rates. As a consequence, prospective randomized studies are needed in future to discover the effective parameters on sperm retrieval in patients with NOA.

CONCLUSIONS

The fertility decreased by both male and female age and for men with NOA. Men with NOA younger than 35.2 years and having a female partner younger than 36.9 years seemed to have the best chance to have a living healthy baby. The early visit to doctor seemed to have positive effect. The other factors were not statistically significant.

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ETHICS STATEMENT

The present study protocol was reviewed and approved by the institutional review board of Gülhane Training and Research Hospital, Ankara, Turkey (approval number: 2020-44).

AUTHORS' CONTRIBUTIONS

BT: Conceptualization, Data curation, Investigation, Methodology, Software, Writing – original draft. **TE:** Conceptualization, Writing – original draft. **SS:** Data curation, Validation. **EK:** Formal analysis, Validation, Writing – review & editing. **UF:** Resources, Writing – review & editing. **CK:** Resources. **STC:** Methodology, Writing – review & editing. **SB:** Software. **MG:** Supervision. **OFK:** Investigation, Visualization.

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