ARTICLE

Sperm concentration and semen volume increase after smoking cessation in infertile men

Deniz Kulaksiz[1], Tuncay Toprak[2]✉, Eda Tokat[3], Mehmet Yilmaz[4], Mehmet Akif Ramazanoglu[5], Asgar Garayev[6], Muhammed Sulukaya[7], Recep Burak Degirmentepe[8], Elnur Allahverdiyev[9], Murat Gul[10] and Ayhan Verit[2]

Smoking has negative reproductive consequences. This study investigated the effect of smoking cessation on the main semen parameters. We included 90 participants who applied to our infertility clinic and smoked at least 20 cigarettes a day for at least 1 year. Of the 90 participants, 48 were in the study group and 42 were in the control group. Semen analysis was performed before and at least 3 months after quitting smoking in the study group. Semen analysis was repeated at baseline and at least 3 months later in the control group. Semen parameters such as volume, sperm concentration, total sperm count, morphology, and motility were evaluated according to the World Health Organization criteria. Patient characteristics as well as the duration of the smoking period, the number of cigarettes smoked per day and the time elapsed since smoking cessation were recorded. The mean age of the participants was 34.69 ± 5.3 years, and the duration of infertility was 34.12 ± 12.1 months ($n = 90$). The number of cigarettes smoked per day was 30.14 ± 6.69, and the smoking time was 8.31 ± 3.53 years. The average time to quit smoking was 104.2 ± 11.51 days ($n = 48$). A significant increase in semen volume, sperm concentration and total sperm count was observed 3 months after smoking cessation (2.48 ± 0.79 ml vs. 2.90 ± 0.77 ml, $p = 0.002$; 18.45×10^6/ml ± 8.56 vs. 22.64×10^6/ml ± 11.69, $p = 0.001$; $45.04 \pm 24.38 \times 10^6$ vs. $65.1 \pm 34.9 \times 10^6$, $p < 0.001$, respectively). This study showed that smoking cessation had a positive effect on sperm concentration, semen volume, and total sperm count. Although smoking cessation contributed positively to sperm motility and morphology, the difference was not statistically significant.

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INTRODUCTION

The World Health Organization reports that approximately one-third of individuals over the age of 15 worldwide smoke [1]. Smoking is a common health problem, and findings show that smoking affects reproductive health in both women and men more than consumption of alcohol or caffeine [2]. Cigarettes contain approximately 600 ingredients, which create over 7000 chemicals when burned. Arsenic, benzene, carbon monoxide, nicotine, and heavy metals are only a few of the toxic ingredients found in cigarettes, although the fundamental ingredient of the particle phase comprises nicotine aggregates [3]. Since cigarette smoke includes over 30 chemicals that are reported to be carcinogens, mutagens or allergens, it is reasonable to hypothesize that smoking exerts direct harmful effects on human germ cells [4]. In fact, smoking can adversely influence the male reproductive system [5], cause attenuated or impaired sperm production in the testicles [6], and/or indirectly cause a hormonal imbalance in the endocrine system [7]. The mechanisms by which tobacco smoke affects spermatozoa are not fully understood. One hypothesis is the production of "oxidative stress" that reduces sperm quality [8]. Cigarette smoke contains various reactive oxygen species, and smoking has been shown to increase leukocyte concentrations in semen by 48% [9]. Smoking can also damage the chromatin structure of sperm, causing endogenous DNA strand breaks and thus impairing the fertilization capacity [10]. DNA damage levels are higher in smokers [11].

Semen analysis is the most common and valid diagnostic test for sperm quality and involves the evaluation of semen volume and total count, sperm concentration, sperm morphology and sperm motility [12]. Smoking may be associated with decreased fertility in men due to decreased sperm motility and concentration and morphologically decreased normal sperm percentage [13]. In another study, smoking only had a negative effect on sperm concentration [14]. A meta-analysis of studies with a large population of 5865 men from 26 regions/countries reported that smoking has an adverse effect on semen quality in both fertile and sterile men and is associated with decreased sperm motility and count, particularly in moderate to heavy smokers [15]. In another study, smoking was associated with a significant decrease in sperm count and deterioration in sperm morphology [7]. Although

[1]Department of Obstetrics and Gynecology, Trabzon Kanuni Training and Research Hospital, University of Health Sciences, Trabzon, MD, Turkey. [2]Department of Urology, Fatih Sultan Mehmet Training and Research Hospital, University of Health Sciences, Istanbul, Turkey. [3]Department of Urology, Ankara Training and Research Hospital, University of Health Sciences, Ankara, MD, Turkey. [4]Department of Urology, Faculty of Medicine, University of Freiburg—Medical Centre, Freiburg, MD, Germany. [5]Department of Urology, Trabzon Kanuni Training and Research Hospital, Trabzon, MD, Turkey. [6]Department of Urology, Istanbul Florence Nightingale Hospital, Istanbul, MD, Turkey. [7]Department of Urology, Malatya Training and Research Hospital, Malatya, MD, Turkey. [8]Department of Urology, Karasu State Hospital, Sakarya, MD, Turkey. [9]Department of Urology, Central Clinic Hospital, Baku, MD, Azerbaijan. [10]Department of Urology, Selcuk University Faculty of Medicine, Konya, Turkey. ✉email: drtuncay55@hotmail.com

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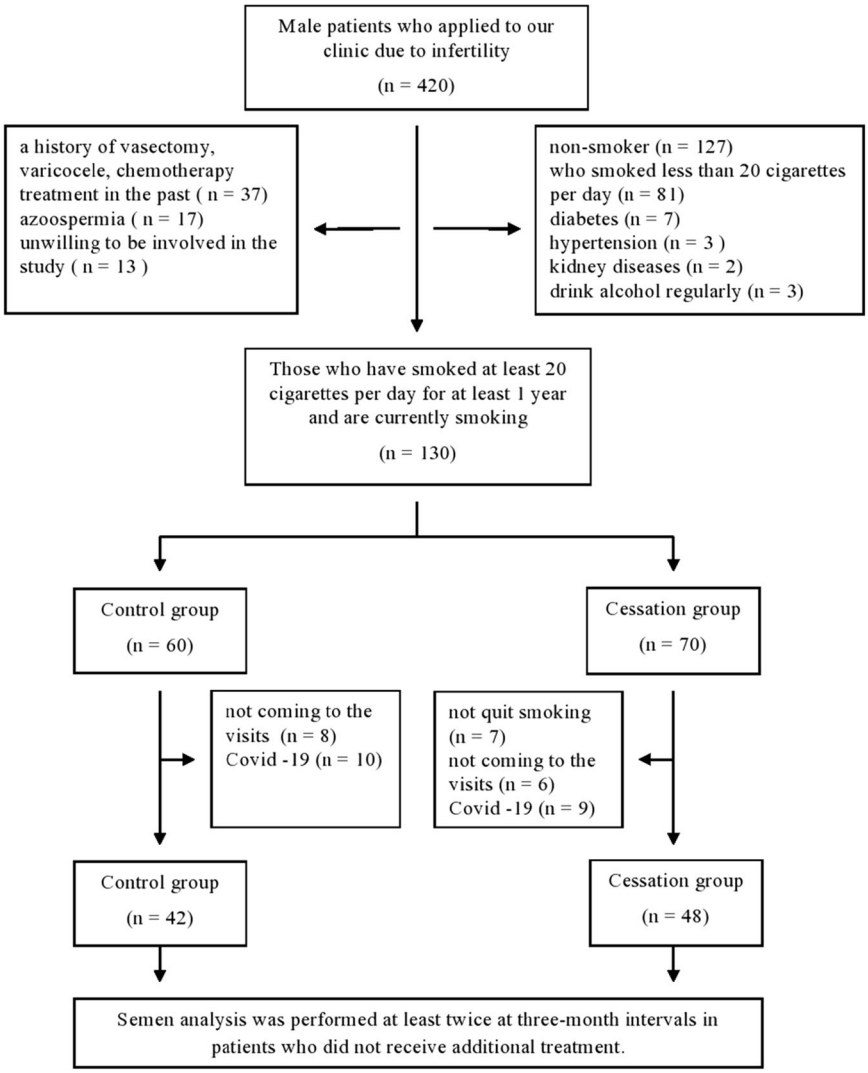

Fig. 1 Participant evaluation flowchart. Disclosure of participants included and excluded from the study. Reasons for drop-outs in groups. Follow-up of the groups.

the negative effect of smoking on semen parameters is described in the literature, contradictory evidence has been reported on exactly which parameters are affected. Most of the studies compare semen parameters of smokers with nonsmokers, and there is only one case report in the literature investigating whether the same individual has improved semen parameters after a 3-month smoking cessation program [16]. We aimed to determine the effects of smoking cessation on semen parameters. To our knowledge, this is the first human study to show the effect of smoking cessation on semen analysis parameters.

MATERIALS AND METHODS

We conducted a pilot study with 8 participants to evaluate a required number of participants at a significance level of 0.05 using version 3.01 of the G* Power software (Franz Foul, Kiel, Germany), and found that at least 40 participants were needed for each group. For the group represented by the sample, the number of men with abnormal semen parameters who smoked in Turkey was analyzed based on reported data. The prevalence of smoking in men in Turkey is 42% [17], and the prevalence of abnormal semen parameters in this population is 18.3% [18]. There are approximately 28 million adult men in Turkey [19]. While the mean total sperm count before smoking cessation was 48.3 million, it was 61.2 million after smoking cessation in the pilot study. The data of 8 participants were included in the study group. We assumed that some participants would not be able to quit smoking; thus, we enrolled 15% more participants in the study group than in the control group. After institutional ethics committee approval (FSMEAH-KAEK 2021/17) 130 men who applied to our outpatient clinic because of infertility problems and who smoked at least 20 cigarettes a day for at least 1 year and were smoking at the time recruitment were enrolled in the study.

Participants who smoked less than 20 cigarettes per day, drank alcohol regularly or used recreational drugs in the previous 3 months, had a history of vasectomy, varicocele, inguinal hernia operation, cryptorchidism, hypospadias, testes injury, orchitis, or chronic urinary tract infection, had radiation or chemotherapy treatment in the past, had chronic diseases (e.g., diabetes, kidney diseases) and had abnormal reproductive organs or azoospermia were excluded from the study. Chronic comorbid diseases may impair semen parameters [20, 21]. Although infertile men with chronic diseases are frequently seen, these patients were excluded to focus on the effect of smoking.

In this prospective nonrandomized controlled trial, smoking cessation was recommended to all patients; 70 patients who accepted smoking cessation were assigned to the study group, and 60 patients who did not accept smoking cessation were assigned to the control group. Eighteen participants in the control group and 22 participants in the study group were excluded from the study due to not being able to continue the follow-ups for reasons such as the COVID-19 pandemic, being out of the city, not being able to quit smoking, and not coming to the 3rd-month visit. The participant evaluation flowchart is given in Fig. 1. We included the remaining 90 men in the study after detailed examination of their medical

Table 1. Demographic characteristics and information about smoking status of patients.

	Cessation Group ($n = 48$)	Control Group ($n = 42$)	p value*
Age (year)	34.3 ± 5.4 (28–40)	35.2 ± 5.3 (29–41)	0.342
Body mass index (kg/m^2)	23.2 ± 3.9 (19.1–27.3)	23.7 ± 3.7 (19.8–27.7)	0.282
Educational level (year)	11.7 ± 4.1 (7–16)	11.2 ± 4.6 (6–16)	0.201
Duration of infertility (month)	34.8 ± 11.5 (23–47)	33.3 ± 12.6 (20–46)	0.323
Smoking time (year)	8.2 ± 3.6 (4–12)	8.4 ± 3.4 (4–12)	0.103
Average number of cigarettes smoked per day	30.7 ± 6.8 (20–40)	29.4 ± 6.6 (20–40)	0.126
Smoking cessation time (day)	104.2 ± 11.51 (90–120)	n.a	n.a

Data are given as "mean ± SD". Range values are given in parentheses.
*Independent *T*-Test.

records and daily routines. We performed a detailed examination to detect conditions that could cause infertility. The duration of smoking, average number of cigarettes smoked per day, alcohol use, drug or substance abuse (yes or no) and demographic data of the participants were recorded in a questionnaire. To better determine the effect of smoking, the subjects who smoked 20 or more cigarettes per day and were defined as heavy smokers in previous studies [14, 15] were included in the study. Spermatogenesis in humans takes approximately 74 days [22]. It is stated that 3 months is usually a sufficient time to see changes in semen analysis results [23–25]. Thus, the period for semen analysis comparison in the present study was determined to be 3 months for semen analysis comparison. The men in the study group were asked to provide samples for semen analysis twice (a total of four times) before and after smoking cessation. There was a difference of at least 1 week between repeated semen analyses. The average of the two semen analysis results was calculated. When 2 semen analyses differed significantly from each other, the third one was performed and the average of the two that were close to each other was accepted as correct. The same follow-up was performed at 3-month intervals in the control group. Semen analysis is an important method for evaluating male fertility [26]. For this reason, semen analysis was evaluated in the present study.

The ejaculate was collected by way of masturbation after a sexual abstinence period of 3 to 5 days and kept in a leak proof sterile wide-mouthed container. The sample was left to liquefy at 37 °C for 20 min and was analyzed according to the World Health Organization's guidelines [27]. Semen analysis included a macroscopic examination of appearance, viscosity, volume, and pH. The semen volume was evaluated by weighing the samples, and the result was recorded after 60 s. We microscopically analyzed the collected samples for sperm motility (a + b), morphology, and density. We evaluated sperm motility and concentration with a Makler's counting chamber. Only progressive mobility (WHO grades a + b) was included so that the results were more accurate and consistent. WHO criteria were used to define oligozoospermia [27].

Statistical analysis

SPSS Statistics 22.0 (IBM, USA) was used for statistical analysis. Normality of distribution of the parameters was evaluated by the Kolmogorov–Smirnov test. Continuous data are presented as the median (min-max) or mean ± standard deviation (SD). Statistical comparisons within groups were made using the Wilcoxon signed-rank test, since the variables were not normally distributed. We made statistical comparisons between groups using the independent samples *t*-test when the data were normally distributed and the Mann–Whitney *U* test when they were not. Changes in semen parameters between the cessation group and the control group were evaluated in terms of age, BMI and duration of infertility with the multivariable logistic regression analysis test. The Yates corrected chi-square test was used when the significance test of the difference between the two percentages was applied. $p < 0.05$ was considered statistically significant.

RESULTS

In terms of age, body mass index, educational level, duration of infertility, smoking time, and average number of cigarettes smoked per day, there were no statistically significant differences between the groups. Patient demographic characteristics and information about smoking status are presented in Table 1. A significant increase in semen volume, sperm concentration and total sperm count was observed 3 months after smoking cessation (2.48 ± 0.79 ml vs. 2.90 ± 0.77 ml, $p = 0.002$; 18.45×10^6/ml ± 8.56 vs. 22.64×10^6/ml ± 11.69, $p = 0.001$; $45.04 \pm 24.38 \times 10^6$ vs. $65.1 \pm 34.9 \times 10^6$, $p < 0.001$, respectively). There was no statistically significant difference in terms of baseline semen characteristics between the groups. Semen volume, sperm concentration and total sperm count were significantly higher in the study group than in the control group in the 3rd month ($p = 0.001$, $p = 0.001$, and $p < 0.001$, respectively). Although sperm motility and morphology increased after smoking cessation, the difference was not statistically significant ($p = 0.190$, and $p = 0.120$, respectively). Changes in semen parameters in the groups are presented in Table 2. Changes in semen parameters were compared with multivariable logistic regression analysis in terms of age, BMI, and duration of infertility (Table 3). One patient in the control group and 8 patients in the study group became normospermic in the 3rd month. While the proportion of oligozoospermic patients decreased significantly from 70.8% to 54.2% in the cessation group ($p = 0.043$), it decreased from 69% to 66.6% in the control group ($p = 0.441$).

DISCUSSION

Smoking has been reported to cause many adverse health outcomes [28]. Evidence is controversial on whether smoking affects semen parameters [29]. In the present study, a significant increase was shown in semen volume 3 months after smoking cessation. This might be an indicator of the effects of smoking on accessory glands and improvement due to smoking cessation. The other hypothesis was that nicotine in cigarettes might affect the functioning of the auxiliary sex glands that control the volume of semen through their secretion. There are studies showing that the decrease in semen volume in smokers is inversely correlated to the number of cigarettes consumed daily [30].

Our study highlights the fact that smoking has negative consequences on semen quality. In our study, smoking cessation improved sperm concentration and total sperm count. In the literature, nicotine has been shown to reduce the total sperm count in smokers [5]. In other studies, semen abnormalities have been observed in smokers, depending on dose [30, 31]. Nicotine negatively influences sperm count and morphology, whereas seminal cotinine negatively affects sperm motility [29].

Although not statistically significant in our study, we found that smoking cessation improved sperm motility. There are studies showing a decrease in sperm motility in smokers [30, 32] because of the toxic effects of nicotine [33] or an increased rate of carbon monoxide in the blood, which leads to a decrease in oxygenated hemoglobin and thus affects sperm mitochondrial oxygen utilization.

In the present study, we found that smoking cessation did not significantly change sperm morphology. One explanation for this

Table 2. Baseline and 3rd month semen parameters of the cessation group and the control group.

		Cessation group ($n = 48$)	Control group ($n = 42$)	p value**
Semen volume (ml)	Baseline	2.48 ± 0.79 (1.5–3.5)	2.46 ± 0.75 (1.5–3.5)	0.257
	3rd month	2.90 ± 0.77 (2.0–4.0)	2.45 ± 0.81 (1.5–3.5)	0.001
	p value*	0.002	0.170	
Sperm concentration (x10^6 /ml)	Baseline	18.45 ± 8.56 (9–28)	17.56 ± 9.61 (7–28)	0.116
	3rd month	22.64 ± 11.69 (10–35)	18.51 ± 9.42 (9–28)	0.001
	p value*	0.001	0.192	
Total sperm count (x10^6)	Baseline	45.04 ± 24.38 (20–70)	44.67 ± 22.7 (20–69)	0.241
	3rd month	65.1 ± 34.9 (29–102)	46.2 ± 21.7 (23–69)	<0.001
	p value*	<0.001	0.090	
Sperm motility (A + B) (%)	Baseline	20.54 ± 15.72 (4–38)	21.47 ± 14.76 (4–38)	0.316
	3rd month	21.41 ± 14.97 (6–38)	20.6 ± 15.92 (4–38)	0.185
	p value*	0.190	0.180	
Sperm morphology (%)	Baseline	2.22 ± 1.69 (0–10)	2.39 ± 1.58 (0–9)	0.211
	3rd month	2.43 ± 1.47 (0–11)	2.26 ± 1.71 (0–10)	0.131
	p value*	0.120	0.140	

Data are given as "mean ± SD". Range values are given in parentheses.
*Wilcoxon signed-rank test, **Independent *T*-Test.

Table 3. Multivariable logistic regression analysis at baseline/3rd month ($n = 90$).

	Semen volume OR (95% CI) p value	Sperm concentration OR (95% CI) p value	Total sperm count OR (95% CI) p value	Sperm motility OR (95% CI) p value	Sperm morphology OR (95% CI) p value
Age	1.20 (1.16–1.24)	1.19 (1.15–1.23)	1.42 (1.37–1.47)	1.11 (1.07–1.15)	1.17 (1.12–1.22)
	0.001	0.001	<0.001	0.186	0.113
Body Mass Index	1.14 (1.10–1.18)	1.13 (1.09–1.17)	1.36 (1.31–1.41)	1.06 (1.01–1.11)	1.13 (1.08–1.18)
	0.002	0.001	<0.001	0.202	0.132
Duration of infertility	1.17 (1.12–1.22)	1.16 (1.12–1.20)	1.39 (1.33–1.45)	1.08 (1.01–1.15)	1.15 (1.11–1.19)
	0.002	0.001	<0.001	0.188	0.115
Smoking cessation	1.16 (1.11–1.20)	1.18 (1.14–1.22)	1.41 (1.35–1.48)	1.06 (1.01–1.11)	1.13 (1.07–1.19)
	0.001	0.001	<0.001	0.196	0.138

OR odds ratio (Increase in semen parameters with smoking cessation).
CI confidence interval.

result is that these parameters probably require more tobacco abstinence to decline to normal values. Another explanation is that the patient's long history of smoking caused irreversible damage to some germ cells, and smoking cessation has no effect on sperm morphology. A third possible explanation may be the small number of participants included in the study. Although some studies have not found any relationship between smoking and sperm morphology, other studies have shown impairment in sperm morphology in smokers [34], even inversely proportional to the number of cigarettes smoked [30].

Our findings are partly in agreement with the meta-analysis of Sharma et al. [15], which found that cigarette smoking has an absolute adverse effect on semen parameters, particularly sperm count and motility. However, this meta-analysis included both fertile and infertile men, and several laboratory methods were used for semen analysis. Ranganathan et al. [35] found a negative effect of smoking on all semen parameters of infertile men, especially on sperm motility, and significant changes in sperm DNA fragmentation. Our results contrasted with those of Trummer et al. [36] who found no significant differences in conventional semen parameters or an increase in leukocytes, which are a main source of reactive oxygen species (ROS) in the ejaculate, in smokers.

To our knowledge, our study is the first to evaluate the effect of smoking cessation on semen quality. Except for a case report [16] that showed better semen parameters after 3 months of smoking cessation, the studies in the literature compared smokers with nonsmokers to describe the effect of smoking on semen quality. Similar data were shown in a recent study showing a benefit of assisted reproductive technology (ART) after the male partner quit smoking. In this study, examining the effect of smoking cessation on ART every passing year since a smoking man quit smoking, the risk of failure during an ART cycle decreased by 4% each year, regardless of the duration and intensity of smoking [37]. Although they showed an improvement in ART after the male partner stopped smoking, they calculated the number of cigarettes used, the time of smoking cessation for former smokers and the planned conservative smoking cessation period of at least 2 months for current smokers. In this respect, this study differs from our study in terms of the duration of smoking cessation, smoking dose, and the purpose. Therefore, the benefit of ART in this study after the male partner quit smoking was open to bias.

Another study that supports our study indirectly found no significant difference in semen quality in any category in men who previously smoked, compared with men who had never smoked. It was indirectly shown that smoking cessation could have a positive effect on semen quality [38]. Experimental animal (rat) studies have shown that nicotine has dose-dependent detrimental effects on sperm and this effect could be improved by nicotine cessation [39].

Study limitations

Our study had some methodological limitations. First, the results reflected the changing semen quality of men with infertility problems who presented to an andrology outpatient clinic; therefore, the study results should not be generalized to the fertile population. Therefore, this situation can cause a selection bias. The second limitation of our study was that self-reported smoking was the only source of information about smoking habits in our study, as in most published studies. There was no information about subjective measures of cigarette exposure, such as types of cigarettes, actual content in different products, smoking methods, and breathing depth, or about objective measures of cigarette smoke exposure such as cotinine levels. In addition, subjects might have underreported their lifestyle habits, particularly regarding the amount of smoking, which may cause a bias in the results. A study showing that the nicotine metabolite cotinine could provide a better measure of smoke exposure than the number of cigarettes smoked per day found a correlation between seminal plasma cotinine levels and decreased sperm motility [40]. Therefore, biochemical evaluation of smoking exposure is more desirable for the determination of smoking effects. However, there are studies showing high accuracy between the self-reported number of cigarettes and the values measured with biochemical elements in the blood [41]. Some studies have found a person's self-report of smoking reliable despite some misclassifications of smokers because of its subjectivity [42]. Because our study did not examine cotinine or other markers, we included only heavy smokers (over 20 cigarettes per day) to observe the effect of smoking. Third, the groups were formed as nonrandomized. Patients who quit smoking were more likely to have a healthier lifestyle with a potential impact on semen analysis. As another limitation, the time could be extended up to 6 months to monitor changes in semen parameters. However, maintaining motivation in the cessation group could be difficult. In addition, no significant difference was observed between 3 months and 6 months in studies comparing the improvement in semen analysis [23–25]. Finally, hormonal evaluation was not performed on the participants in our study. Hormone profile evaluation is recommended when the sperm concentration is <15 million/ml [43]. Since we did not include azoospermic participants in our study, we did not evaluate the hormone profile.

CONCLUSIONS

Our study showed that smoking cessation may improve semen quality and reduce the number of patients with abnormal parameters by improving semen volume, sperm concentration and total sperm count. Therefore, infertile patients should be strongly advised to quit smoking before any treatment.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author (TT) upon reasonable request.

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AUTHOR CONTRIBUTIONS

Conceptualization, statistical analysis, writing—original draft, writing—review and editing: DK, TT, ET, MY, MAR. Conceptualization, investigation, data curation, resources: TT, MY, MAR, AG, MS Conceptualization, formal analysis, review: AG, MS. Paper editing, review: DK, EA, RBD, MG, AV Supervision: MG, AV. All authors read and approved the final paper.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

Fatih Sultan Mehmet Training and Research Hospital Clinical Research Ethics Committee 2021/17 approval was obtained.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Tuncay Toprak.

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