Clinical Research

A Single-Center Study Assessing the Relationship Between Smoking Habits and sperm Parameters in Men with Suspected Infertility

Muhamet AFŞİN[1,a], Ayşe Feyda NURSAL[2], Dilek YAVUZ[1], Hasan AKKOÇ[3]

[1]*Health Sciences University, Gazi Yaşargil Training and Research Hospital, Andrology Clinic, Diyarbakır, Turkey*
[2]*Hitit University Faculty of Medicine, Department of Medical Genetics, Çorum, Türkiye*
[3]*Dicle University Faculty of Medicine, Department of Medical Pharmacology, Diyarbakır, Türkiye*

ABSTRACT

Objective: Many studies have linked smoking to male infertility. Therefore, we aimed to investigate the effect of smoking on sperm parameters in men with suspected infertility in the Turkish population. We also examined the effect of daily smoking amount and smoking duration on sperm parameters.

Material and Method: This study consisted of 1005 men (smokers= 599, non-smokers= 406) with suspected infertility. It evaluated sperm parameters, including, leucocyte count, sperm concentration, total sperm count, motility, and the total progressive motile sperm count in these men.

Results: In our study group, 59.60% were smokers and 40.40% were non-smokers. Body mass index (BMI) was higher in non-smoker males. Sperm characteristics were similar in smokers and non-smokers. We evaluated the smoker's group according to the number of cigarettes smoked per day. There was no significant difference in sperm parameters between the group that smoked up to 30 cigarettes a day and the group that smoked more than 30 cigarettes. Then, we examined the smokers in 3 groups according to the duration of smoking: 0-10 years, 11-20 years, and 20 years and over. It was observed that non-progressive motility was the lowest and immotility was the highest in smokers who had been smoking for 20 years or more.

Conclusion: This study is the most comprehensive study in Turkey examining the relationship between smoking and sperm parameters to the best of our knowledge. Our results show that the duration of smoking affects sperm functions. Our evidence indicates that men with suspected infertility should quit smoking to optimize their successful conception.

Keywords: *Smoking, Male Infertility, Sperm Count.*

ÖZ

İnfertilite Şüphesi Olan Erkeklerde Sigara İçme Alışkanlıkları ile Sperm Parametreleri Arasındaki İlişkiyi Değerlendiren Tek Merkezli Bir Çalışma

Amaç: Birçok çalışma sigara içmeyi erkek kısırlığıyla ilişkilendirmiştir. Bu nedenle Türk popülasyonunda infertilite şüphesi olan erkeklerde sigara içmenin sperm parametrelerine etkisini araştırmayı amaçladık. Ayrıca günlük sigara içme miktarı ve içme süresinin sperm parametrelerine etkisini inceledik.

Gereç ve Yöntem: Bu çalışmaya infertilite şüphesi olan 1005 erkek (sigara içen= 599, sigara içmeyen= 406) dahil edildi. Bu erkeklerde lökosit sayısı, sperm konsantrasyonu, toplam sperm sayısı, hareketlilik ve toplam ilerleyici hareketli sperm sayısı gibi sperm parametreleri değerlendirildi.

Bulgular: Çalışma grubumuzun %59,60'ı sigara içiyordu ve %40,40'ı sigara içmiyordu. Vücut kitle indeksi (VKİ) sigara içmeyen erkeklerde daha yüksekti. Sperm özellikleri sigara içen ve içmeyenlerde benzerdi. Sigara içen grubu günlük içilen sigara sayısına göre değerlendirdik. Günde 30 adete kadar sigara içen grup ile 30 adetten fazla sigara içen grup arasında sperm parametreleri açısından anlamlı fark yoktu. Daha sonra sigara içenleri sigara içme sürelerine göre 0-10 yıl, 11-20 yıl ve 20 yıl ve üzeri olmak üzere 3 grupta inceledik. 20 yıl ve üzeri sigara içenlerde nonprogresif motilitenin en düşük, immotilitenin ise en yüksek olduğu görüldü.

Sonuç: Bu çalışma bildiğimiz kadarıyla sigara içme ve sperm parametreleri arasındaki ilişkiyi inceleyen Türkiye'deki en kapsamlı çalışmadır. Sonuçlarımız sigara içme süresinin sperm fonksiyonlarını etkilediğini göstermektedir. Kanıtlarımız, infertiliteden şüphelenilen erkeklerin başarılı döllenmeyi optimize etmek için sigarayı bırakmaları gerektiğini göstermektedir.

Anahtar Sözcükler: *Sigara İçme, Erkek İnfertilitesi, Sperm Sayısı.*

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ORCID IDs: M.A. 0000 0002 7625 3982, A.F.N. 0000 0001 7639 1122, D.Y. 0000 0002 2877 533x, H.A. 0000 0003 2836 2452.

According to the World Health Organization **(**WHO), about 8% of the couples in the world and 10-15% of the couples in the industrialized countries have infertility, and 30% to 35% of all cases include male factor infertility (1). Although smoking has generally detrimental impacts on health, particularly on the male reproductive system (2), smoking is a global phenomenon. The World Health Organization (WHO) reports that 30% of the men who are 15 years of age and older are smokers (3) . About 46% of smokers are reproductive-age men (20-39 years of age) (4) .

The most important diagnostic tool is semen analysis

[a]Yazışma Adresi: Muhamet AFŞİN, Health Sciences University, Gazi Yaşargil Training and Research Hospital, Andrology Clinic, Diyarbakır, Turkey
Tel: 0412 258 0060 e-mail: afsin.muhammet21@hotmail.com
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for assessing fertility, which includes parameters such as sperm concentration, sperm count, sperm motion, semen volume, and sperm motility (5). Semen quality, including sperm density, viability, semen volume, motility, and normal morphology, is adversely affected by toxins from tobacco smoking, leading to male infertility (6). Aside from its link to male fertility impairment, smoking increases aneuploidies, sperm mutations (7), DNA damage, and spermatogenic cell apoptosis (8). The possible relationships between smoking and male infertility have been identified in different studies, some of which have reported contradictory results. Several studies have shown that smoking has negatively affected parameters of semen analysis and male infertility (9-12), while other studies have not found such effects. In some cases, even positive effects on sperm motility (13), and the extent of nuclear DNA damage in sperm (14), have been found (14). A study in Turkey found that cigarette and alcohol users had significantly lower follicle stimulating hormone (FSH) levels than smokers and non-smokers, and smokers had significantly decreased sperm motility (15). Furthermore, sperm counts dramatically decreased in smokers and alcohol abusers after washing, but especially the sperm rates of smokers were shown to decrease significantly only when the semen analysis of the smokers was evaluated.

Since the literature on the effects of smoking on human fertility has inconsistencies, we aimed to investigate the effect of smoking on sperm parameters in men with suspected infertility in a Turkish population. We also examined the effect of daily smoking and smoking duration on sperm parameters.

MATERIAL AND METHOD

Study population

The present case-control study was carried out from January 2021 to October 2021 at University of Health Sciences, Gazi Yaşargil Training and Research Hospital, Andrology Laboratory, Diyarbakır. One thousand five males (599 smokers, mean age: 30.67±7.21 and 406 non-smokers, mean age: 30.41±7.49) with suspected infertility aged between 16 and 70 years were included in the study. The smoker group consisted of active smokers. These subjects were defined as those who had previously smoked more than one cigarette per day. Non-smokers were the only subjects who had never smoked during their lifetime. All participants were informed of the aim of this research, and they provided their written informed consent. The principles outlined in the Declaration of Helsinki were followed. The local ethics committee for human research at the same hospital approved the study (date and number of approval: 2021/868).

Semen analysis

We collected the sperm samples from those who were sexually abstinent for 2-7 days without using any lubricant by masturbating into the sterile disposable plastic cups. WHO criteria were applied to examine the semen samples taken from the participants after liquefaction. Based on the WHO criteria regarding viscosity, in normal semen, distinct small drops fall out of the pipette. The drop forms a strand longer than 2 cm in length due to the abnormal viscosity. The WHO definition of leukocytospermia (million/ml) was used in this study to define leukospermia. We first homogenized the semen samples by pipetting with a Pasteur pipette. We pipetted about 10 µl of semen and placed it on the Makler Camera (Makler caunting chamber; Sefi Medical Instruments) and sealed it with a glass lid to determine motility and the count. We counted spermatozoa in 10 squares through the light microscope's x20 lens (Olympus CX31), and expressed the result in millions. We evaluated the sperm parameters such as viscosity, ejaculate volume, sperm concentration, leucocyte count, motility, immotility, total sperm count, and total progressive motile sperm count (TPMSC).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 21.0 was used for statistical analysis. The Shapiro-Wilk test was used to determine whether numerical data conformed to a normal distribution. Numerical data were expressed as median (min-max), and qualitative data were expressed as frequency and percentage values. In the analysis of numerical data, the Mann-Whitney U test was used to compare the two groups. Kruskal-Wallis post hoc Dunn-Bonferroni tests were used to compare multiple groups. The chi-square test was used in the analysis of categorical data. The significance level was taken as $p < 0.05$.

RESULTS

The study population consisted of 1005 male participants. Smoking status was classified as follows: 599 (59.60%) were smokers, and 406 (40.40%) were non-smokers. The mean age was similar among smokers and non-smokers. Body mass index (BMI) was higher in non-smoker males (p= 0.036). While smoking was more common among primary school graduates, it was less common among university graduates (p= 0.002). The demographical findings are demonstrated in table 1.

Table 1. *Comparison of demographic characteristics of smokers and non-smokers.*

Demographic characteristics	Smoker (n= 599)	Non-smoker (n= 406)	p
Age (year), median (min-max)	30.0 (17.0-63.0)	30.0 (15.0-30.0)	0.816
BMI (kg/m^2), median (min-max)	25.2 (14.1-45.1)	25.4 (18.8-75.1)	**0.036**
Education level			
Primary School, n (%)	310 (51.7)	175 (43.2)	**0.002**
High School n (%)	143 (23.9)	87 (21.1)	
University, n (%)	146 (24.4)	147 (35.7)	
Residential area			0.656
Rural, n (%)	108 (18)	69 (17)	
Urban n (%)	491 (82)	337 (83)	
Infertility type			
Primary, n (%)	403 (67.3)	286 (69.9)	0.664
Secondary, n (%)	196 (32.7)	123 (30,1)	
Infertility period (year), mean±SD	3.01±3.03	2.93±3.07	0.681
Varicocele			0.530
Yes/no, n (%)	187/412 (31.2/68.8)	119/287 (29.3/70.7)	
Chronic diseases			0.149
Yes/no, n (%)	81/518 (13.2/86.8)	43/363 (10.6/89.4)	
Stress			0.072
Yes/no, n (%)	439/160 (73.3/26.7)	282/124 (69.4/30.6)	

BMI: Body Mass Index, SD: Standard deviation, The results that are statistically significant are shown in boldface.

We evaluated sperm parameters, including ejaculate volume, leucocyte count, sperm concentration, total sperm count, motility, immotility, and TPMSC, between the smoker group and non-smoker group. Both groups had similar ejaculate volume, leucocyte count viscosity, sperm concentration, total sperm count, motility, immotility, and TPMSC ($p > 0.05$). Sperm parameters in smokers and non-smokers are shown in table 2.

Table 2. *Comparison of sperm parameters in smokers and non-smokers.*

Sperm parameters	Smoker (n= 599) Median (min-max)	Non-smoker (n= 406) Median (min-max)	p
Abstinence time (days)	3.0 (1.0-5.0)	3.0 (2.0-5.0)	0.112
Ejaculate volume (ml)	3.0 (0.2-10.0)	3.0 (0.1-10.0)	0.151
Leucocyte concentration (million/mL)	0.0 (0.0-4.0)	0.0 (0.0-3.0)	0.165
Sperm concentration (million/mL)	30.0 (0.01-240.0)	27.0 (0.02-250.0)	0.255
Total sperm count (million)	90.0 (0.04-513.0)	78.0 (0.04-525.0)	0.409
Motility			
Progressive (%)	54.5 (0.0-100.0)	55.0 (0.0-92.0)	0.372
Nonprogressive (%)	5.0 (0.0-50.0)	5.0 (0.0-50.0)	0.532
Immotility (%)	38.0 (0.0-100.0)	40.0 (5.0-100.0)	0.668
TPMSC (million)	44.0 (0.0-535.0)	44.3 (0.0-410.0)	0.439

TPMSC: Total Progressive Motile Sperm Count, SD: Standard deviation. The results that are statistically significant are shown in boldface.

Then we evaluated the smoker group in two groups based on the number of cigarettes smoked per day up to 30 and from 30 upwards. The results are presented in table 3.

Table 3. *Comparison of smokers' sperm parameters according to the number of cigarettes smoked per day.*

Sperm parameters	Up to 30 (n= 539) Median (min-max)	30 upwards (n= 60) Median (min-max)	p
Abstinence time (day)	3.0 (1.0-5.0)	3.0 (2.0-4.0)	0.497
Ejaculate volume (ml)	3.0 (0.8-9.5)	3.0 (0.2-10.0)	0.845
Leucocyte concentration (million/mL)	0.0 (0.0-3.0)	0.0 (0.0-4.0)	0.640
Sperm concentration (million/ml)	33.0 (0.20-110.0)	30.0 (0.01-240.0)	0.675
Total sperm count (million)	10.0 (0.80-513.0)	90.0 (0.04-486.0)	0.623
Motility (%)			
Progressive (%)	55.0 (0.0-90.0)	52.5 (1.0-100.0)	0.788
Non-progressive (%)	5.0 (0.0-50.0)	5.0 (0.0-20.0)	0.218
Immotility (%)	38.0 (8.0-100.0)	41.5 (0.0-95.0)	0.676
TPMSC (million)	44.0 (0.20-410.0)	44.3 (0.0-405.0)	0.875

TPMSC: Total Progressive Motile Sperm Count, SD: Standard Deviation.

There was no significant difference in sperm parameters, including ejaculate volume, leucocyte count, sperm concentration, total sperm count, motility, immotility and TPMSC, between the group that smoked up to 30 cigarettes per day and those that smoked more than 30 cigarettes ($p > 0.05$).

Then, we examined the smokers in 3 groups according to their smoking duration: 0-10 years, 11-20 years, and 20 years and older. Non-progressive motility was the lowest in smokers for 20 years or more ($p = 0.003$). Sperm immotility was highest in those who smoked for 20 years or more ($p = 0.026$). Sperm parameters 0-10

years, 11-20 years, and 20 years above are shown in table 4.

Table 4. *Comparison of sperm parameters of smokers based on smoking duration.*

Sperm paramaters	**0-10 years (n= 260)** Median (min-max)	**11-20 years (n= 240)** Median (min-max)	**20 years and more (n= 99)** Median (min-max)	**p**
Abstinence time (days)	3.0 (1.0-5.0)	3.0 (2.0-5.0)	3.0 (3.0-5.0)	0.690
Ejaculate volume (ml)	3.0 (0.3-10.0)	3.0 (0.7-7.0)	3.0 (0.2-10.0)	0.355
Leucocyte concentration (million/mL)	0.0 (0.0-4.0)	0.0 (0.0-3.0)	0.0 (0.0-3.0)	0.239
Sperm concentration (million/ml)	32.5 (0.01-150.0)	30.0 (0.02-150.0)	28.0 (0.11-240.0)	0.918
Total sperm count (million)	85.5 (0.04-513.0)	102.5 (0.04-480.0)	85.0 (0.29-300.0)	0.629
Motility (%)				
Progressive (%)	57.0 (0.0-90.0)	54.0 (0.0-100.0)	50.0 (1.0-90.0)	0.147
Non-progressive (%)	5.0 (0.0-30.0)	5.0 (0.0-50.0)	3.0 (0.0-22.0)*	**0.003**
Immotility (%)	37.0 (9.0-100.0)	40.0 (0.0-98.0)	45.0 (8.0-95.0)**	**0.026**
TPMSC (million)	44.5 (0.0-410.0)	47.0 (0.0-405.0)	37.3 (0.08-264.0)	0.537

p <0.01 vs 0-10 years group, **p <0.05 vs 0-10 years group;* ***TPMSC:* *Total Progressive Motile Sperm Count,* ***SD****: Standard Deviation, The results that are statistically significant are shown in boldface.*

DISCUSSION

Smoking is a health hazard that has been widely recognized. Despite global antismoking campaigns, some people continue to smoke regularly. Smoking is divided into two phases: the particulate and gaseous phases. A lit cigarette generates approximately 4000 compounds through various processes such as pyrolysis, hydrogenation, decarboxylation, oxidation, and dehydration. The main constituents affecting health are carbon monoxide in the gaseous phase, nicotine, and tar in the particulate phase (16). Evidence also shows that the toxins found in cigarette smoke may not be exclusively responsible for the adverse effects of smoking. In fact, a study that investigated the impacts of oral nicotine on male rats showed that rats exposed to oral nicotine experienced a significant reduction in sperm count and sperm motility (17). Therefore, nicotine may also adversely affect fertility and is not dependent on the toxins found in cigarette smoke.

It is interesting that there was an improvement in the parameters that were impacted by oral nicotine after 30 days of cessation, showing a component of reversibility to these impacts. In addition, a study on 210 men showed that men with higher concentrations of cotinine in the seminal plasma also had a higher percentage of abnormal sperm morphology (18). Since cotinine is a nicotine metabolite, these findings support the hypothesis that nicotine itself may possibly lead to the adverse effects of smoking on fertility. In a society where males dominate, females usually carry the burden of infertility, though in most cases, the male counterpart also contributes to infertility. Soares et al. (19), who reviewed the literature on the association between smoking and reproductive function, focused on strong evidence indicating that cigarette smoking negatively affected female and male fertility. Ramlau-Hansen et al. (20) conducted a cross-sectional analysis of 2542 healthy men during 1987–2004 and found that the semen analysis showed that cigarette smokers had lower sperm counts, semen volumes, and motile sperm percentages than the men who did not smoke. It was also suggested that the association between smoking and sperm concentration was dose-dependent. In a case-control study, there was an association between smoking and impaired motility of spermatozoa, reduced semen concentration, and a higher morphology defect (21). In addition, an article published by the Canadian Society of Clinical Chemists showed that abnormal structural defects of spermatozoa, especially round head defects, were associated with tobacco smoking due to a lack of sufficient scavenging antioxidant enzymes and higher oxidative stress in the infertile men's seminal fluids (22). Another study that evaluated the impact of smoking on vital seminal parameters influencing fertility showed that smoking impaired motility more than impaired sperm count (23). A meta-analysis assessing human semen found that tobacco smoking negatively affected semen parameters (24). The analysis of 5865 infertile and fertile men found impaired motility and a lower sperm count in the semen samples of these young men. In summary, the possible mechanisms involved with the impact of smoking on semen parameters are that the toxic contents of cigarettes harmfully affect the male germ cells and their developmental processes (25).

In a meta-analysis evaluating 16 studies with a total of 10823 infertile men (5 257 smokers and 5 566 non-smokers) as participants, it was found that oligozoospermia and morphological defects of spermatozoa were more common in smokers compared with non-smokers (26). In a retrospective study of 296 infertile men in our country, Ozgur et al. (27) reported that morphologic evaluation revealed better results for the nonsmokers than the heavy smokers in terms of tail anomalies and percent of coiled tails.

In the current study, we examined the effects of smoking on sperm parameters in 1005 men with suspected infertility. As far as we know, this study is the most extensive one conducted in the Diyarbakir region. BMI was higher in the non-smoker group than in the smoker group (Table 1). Comparing sperm parameters between smokers and non-smokers, there was no significant difference between groups (Table 2). When we examined the effects of daily smoking on sperm parameters, we found no significant difference between the group that smoked up to 30 cigarettes per day and the group

that smoked more than 30 cigarettes (Table 3). We also analyzed the smoker group by separating it according to the duration of smoking. We observed that non-progressive motility was the lowest and immotility was the highest in smokers who had been smoking for 20 years or more (Table 4).

Study Limitations

Our study is not devoid of limitations. In this study, the results of individuals with a suspicion of infertility who applied to the hospital are shown. Consequently, this study's participants have male infertility problems. These results may not show the association between the individuals' smoking status in the entire population and the sperm parameters. Furthermore, one of the limitations of this study is that it may not evaluate parameters thought to affect the participants' smoking habits, such as anxiety and depression. There should be a multidisciplinary approach to identify and inform those exposed to infertility, which has an important place in society.

Conclusion

Consequently, this study is the most comprehensive one in Turkey, examining the relationship between smoking and sperm parameters. Our results show that smoking frequency and duration affect sperm functions. Based on the data obtained from this study, it is shown that smoking has long-term effects on sperm functions. Our evidence indicates that men with suspected infertility should quit smoking to optimize their successful conception.

Ethics Committee Approval

This study was approved by the Ethics Committee of the University of Health Sciences Gazi Yaşargil Training and Research Hospital Clinical Research (date and number of approval: 2021/868).

Conflict of interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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