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Research Article

Evaluation of spermatic DNA fragmentation in smoking normospermic infertile individuals

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Abstract

Aim: In approximately 20% of infertile couples, the male factor is the only main cause. In recent years, it has been hypothesized that sperm DNA integrity may be a better indicator than routine semen analysis. Although the effects of smoking on male infertility have not yet been proven, smoking is considered a reasonable risk factor for infertility. In our study, we aimed to evaluate DNA damage in normospermic infertile men who smoke and do not smoke using the acridine orange method.

Material and Methods: This study was conducted on semen samples from 50 male cases, 25 of whom were smokers and non-smokers, who met the infertility criteria and applied to the urology clinic of Dicle University Medical Faculty Hospital. Microscopic examinations and morphological evaluations were performed in accordance with the WHO 2010 criteria. The acridine orange method was applied to evaluate DNA fragmentation and DNA fragmentations were calculated. Statistical analysis of the obtained data was performed using the Mann-Whitney U Test.

Results: In our study, in the smoking group, age was significantly associated with the duration of infertility, concentration with total motile sperm count, total sperm count with total motile sperm count, and amorphous head anomaly ($p<0.01$). In the non-smoking group, age and infertility were significantly associated with the duration, volume and total count, concentration and total count, total motile sperm count, and DNA fragmentation ($p<0.01$). However, there was no statistical difference between the smoking and non-smoking groups in terms of semen parameters and DNA fragmentation ($p>0.05$).

Conclusion: In order to obtain more reliable results, it is thought that large-scale studies are needed in the same groups and in the general population.

Keywords: sperm, infertility, DNA fragmentation, smoking

INTRODUCTION

In approximately 20% of infertile couples, the male factor is the only main cause [1]. If the female and male factors are included, this rate reaches 40%. Today, routine semen analysis is used in the evaluation of male infertility [2]. However, since semen analysis results are normal in 15% of infertile men, a definitive diagnosis of fertility cannot be made with routine semen analysis in these cases. Therefore, the need for new tests that will definitely distinguish fertile and infertile men from each other and predict pregnancy outcomes has increased, and studies have focused on sperm DNA integrity [3]. In recent years, studies investigating sperm nuclear DNA integrity in male infertility have increased. In these studies, it has been argued that sperm DNA integrity may be a better indicator than routine semen analysis in predicting male infertility [4,5].

Smoking is one of the most widely used and potentially dangerous social habits in the world. Men smoke more than women. Cigarettes contain mutagens and carcinogens [6]. It has raised concerns due to its negative effects on the male reproductive system. Studies have also shown that there is a relationship between tobacco chewing, smoking and semen criteria. In a study conducted on infertile Turkish men, it was observed that sperm tail defects were more common in those who smoked twenty or more cigarettes per day, but interestingly, it was found that the number of progressively motile sperm was higher in those who smoked more than twenty cigarettes per day compared to light smokers [7].

A significant increase in the rate of DNA-damaged spermatozoa and a high risk of childhood cancer and birth defects were observed in children whose fathers smoked. In a small number of studies, no relationship was found between sperm nuclear DNA damage or sperm function and sperm quality [8]. Although the effects of smoking on male infertility are inconclusive, smoking can be considered a reasonable infertility risk factor, as evidence of the negative effects of smoking on semen parameters suggests. Especially when marginal or

abnormal semen parameters are clearly documented, smoking should be stopped in both male and female partners in couples with a history of recurrent pregnancy loss or infertility [9]. It has been reported that there is a positive relationship between active smoking and sperm DNA fragmentation, as well as axonemal damage and decreased sperm count. It has been found that sperm from smokers is significantly more sensitive to acid-induced DNA denaturation than from non-smokers. Because DNA strand breaks have been observed at higher levels in the sperm of smokers [10].

This study shows whether DNA damage is caused by smoking in infertile men who smoke and do not smoke using the acridine orange staining method.

MATERIAL AND METHODS

Study Design

This study was conducted on semen samples from a total of 50 men who applied to the Urology Clinic of Dicle University Medical Faculty Hospital; who smoked 25 cigarettes at least 10/day and 25 non-smokers, who met the infertility criteria, who did not have another chronic disease (diabetes, hypertension, rheumatic disease, etc.) or a medical history of urological surgery, infection, and undescended testicles. Semen samples that met the infertility criteria but were evaluated as azoospermic after microscopic examination were excluded from the study. Semen samples were collected by masturbation into sterile containers after at least three days of sexual abstinence. For macroscopic examination, semen samples were allowed to liquefy in an oven at 370C for 20-40 minutes [11]. After the preparations prepared for morphological evaluation dried, they were stained with spermac dyes and evaluated according to WHO 2010 criteria.

Sperm Staining and Morphology Evaluation

A drop of semen sample was dropped onto a slide for morphological evaluation. The amount of dropped semen was adjusted depending on the number of spermatozoa. This drop was spread on the slide with the help of a second slide and dried with air. It was stained with the Spermac staining (Ferti Pro NV, Industriepark Noord, Belgium) method. The preparations spread on the slide and dried were kept in the fixative solution for 10 minutes. After the fixative was purified with water, the water was filtered and the staining process was carried out by waiting for 1.5 minutes in A and B (Ferti Pro NV, Industriepark Noord., Belgium) solutions and 30 seconds in C solution. It was waited to dry and examined using immersion oil at x100 magnification. 100 spermatozoa were taken into account when evaluating morphology. Each observed anomaly was noted separately [12].

Swim Up Method

After the normozoospermic samples are evaluated with the Makler camera, they are added to 15 ml Falcon round-bottom tubes and 2 ml of sperm preparation medium (G-IVF™ PLUS) is added. The prepared tube is incubated at 37°C, 5% CO2 and 95% humidity in a Herause Heracell 240 incubator for 30-60 minutes at a 45° angle. The upper medium is aspirated without touching the semen at the bottom and 5 ml of sperm washing medium is added and mixed. It is centrifuged at 300g-600g. The supernatant is discarded after centrifugation [12].

Acridine Orange Staining

Acridine orange is a dye that allows the staining of denatured DNA under acidic conditions. Acridine orange changes from green to orange under acidic conditions. Based on this principle, it enables the evaluation of DNA denaturation under a fluorescent microscope. When the dye binds to normal DNA, it gives green fluorescence, and when it binds to denatured DNA, it gives red fluorescence. A smear preparation was prepared by taking 20 µl from the semen sample, which was washed with special solutions to remove plasma, dead sperm and other cells. After waiting for about 1 hour in Carnoy's fixative, it was dried in air and taken to acridine orange dye. After waiting for 5 minutes in the dye in the dark, it was washed with distilled water and examined under a fluorescence microscope at a wavelength of 450-490 nm. Sperms that gave a green fluorescent image were considered normal, and sperms that gave a yellow-orange fluorescent image were considered to have damaged DNA, and an average of 100 sperm cells were counted to calculate the DNA fragmentation percentage [12].

RESULTS

DNA fragmentation rate was 4.8% (±2.7) in the smoking group; 5.96% (±4.2) in the non-smoking group and no statistical difference was found between the two groups. The highest sperm DNA fragmentation rate was 11% in the smoking group; this value was 14% in the non-smoking group. When the cases with the lowest and highest DNA fragmentation rates were compared; in the smoking group, in cases with low DNA fragmentation rates: concentration, total number and total motile sperm counts were high; while these values were lower in those with high DNA fragmentation rates (concentration 130;20, total number 416 million;56 million, TPMSS 299.52;31.36). In the non-smoking group; when we compared cases with high and low DNA fragmentation rates: no difference was found as in the smoking group.

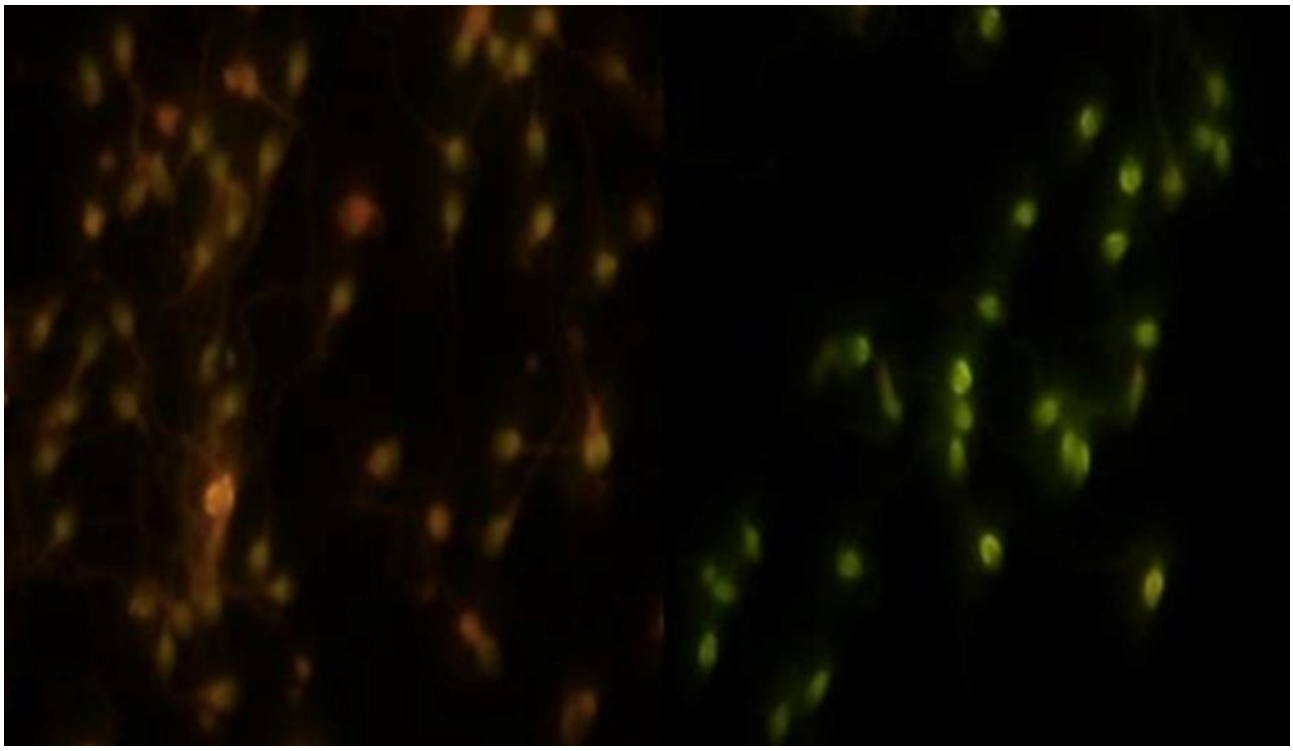

Figure 1: Positive (orange - defective) and negative (green - normal) representations of sperm DNA fragmentation in acridine orange staining (100x immersion objective fluorescence microscope image)

DISCUSSION

In approximately 20% of infertile couples, the male factor is the only main cause. If the female and male factors are included, this rate reaches 30%-40% [13]. In the literature, there is a suspicion of a causal relationship between smoking and impaired reproductive function [14]. However, the real effect of tobacco on male infertility is still controversial. Numerous mechanisms have been suggested, such as impaired spermatogenesis, induction of ultrastructural structural anomalies and apoptosis). Studies on the relationship between smoking and sperm quality reveal conflicting results. In the study conducted by Özgür et al. [6], it was reported that age, duration of sexual abstinence, semen volume, sperm density, liquefaction time and number of round cells per milliliter were similar in non-smokers, low-smokers and high-smokers.

Many other studies also reported that there was no significant difference in sperm parameters between smoking and non-smoking groups [3, 10]. In parallel with the results of this study, our results also showed that there was no statistical difference in semen parameters and morphology between smoking and non-smoking cases. However, many studies are being conducted on this subject and the results seem to be contradictory. We believe that large-scale studies with increased sample sizes in the general population will reveal more clearly whether smoking has an effect on semen parameters.

Agarwal et al. [16] reported in a study that smoking has negative effects on sperm count, motility and morphology, and one of the mechanisms was seminal oxidative stress-induced ROS, which has destructive effects on sperm quality and function. The oxidative stress status in the semen of smoking men is higher than that of non-smoking men. Studies show that smoking increases seminal oxidative stress through various mechanisms. In line with these data, in another study; It has been reported that alcohol consumption, as well as smoking, has a detrimental effect on seminal parameters such as seminal volume and sperm concentration, with significant changes in sperm morphology [17, 18]. In this study, where we evaluated DNA fragmentation with acridine orange, no statistical difference was found when we compared both groups (smokers and non-smokers).

CONCLUSION

In light of the above literature, most of our results are consistent with the literature. However, in order to obtain more reliable results, it is thought that large-scale studies are needed to be conducted on the same groups and the general population.

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