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The Effect of Smoking on the Apoptotic Marker (Caspase-3) of Human Sperm

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ABSTRACT

The ability of human sperm to fertilize can be compromised both in vivo and in vitro by the toxins found in cigarette smoke, which can lower sperm mitochondrial activity and harm chromatin structure. The aim is to study the effect of smoking on the apoptotic marker (caspase-3) of human sperm. A cross-sectional study of 80 semen samples of sub-fertile men, were divided into smokers (35 patients) and non-smokers (45 patients). All the semen samples after seminal fluid analysis underwent HOST test and grading (A-G) according to the WHO 2021 criteria then the slides were smeared and stained with an immunocytochemistry kit for Caspase-3 apoptotic marker. There was no significant effect of smoking in the concentration of caspase-3 expression, in spite of the high presentation of both grades A and G in the smoker group (p = 0.57, 0.56 respectively). Smoking has a negative impact on the DNA integrity of sperm as it increases the apoptotic marker within the smoker group.

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KEYWORD

Smoking, apoptosis, caspase-3, HOST

1. Introduction

Smoking has been linked to a decline in sperm quality, particularly in terms of motility, concentration, and shape, the three factors that are most commonly applied in clinical settings to evaluate fertility. The evidence is not conclusive, though, and several research did not find any impact on semen quality **(Sharma et al., 2016 [1]).**

Around 4000 chemical compounds are produced when tobacco is burned, and smokers inhale a variety of carcinogens such as nicotine, carbon monoxide, cadmium, and other mutagenic substances, all of which have the potential to harm male germ cells **(Sharma et al., 2016 [1])**.

The ability of human sperm to fertilize can be compromised both in vivo and in vitro by the toxins found in cigarette smoke, which can lower sperm mitochondrial activity and harm chromatin structure **(Sharma et al., 2013 [2]).**

Numerous studies have demonstrated that smoking causes semen characteristics such sperm concentration, viability, progressive motility, and morphology to decrease. Smoking metabolites, such as polycyclic aromatic hydrocarbons, can act as chemotactic cues, causing leukocyte recruitment, an inflammatory response, and the production of reactive oxygen species as a result **(Sreenivasa et al., 2016 [3]).**

According to biological, scientific, and epidemiological research, smoking cigarettes may be responsible for up to 13 % of infertility. Smokers also tend to have lower-quality sperm function tests than non-smokers **(Sreenivasa et al., 2016 [3]).**

Intracytoplasmic sperm injection (ICSI) may have a possible downside in that selected spermatozoa have an elevated risk of DNA damage. The existing diagnostic techniques for this are mostly invasive, making them

unsuitable for use in sperm selection. A reliable, non-invasive alternative selection method is HOST **(Stanger et al., 2010 [4]).**

Caspases trigger DNA fragmentation in response to significant DNA damage, which is a biological characteristic of apoptosis. Due to the disruption of sperm. Biochemical, and morphological integrity, oxidative stress can also result in apoptotic cell death. An essential part of apoptosis in many cell types, including spermatozoa, is played by a kind of caspase called final executioner caspase-3 **(Fatima et al., 2020 [5])**.

2. Patients and Methods

80 semen samples were collected for the current comparative cross-sectional study from 40 asthenozoospermic infertile men and 40 normozoospermic sub-fertile men who visited the male infertility consultant at the High Institute for Infertility Diagnosis and ART/Al-Nahrain University between November 2022 and April 2023. The High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University's ethical committee gave the approval to this study.

The patients' age range from 22 to 70 years, they were divided into smokers (35 patients) and non-smokers (45 patients). All the semen samples after seminal fluid analysis underwent HOST test and grading (A-G) according to the WHO 2021 criteria then the slides smeared and stained with immunocytochemistry kit for Caspase-3 apoptotic marker.

3. Results

The mean age of the studied sample men was 34.36±9.11 years. Thirty-five patients (43.75%) were smokers while 45 (56.25%) were not, 52.8% were normozoospermic, while 36.4% were asthenozoospermic patients. Table 1 revealed no statistically significant differences in

the expression of caspase-3 in sperm cells across all HOST grades between men who smoked and those who did not (p = 0.57; 0.09; 0.45; 0.59; 0.57; 0.54; and 0.56).

(Table 1): Comparing the expression of caspase-3 in sperm cells between men who smoke and those who don't, for each HOST grade

HOST grades	Smoking	No.	Mean ± SD	*p* value
Grade A	Smoker	35	25.79 ± 8.82	0.571
	Non-smoker	45	24.64 ± 9.94	
Grade B	Smoker	35	1.66 ± 1.71	0.091
	Non-smoker	45	1.37 ± 1.19	
Grade C	Smoker	35	2.06 ± 1.64	0.450
	Non-smoker	45	1.99 ± 1.57	
Grade D	Smoker	35	1.97 ± 2.22	0.591
	Non-smoker	45	2.05 ± 1.82	
Grade E	Smoker	35	4.30 ± 3.13	0.570
	Non-smoker	45	3.67 ± 2.98	
Grade F	Smoker	35	1.66 ± 1.56	0.540
	Non-smoker	45	1.76 ± 1.58	
Grade G	Smoker	35	22.92 ± 7.49	0.561
	Non-smoker	45	22.57 ± 8.81	

4. Discussion

One major risk factor for reproductive health is smoking. It results in problems with reproductive hormones, spermatogenesis deficits, sperm maturation, and impaired spermatozoa functioning **(Rehman et al., 2019 [6]).** Between the groups of smokers and non-smokers, there was no significant different in normozoospermia and asthenozoospermia (p = 0.11). These outcomes are in line with the findings of **(Rehman et al., 2019 [6])**, who proved smoking has no significant impact on motility. Additionally, the outcomes of this study concur with those of another research. **(Mariappen et al., 2018 [7]) . Also, (Bui et al., 2018 [8])** demonstrated that while there was a much-increased frequency of morphological abnormalities in smokers' spermatozoa, motility did not differ between smokers and non-smokers. Additionally, a meta-analysis was carried out by **(Omolaoye et al., 2022 [9])** discovered that there was no impact of tobacco use on motility.

Contrary to these results, studies by **(Sharma et al., 2016 [1]; Asare-Anane et al., 2016 [10]; Boeri et al., 2019 [11] and Mostafa et al., 2018 [12])** all demonstrated the detrimental effects of smoking on sperm motility.

The number of cigarettes and duration of smoking among the research groups may account for the discrepancy between the results of the current study and previous studies. The patients in these studies were classified as mild, moderate, or severe smokers; the mild and moderate groups did not exhibit any noteworthy outcomes.

Result revealed that there is no statistically significant difference in the concentration of caspase-3 expression between the smoker and non-smoker groups, despite the smoker group having a higher presentation of both grades A and G (p = 0.57, 0.56, respectively).

Numerous studies have shown how smoking affects DNA fragmentation.

According to **(Cui et al., 2016 [13]),** there have been conflicting findings from clinical studies regarding how smoking affects sperm DNA fragmentation.

Also, a study by **(Le et al., 2019 [14])** found that smoking dramatically raised the rate of DNA fragmentation in smokers' sperm when the DFI of smokers and non-smokers was compared. **(Kumar et al., 2015 [15])** found that higher levels of Sperm DNA fragmentation index and 8-hydroxy-2-deoxyguanosine (8-OHdG) were shown to be associated with an increase in seminal ROS in the semen samples taken from men who smoked.

Numerous studies have revealed that nicotine, one of the components of tobacco smoke, can cross the blood-testis barrier and impact spermatogenesis by changing the synthesis of hormones or the integrity of the DNA **(Kumar et al., 2015 [15]; Aprioku JS. and Ugwu TC., 2016 [16])** DNA fragmentation, a biological indicator of apoptosis, is induced by extensive DNA damage and is caused by caspase activities **(Fatima et al., 2020 [5]).**

5- Conclusion

Smoking has a negative impact on the DNA integrity of sperm as it increases the apoptotic marker within the smoker group, as most defective grades (A and G) were mostly presented in the smoker group.

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Author Contribution

Mohanad Mohammed performed the study, Muayad Saribet, Rehab Al-Maliki, supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee

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