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Influence of different types of tobacco smoking on seminal fluid parameters and sex hormones among males from the Gaza Strip

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Abstract:

Background: Smoking is a global problem that negatively affects human health and fertility and the endocrine functions of the male reproductive system.

Objectives: The present study aims to investigate the influence of the different types of tobacco smoking (cigarette, hookah, and electronic) on semen parameters and sex hormones in males from the Gaza Strip.

Methods and Materials: A total of 206 samples were classified into 156 cases and 50 controls. The cases have been classified into three groups depending on the type of tobacco smoking (cigarette smoking "n=52", hookah "n=55", and electronic "n=49"). Semen samples were collected to investigate the semen parameters in smokers compared to non-smokers. Additionally, blood samples were also collected to assess the level of sex hormones (testosterone, follicular stimulating hormone (FSH), and luteinizing hormone (LH)).

Results: A significant increase in FSH and LH levels has been found in smokers compared to non-smokers ($P < 0.001$), while a significant decrease in testosterone level has been observed in smokers' men compared to non-smokers ($P < 0.001$). Significant variations have been shown in the sperm concentration ($P < 0.001$) and the percentages of sperm total motility ($P < 0.001$), active progressive motility ($P < 0.001$), slow progressive motility ($P = 0.004$), and immotile spermatozoa ($P < 0.001$) among the different tobacco smoking groups. Furthermore, significant variations have been found in the levels of testosterone, FSH, and LH among the different tobacco smoking groups ($P < 0.001$). The results showed significant positive correlations between sperm concentration, total motility, active progressive motility, slow progressive motility, and testosterone level ($P < 0.001$) in smokers.

Conclusions: The different types of tobacco smoking have negative influences on semen parameters and the levels of testosterone, FSH, and LH of smokers in the Gaza Strip.

Keywords:

Cigarette; Hookah; Electronic smoking; Male fertility; Sexual hormones; Gaza Strip

Background

The World Health Organization (WHO) reported that approximately 33.33% of the world's population, over 15 years of age, smoke cigarettes (WHO, 2017). Tobacco smoking is one of the most significant lifestyle-associated risk factors that may induce pulmonary disorders, cardiovascular disorders, and cancer (Fragou et al., 2019; Gaudet et al., 2017; Kamimura et al., 2018). Tobacco smoke contains more than 400 types of ingredients, such as nicotine, tar, carbon monoxide (CO), polycyclic aromatic hydrocarbons, heavy metals, and irradiated substances (Dai et al., 2015). Nicotine is classified as one of the major dangerous and lethal toxins in tobacco, and it has been observed in the blood and semen fluid of smokers with strong correlations between the smoking dose, duration, and nicotine level (Haque et al., 2014). Alvarez (2015) showed that tobacco smoking is correlated with significant adverse impacts on male fecundity. Other investigational studies related to animals have reported that tobacco smoke could be directly or indirectly toxic to spermatogenesis, spermiogenesis, and sperm capacity (Sharma et al., 2016; Yu et al., 2014). Several factors have been used to assess the impact of tobacco smoking on male fecundity, including sperm parameters (Amor et al., 2022; Asare-Anane et al., 2016; Laqqan and Yassin, 2021), spermatozoa membrane integrity (Belcheva et al., 2004), and epigenetic effects (Laqqan et al., 2017; Xie et al., 2021). On the other hand, a study prepared by Oyeyipo and his colleagues found that alterations in male sex hormones were induced by tobacco smoking, and the nicotine inhalation during smoking leads to a significant reduction in the level of testosterone in rats, which has a negative effect on fertility. (Oyeyipo et al., 2013). In addition, a previous study found that the estrogen level has increased and the levels of follicular stimulating hormone (FSH), luteinizing hormone "LH", and prolactin "PRL" have declined in tobacco smokers (Harlev et al., 2016; Ochedalski et al., 1994).

In the last ten years, hookah smoking has become a widespread method of tobacco use over the entire globe, especially among young, adults and teenagers (Kim et al., 2016). Studies revealed that smoking hookah is very widespread in several regions, such as the Middle East and North Africa (Maziak, 2015). Hookah smoking has also increased significantly in the United States, Europe, Asia, and Latin America (Singh et al. 2017). Globally, a study reported that the rate of hookah smoking is increasing more than cigarette smoking (Qasim et al., 2019). In recent years, a new type of tobacco smoking has appeared called electronic cigarettes (e-cigarettes), which are electronic devices, consisting basically of a cartridge, filled with an e-liquid, a heating element/atomizer necessary to heat the e-liquid to create a vapor that can be inhaled through a mouthpiece, and a rechargeable battery (Hiemstra & Bals, 2016). The electronic fluid typically consists of humectants and flavorings, with or without nicotine; once vaporized by the atomizer, the aerosol provides a feel identical to tobacco smoking, but allegedly without toxic outcomes (Rowell & Tarran, 2015). A previous study reported that the potential hazard of electronic cigarettes is unknown and cannot be identified as harmless for inhalation (Farsalinos et al., 2015). Various types of smoking, including

cigarettes, hookah, and electronic, have a significant negative impact on male fecundity by affecting the same parameters (sperm concentration, sperm motility, normal shapes of spermatozoa, and other parameters) and male sex hormones "FSH, LH, and testosterone" (Fawzy et al., 2011). According to our knowledge, there are a few studies investigating the impact of different types of tobacco smoking on fertility and sex hormones in men that seem to be few and modest in the Gaza Strip. Consequently, this study was performed to investigate the effects of various types of smoking (cigarette, hookah, and electronic) on human seminal fluid parameters and sex hormones (FSH, LH, and testosterone), and to study the relationship between seminal fluid parameters and sex hormones level in smokers.

Material and Methods

Study population and participation criteria

This study is a prospective case-control study and consisted of 206 males. Participants were classified as follows: 156 cases and 50 controls. The case groups were classified into three groups according to types of tobacco smoking (cigarette smokers "n = 52", hookah smokers "n = 55", and electronic cigarette smokers "n = 49"). The control group consisted of males who had never smoked and had at least one child. On the other hand, the cases suffering from unknown infertility problems. Both the control and the case groups were matched in age and body mass index (BMI). Males in the study must meet the following criteria: be between the ages of 20 and 45, have never received chemotherapy or radiotherapy, have never had genital organ surgery, and have never been exposed directly to heavy metals. On the other hand, the common exclusion criteria include smoking for less than three years, varicocele, diabetes mellitus, drinking alcohol, anti-sperm antibodies, and Y chromosome microdeletion.

Ethical Considerations

The current study was approved by the Palestinian Health Research Council (No. PHRC/HC/987/21), and permission was granted according to the Declaration of the Helsinki Committee. All males signed a consent form to participate in this study.

Semen Collection and Analysis

After three days of sexual abstinence, seminal fluid was collected by masturbation at the Al-Dakhkhni Medical Laboratory, Gaza City. The seminal fluid was allowed to liquefy at 37°C for thirty minutes. Then a Meckler counting chamber was used to assess the sperm concentration, and all the parameters of the semen, such as sperm concentration, motility, and morphology, were evaluated according to the guidelines of the WHO (WHO, 2010). Additionally, five millilitres of blood were collected from each male under quality and safety control procedures, and then the serum was separated by centrifugation at 2500 rpm for 20 minutes. Serum samples were stored at -20°C for the hormone assay (FSH, LH, and testosterone) by using an ELISA kit from the "AccuBind company".

Data Analysis

All the data included in this study were analysed using SPSS version 24.0 (SPSS, Inc., Chicago, IL, USA). The Kruskal-Wallis "H" test and the Mann-Whitney "U" test were applied to compare quantitative variables between study groups. Spearman's correlation coefficient was used to determine the association between the various parameters. All results were considered statistically significant when the P-value was ≤ 0.05 .

Results

This study consists of 206 males with a mean age of 30.6 ± 4.6 years. The samples were classified according to smoking status into smokers ($n = 156, 75.7\%$) and non-smokers ($n = 50, 24.3\%$), with mean ages of 30.6 ± 4.5 and 30.6 ± 4.9 years, respectively. Table 1 illustrates the descriptive characteristics and clinical parameters of smokers compared to non-smokers. The results showed significant reductions in sperm concentration ($P < 0.001$), percentage of sperm total motility ($P < 0.001$), active progressive motility ($P < 0.001$), and slow progressive motility ($P = 0.01$) in smokers compared to non-smokers. In contrast, a significant increase has been observed in the percentage of immotile spermatozoa and abnormal forms of spermatozoa ($P < 0.001$ and $P = 0.03$) in smokers compared to non-smokers. However, no significant differences in semen volume, spermatogenic cell count, or white blood cells (WBCs) and red blood cells (RBCs) were found in smokers versus non-smokers ($P = 0.97, P = 0.06, P = 0.25,$ and $P = 0.95,$ respectively). Additionally, there was no significant variation between smokers and non-smokers in age, height, and weight ($P = 0.77, 0.66,$ and $0.37,$ respectively).

Hormone levels in smokers compared to non-smokers

As indicated in Table 2, a significant increase has been found in the levels of FSH and LH in smokers compared to non-smokers (22.33 ± 10.66 vs. $8.79 \pm 2.70, P < 0.001,$ and 18.10 ± 7.90 vs. $5.15 \pm 3.17, P < 0.001,$ respectively). Conversely, smokers have a significant reduction in the level of testosterone compared to non-smokers (3.68 ± 1.48 vs. $6.80 \pm 1.48, P < 0.001$).

Descriptive characteristics and semen parameters among the different study groups

As illustrated in Table 3, significant variations have been observed in the sperm concentration ($P < 0.001$), percentage of sperm total motility ($P < 0.001$), active progressive motility ($P < 0.001$), slow progressive motility ($P = 0.004$), and immotile spermatozoa ($P < 0.001$) among the study groups. On the other hand, there was no significant difference among the study groups in age ($P = 0.971$), height ($P = 0.913$), weight ($P = 0.593$), percentage of sperm non-progressive motility ($P = 0.308$), spermatozoa abnormal form ($P = 0.155$), number of spermatogenic cells ($P = 0.311$), number of WBCs ($P = 0.670$), and number of RBCs ($P = 0.781$).

Hormones levels among the different study groups

As represented in Table 4, the study showed significant variations in the levels of testosterone, FSH, and LH among the different study groups ($P < 0.001$). The levels of testosterone and FSH in hookah smokers were the highest (3.92 ± 1.45 and 27.81 ± 10.26), compared to cigarette smokers (3.83 ± 1.45 and 18.35 ± 8.44) and electronic cigarette smokers (3.24 ± 1.48 and 20.40 ± 10.84). In contrast, electronic cigarette smokers have the highest LH level (22.18 ± 10.89) compared to other groups.

Discussion

The reports revealed that about 1.1 billion people use tobacco products globally, with cigarettes being the most widespread product (82%), and the WHO reported that tobacco kills approximately 8 million people per year (Giovino et al., 2012). The percentage of individuals who smoke one or more tobacco products in Palestine increased to about 31% of the total individuals aged 18 years and above in 2021. The prevalence of smoking in the Gaza Strip was 17.0%, according to the Palestinian Central Bureau of Statistics (PCBs) (2022). The American Heart Association has recommended many steps and policies for tobacco control and mitigating the use of tobacco (Benjamin et al., 2018). Nevertheless, the increasing trend of tobacco hookah smoking is yet to be handled. Hookah was used to reduce the harm of traditional tobacco cigarette smoking, a belief that is still found among hookah smokers now (WHO, 2015). The increased smoking prevalence of various types of tobacco prompted more research into its relationship with human infertility issues. For this purpose, this study was performed. Several studies have reported that tobacco consumption is considered one of the main causes of male infertility (Daumler et al., 2016; Laqqan and Yassin, 2022; Mima et al., 2018; Yang et al., 2017).

The present study revealed significant declines in sperm concentration, percentage of sperm total motility, sperm active progressive motility, and sperm slow progressive motility in tobacco smokers compared to non-smokers. Conversely, smokers have significant elevations in the percentage of immotile and abnormal forms of spermatozoa compared to non-smokers. These findings match with other studies that showed significant decreases in sperm concentration, sperm total motility, and sperm abnormal forms in smokers compared to non-smokers (Mostafa et al., 2018; Rehman et al., 2019). Additionally, a study observed a detrimental effect of cigarette smoking associated with varicocele on sperm motility and morphology (Collodel et al., 2009). Several previous studies reported that exposure to cigarette smoking leads to a decline in sperm count, sperm motility; sperm quality, and these findings support the results of this study (Künzle et al., 2003; RamlauHansen et al., 2007; Sharma et al., 2016; Taha et al., 2012).

On the other side, the present study exhibited no significant alterations between smokers and non-smokers in semen volume, number of spermatogenic cells, WBC count, and RBC count. These findings are in agreement with previous studies (Hamad et al., 2018; Laqqan & Yassin,

2021a; Trummer et al., 2002). In contrast, other studies showed a significant variation in smokers and non-smokers in semen volume and WBC count (Bauer et al., 2016; Laqqan & Yassin, 2021; Shipa et al., 2017; Smith et al., 2021), and this variation can be explained as a response to the effect of nicotine that stimulates immune cells as the smoker's body treats these substances as foreign bodies.

Regarding sex hormones, the present study found that tobacco smokers have significant elevations in LH and FSH compared to non-smokers, and these results agree with a study performed by Bassey et al. that revealed a decline in the level of testosterone hormone in smoking compared to non-smokers (Bassey et al., 2018). Additionally, decreased testosterone and increased FSH and LH levels in smokers were pointed out by several studies (Bassey et al., 2018; Elmleeh et al., 2013; Oyeyipo et al., 2010; Park et al., 2012). Nicotine, one of the toxic components in tobacco smoke, had been shown to inhibit androgen biosynthesis and Leydig-cell growth (Duca et al., 2019; Funabashi et al., 2005), which could explain the observed drop in testosterone among smokers. The lower level of testosterone in smokers may translate to a relative decrease in fertility because testosterone is a very vital male reproductive hormone. Additionally, the higher levels of LH and FSH in smokers observed are indicators of testicular dysfunction (Araujo & Wittert, 2011). Elevated levels of FSH and LH in males usually reflect a lack of male steroid hormone-negative feedback (Fraietta et al., 2013; Sansone et al., 2018).

Regarding semen parameters among different smoking groups, the current results found that cigarette smokers have the highest reduction in spermatozoa count, percentage of spermatozoa total motility, active progressive motility, and slow progressive motility compared to other study groups. These results agree with other studies reporting that tobacco cigarette smoke contains carcinogens and mutagens that can lead to poor seminal fluid quality (Elshal et al., 2009). Briefly, the possible mechanisms that explain the effect of tobacco cigarette smoking on semen parameters are as follows: toxic contents found in cigarette smoking might have harmful effects on male germ cells and their developmental processes (Zenzes, 2000). The negative effects of nicotine on semen parameters have also been mentioned in a previous study (Beal et al., 2017). but they do not agree with other studies claiming that hookah smoking harms semen parameters more than other types of smoking (Fawzy et al., 2011, Noonan & Kulbok, 2009). They explained that hookah smoke causes a dramatic increase in oxidative stress in vitro that negatively affects semen parameters (Cobb et al., 2012; Wolfram et al., 2003). Despite the fact that several studies have found hookah smoking to be more harmful and a growing threat to public health (Jukema et al., 2014; Wong et al., 2016). This is due to each hookah smoking session lasting 30 minutes and being inhaled approximately 200 times per single cigarette, resulting in 30 times of exposure to CO (Daher et al., 2010).

The present study found a large variation in the levels of testosterone, FSH, and LH hormones among the different study groups. While hookah smokers have the highest levels of

testosterone and FSH compared to other types of tobacco smokers; e-cigarette smokers have a higher level of LH than other types of tobacco smokers. These findings coincide with studies that found a significant elevation of testosterone and FSH levels in hookah smokers compared to other smokers and non-smokers (Fawzy et al., 2011; Trummer et al., 2002). These results could be explained by the fact that hookah smoke has high concentrations of carbon monoxide (CO), nicotine, "tar," and heavy metals (Knishkowsky and Amitai, 2005; Shihadeh, 2003). Also, commonly used heat sources like charcoal may increase health risks because they produce toxicants such as CO, metals, and carcinogens (Shihadeh and Salehm, 2005). Also, the style of hookah smoking results in a dramatically higher exposure volume to smoke, more tobacco cigarette consumption per smoking event, and a longer smoke inhalation period (Eissenberg and Shihadeh, 2009; Cobb et al., 2010).

Conclusion

The present study showed that the different types of tobacco smoking have negative influences on semen parameters and sex hormone levels (FSH, LH, and testosterone) among males from the Gaza Strip. Where the highest decline in sperm concentration, percentage of sperm total motility, progressive motility, FSH, and LH levels have been found in males smoking tobacco cigarettes compared to other types of tobacco smoking.

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Table 1. Descriptive and semen parameters in smokers compared to non-smokers (n = 206)

Variables	Non-Smokers		Smokers		P-value
	(n = 50)		(n= 156)		
	Mean	SD	Mean	SD	
Age (Years)	30.60	4.97	30.64	4.47	0.77
Height (cm)	175.72	6.67	175.67	7.28	0.66
Weight (Kg)	79.88	9.87	81.97	11.48	0.37
Duration of smoking (Years)	0.00	0.00	12.41	6.15	< 0.001
A number of children	0.40	0.70	0.62	0.81	0.09
Semen Volume (mL)	3.40	1.05	3.51	1.39	0.97
Sperm count (10 ⁶ /mL)	43.62	16.87	22.81	15.20	< 0.001
Sperm total motility (%)	57.40	17.05	39.27	19.16	< 0.001
Sperm active progressive motility (%)	26.56	12.94	12.96	9.97	< 0.001
Sperm slow progressive motility (%)	19.26	8.84	15.06	8.63	0.01
Sperm non-progressive motility (%)	10.82	4.15	11.72	4.79	0.23
Sperm Immotile (%)	40.94	18.30	60.25	19.19	< 0.001
Sperm abnormal form (%)	38.20	11.90	45.96	17.41	0.03
Number of spermatogenic cells	4.10	3.12	3.29	2.49	0.06
Number of WBCs	8.32	8.97	7.44	9.66	0.25
Number of RBCs	2.78	1.92	3.56	4.47	0.95

The values are expressed as mean \pm SD; SD: A standard deviation; %: Percentage; WBCs; *White blood cells*; RBCs: Red blood cells; P > 0.05, Not significant; P \leq 0.05, Significant.

Table 2. Hormone levels in smokers compared to non-smokers (n = 206)

Hormones	Non-Smokers		Smokers		P-value
	(n = 50)		(n= 156)		
	Mean	SD	Mean	SD	
Testosterone level (ng/mL)	6.80	1.48	3.68	1.48	< 0.001
FSH level (mIU/mL)	8.79	2.70	22.33	10.66	< 0.001
LH level (IU/L)	5.15	3.17	18.10	7.90	< 0.001

The values are expressed as mean \pm SD; SD: A standard deviation; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; P > 0.05, Not significant; P \leq 0.05, Significant.

Table 3. Comparison of semen parameters among different study groups (n = 206)

Variables	Non-Smoking (n=50)		Cigarette smoking (n=52)		Hookah smoking (n=55)		E-cigarette smoking (n=49)		P-value among study groups
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Male Age (Years)	30.60	4.97	30.77	4.68	30.45	4.49	30.71	4.30	0.971
Post Hoc	0.998 ^a	0.999 ^b	0.999 ^c	0.985 ^d	1.000 ^e	0.992 ^f			
Height (cm)	175.72	6.67	176.06	7.19	175.45	7.42	175.51	7.34	0.913
Post Hoc	0.995 ^a	0.998 ^b	0.999 ^c	0.972 ^d	0.981 ^e	1.000 ^f			
Weight (Kg)	79.88	9.87	80.71	10.66	82.13	11.58	83.14	12.28	0.593
Post Hoc	0.982 ^a	0.730 ^b	0.465 ^c	0.913 ^d	0.692 ^e	0.967 ^f			
Duration of smoking (Years)	0.00	0.00	12.87	6.14	11.87	6.37	12.53	5.98	< 0.001
Post Hoc	< 0.001 ^a	< 0.001 ^b	< 0.001 ^c	0.775 ^d	0.989 ^e	0.924 ^f			
A number of children	0.40	0.70	0.63	0.86	0.60	0.83	0.61	0.76	0.383
Post Hoc	0.443 ^a	0.569 ^b	0.544 ^c	0.996 ^d	0.999 ^e	1.000 ^f			
Semen Volume (mL)	3.40	1.05	3.52	1.46	3.52	1.37	3.48	1.37	0.996
Post Hoc	0.971 ^a	0.964 ^b	0.992 ^c	1.000 ^d	0.999 ^e	0.998 ^f			
Sperm count (Mill/mL)	43.62	16.87	19.17	10.44	25.49	17.69	23.65	15.97	< 0.001
Post Hoc	< 0.001 ^a	< 0.001 ^b	< 0.001 ^c	0.155 ^d	0.470 ^e	0.931 ^f			
Sperm total motility (%)	57.40	17.05	34.38	15.16	43.51	19.45	39.69	21.67	< 0.001
Post Hoc	< 0.001 ^a	0.001 ^b	< 0.001 ^c	0.055 ^d	0.474 ^e	0.719 ^f			
Sperm active progressive motility (%)	26.56	12.94	10.69	8.48	14.08	10.28	14.11	10.82	< 0.001
Post Hoc	< 0.001 ^a	< 0.001 ^b	< 0.001 ^c	0.362 ^d	0.380 ^e	1.000 ^f			
Sperm slow progressive motility (%)	19.26	8.84	12.92	7.13	16.87	9.34	15.31	8.92	0.004

Post Hoc	0.001 ^a	0.488 ^b	0.105 ^c	0.085 _d	0.506 ^e	0.790 _f			
Sperm non-progressive motility (%)	10.82	4.15	11.33	5.05	12.45	4.47	11.33	4.85	0.308
Post Hoc	0.946 ^a	0.275 ^b	0.948 ^c	0.592 _d	1.000 ^e	0.604 _f			
Sperm Immotile (%)	40.94	18.30	66.00	15.14	55.91	19.47	59.02	21.46	< 0.001
Post Hoc	< 0.001 ^a	< 0.001 ^b	< 0.001 ^c	0.029 _d	0.243 ^e	0.832 _f			
Sperm abnormal form (%)	38.20	11.90	46.08	14.87	45.27	18.77	46.61	18.60	0.155
Post Hoc	0.074 ^a	0.122 ^b	0.054 ^c	0.994 _d	0.998 ^e	0.975 _f			
Number of spermatogenic cells	4.10	3.12	3.33	2.52	3.29	2.41	3.27	2.59	0.311
Post Hoc	0.462 ^a	0.408 ^b	0.406 ^c	1.000 _d	0.999 ^e	1.000 _f			
Number of WBCs	8.32	8.97	7.83	10.39	6.82	7.66	7.71	10.95	0.670
Post Hoc	0.994 ^a	0.852 ^b	0.989 ^c	0.947 _d	1.000 ^e	0.964 _f			
Number of RBCs	2.78	1.92	3.81	4.70	3.11	3.94	3.80	4.81	0.781
Post Hoc	0.569 ^a	0.975 ^b	0.590 ^c	0.805 _d	1.000 ^e	0.820 _f			

The values are expressed as mean \pm SD; SD: A standard deviation; %: Percentage; WBCs; White blood cells; RBCs; Red blood cells; E-cigarette: Electronic cigarettes; P > 0.05, Not significant; P \leq 0.05, Significant.

^a: Cigarette smoking vs Non-smoking; ^b: Shisha smoking vs Non-smoking; ^c: E-cigarette smoking vs Non-smoking; ^d: Cigarette smoking vs. Shisha smoking; ^e: Cigarette smoking vs. E-cigarette smoking; ^f: Shisha smoking vs. E-cigarette smoking.

Table 4. Hormone levels among different smoker groups (n = 206)

Hormones	Non-Smoking (n=50)		Cigarette smoking (n=52)		Hookah smoking (n=55)		E-cigarette smoking (n=49)		P- value among study groups
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Testosterone Level (ng/mL)	6.80	1.48	3.83	1.45	3.92	1.45	3.24	1.48	< 0.001
Post Hoc	< 0.001 ^a	< 0.001 ^b	< 0.001 ^c	0.988 ^d	0.180 ^e	0.085 ^f			
FSH Level (mIU/mL)	8.79	2.70	18.35	8.44	27.81	10.26	20.40	10.84	< 0.001
Post Hoc	< 0.001 ^a	< 0.001 ^b	< 0.001 ^c	< 0.001 ^d	0.638 ^e	< 0.001 ^f			
LH Level (IU/L)	5.15	3.17	14.50	3.83	17.87	5.67	22.18	10.89	< 0.001
Post Hoc	< 0.001 ^a	< 0.001 ^b	< 0.001 ^c	0.041 ^d	< 0.001 ^e	0.005 ^f			

The values are expressed as mean ± SD; SD: A standard deviation; E-cigarette: electronic cigarettes; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; P > 0.05, Not significant; P ≤ 0.05, Significant.

^a: Cigarette smoking vs Non-smoking; ^b: Shisha smoking vs Non-smoking; ^c: E-cigarette smoking vs Non-smoking; ^d: Cigarette smoking vs. Shisha smoking; ^e: Cigarette smoking vs. E-cigarette smoking; ^f: Shisha smoking vs. E-cigarette smoking.