Effect of smoking on sex hormones, inflammatory markers and semen parameters in infertile men

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Abstract:

Background

Infertility is a common social and health problem. Male factor is a major cause in 30% of infertile couples. Smoking is common at reproductive age and its effect on fertilitiy is still in controversy. Studying its effect on fertility helps in the management of infertility.

Objectives

To assess the effect of smoking on serum sex steroidal hormones as well as on seminal plasma (SP) IL-2, IL-6 and conventional semen parameters in infertile men and their correlations between each other.

Methods

Seventy male partners of infertile couples (30 smokers and 40 non-smokers) were involved in this study. The man was considered smoker if he had history of smoking of more than 10 cigarettes per day for at least one year. Semen analysis was done according to world health organization (WHO) 2010. Specific kits were used for measurement of serum testosterone, serum estradiol II, SP IL-2, and SP IL-6.

Results

The median of SP IL-2 was significantly ($p < 0.05$) higher in smokers than in non-smokers group and positively correlated with SP IL-6 in them. In addition, IL-2 was negatively correlated with semen volume, sperm motility and morphology, while IL-6 was negatively correlated only with sperm motility. However, there was no significant difference of serum sex hormones level or on semen parameters between smokers and non-smokers.

Conclusion

Cigarette smoking affect cytokine levels in the seminal plasma but has no effect on serum sex hormones level or semen parameters.

Keywords: Male infertility, smoking, seminal plasma, IL-2, IL-6, testosterone, estradiol II.

Introduction

Infertility is a common problem affecting about 15% of couples, The male factor, in the form of defective sperm quality, is a major cause in 30% of infertile couples (1).
Smoking is still a major health problem especially in reproductive age of males and females (2). Many literatures support the hypothesis that a significant correlation exists between tobacco smoking and altered reproductive physiology (3).
Testosterone (T) is a steroidogenic hormone produced by Leydig cells under control of luteinizing hormone (LH) from anterior pituitary gland (4). Testosterone is essential to maintain spermatogenesis and male fertility; it increases germ cell attachment to Sertoli cells and permits the release of mature sperms. In the absence of testosterone, spermatogenesis does not proceed beyond the meiosis stage. After withdrawal of testosterone, germ cells that have progressed beyond meiosis detach from supporting Sertoli cells and die, whereas mature sperm cannot be released from Sertoli cells resulting in infertility (5).
Over 80% of the 17β -estradiol (E2) in the plasma of adult men is produced by extragonadal and extraadrenal arom. atization of circulating testosterone and androstenedione by the enzyme aromatase particularly in the adipose tissue. The remainder (20%) comes from Leydig cells (6). Some

17β -estradiol is also produced by aromatization of androgens in testicular germ cells, sperms and Sertoli cells (7).

It is observed that estrogen content of the fluid in the rete testis is high, and the walls of the rete testis contain numerous estrogen receptors (ERα). In this region, fluid is reabsorbed and the spermatozoa are concentrated. If this does not occur, the sperm entering the epididymis are diluted in a large volume of fluid, and infertility results (8). Estradiol also has a role in spermatogonial stem cell renewal so it is considered as an indispensable "male hormone" in the early spermatogenetic cycle (9).

Most clinical trials with aromatase inhibitors have resulted in a tendency to improve seminal parameters through suppression of estrogen-testosterone ratio, with an associated increase of FSH (9). Additionally, estradiol and testosterone also inhibit LH in a negative feedback loop (10).

Several lines of evidence indicate that cytokines are involved in male infertility; they are secreted by different parts of the male genital tract and may exert effects on the steroidogenesis, spermatogenesis and sperm function (11).

Interleukin 2 (IL-2) "a glycoprotein with a molecular weight of 15, 400" is one of the major cytokines that exerts numerous immunological effects by stimulating the proliferation and growth of **T, B** and natural killer (NK). Moreover, almost any cell possessing IL-2 receptor will be stimulated to grow by IL-2 (12).

IL-6 is a 26 kD protein produced by a number of cell types; including Sertoli cells (13). IL-6 is expected to cooperate with IL-1α to strengthen the local inflammatory reaction directly or through affecting some immune cells response in testis during the early infection (14)

Naz and Kaplan (15) showed that IL-6 was present in significantly higher levels in seminal plasma of infertile and immnunoinfertile men compared to those of fertile men, and showed significant negative correlation with sperm number and motility.

Objectives of the study:

To assess the effect of smoking on serum sex steroidal hormones as well as seminal plasma (SP) inflammatory markers (IL-2, IL-6), and conventional semen parameters in infertile men and their correlations with each other.

Materials and methods

Subjects

Seventy male partners of infertile couples attending to High Institute for Infertility Diagnosis and Assisted Technologies for Reproduction were involved in this study. The couples had no history of pregnancy or abortion for more than one year from marriage with regular unprotected sex. Their age range was (18-58 years with a mean of 32.5±7.81 years). The smokers involved in this study were having a history of smoking of at least 10 cigarettes per day for at least one year. All subjects included in this study had no history of recent or previous medical history of chronic disease.

Two ml of venous blood were drawn from anticubital fossa for the assay of serum testosterone and estradiol II using the following kits.

VIDAS testosterone kit (Ref. 30 418, BioMérieux SA, France), which is an automated quantitative test for use on the VIDAS instruments for the enzyme immunoassay measure of total testosterone in human serum (lithium heparinate), using the ELFA (Enzyme Linked Fluorescent Assay) technique.

VIDAS® estradiol II kit (Ref. 30 431, BioMérieux SA, France), which is an automated quantitative test for use on the VIDAS instruments for the enzyme immunoassay measure of total 17β-estradiol in human serum (lithium heparinate), using the ELFA technique (Enzyme Linked Fluorescent Assay).

Semen sample was taken from each subject by masturbation after 2-7 days of abstinence. Conventional semen analysis was done for each sample according to the protocol of WHO (16), after incubation and liquefaction period (30-60 min). From 106 subjects enrolled in the study, only 70 subjects included and divided into two groups (30 smokers) and (40 non-smokers); while those who were azoospermic or with severe oligospermia (sperm concentration less than 5 million sperm/ml) were excluded from the study. Semen plasma was collected after centrifugation of semen for 15 min, 3000 rpm; and then frozen at -20 C°. till analysis of interleukins (2 and 6) was done.

Seminal plasma IL-2 and IL-6 were measured using IL-2 and IL-6 bioAssay TM ELISA kits (US biological, Catalog No.17663-28E and 18428-04) respectively. The kits were stored in refrigerator at 4 C° until time of use.

Statistical analysis:

Data were analyzed using Microsoft Excel 2010 and SPSS Version 18. The results were presented as mean ± standard deviation (SD). Normally distributed data were analyzed using unpaired student t-test while abnormally distributed data (skewed) were statistically analyzed using Mann-Whitney U test. Pearson correlation coefficient was used for correlations. A value of $p < 0.05$ was considered to be significant.

Results

Table (1) reveals that there was no significant difference in age, body mass index (BMI) and sex steroidal hormones between smokers and non-smokers.

The median of seminal plasma IL-2 was significantly ($p < 0.05$) higher in smokers than in non-smokers group; while there was no significant difference between both groups in seminal plasma IL-6 values (table 2).

Table (1): Age, BMI, testosterone and E2 of smokers and non-smokers infertile

Parameters	Smokers (n=30) mean±SD	Non-Smokers (n=40) mean±SD	P value
Age (yr)	33.27±8.22	31.93±7.55	0.4869
BMI (kg/m^2)	27.24±4.12	28.78±7.17	0.2624
Testosterone (ng/ml)	5.57±2.88	5.67±3.22	0.8906
E2 (pg/ml)	37.82±22.57	39.65±21.4	0.7327
Testosterone/E2 ratio	168.46±90.14	151.51±67.44	0.3915

BMI: Body mass index, E2: Estradiol II, p value for student t-test

Table (2): Inflammatory markers (IL-2 and IL-6) in infertile smokers and non-

Parameters	Smokers (n=30)		Non-Smokers (n=40)		P value Median
	Range	median	Range	median	
IL-2 (pg/ml)	0.0-282.0	177.0	0.0-241.0	132.5	0.02*
IL-6 (pg/ml)	0.0-291.5	5.5	0.0-267.0	2.0	0.967

* Significant (p value <0.05), p value for Mann-Whitney U test

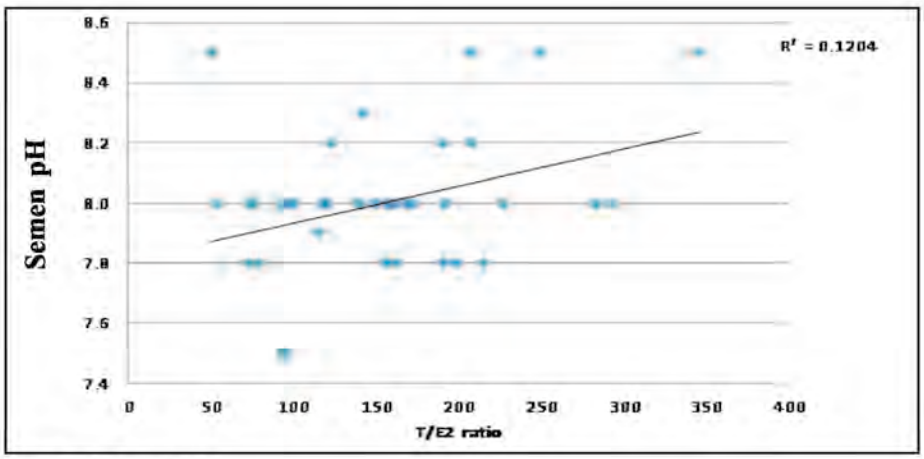
The results showed that there was no significant difference between all semen analysis parameters (volume, concentration, motility, morphology, agglutination and round cells) between smokers and non-smokers groups (table 3).

Table (3): Semen parameters of infertile smokers and non-smokers men

Parameters	Smokers (n=30) mean±SD	Non-Smokers (n=40) mean±SD	P value
Volume (ml)	2.25±0.82	2.09±0.78	0.4108
pH	8.01±0.13	8.0±0.24	0.7222
Concentration (ml)	39.07±26.29	48.8±30.83	0.1595
Progressive motility %	33.53±17.83	31.28±16.58	0.593
Non-progressive motility %	21.0±12.63	20.88±9.38	0.9638
Immotile %	45.47±23.65	47.88±20.97	0.6599
Total sperm count/ejaculate	83.94±65.34	104.24±83.66	0.2586
Normal morphology %	1.27±4.38	1.2±3.02	0.9432
Agglutination %	24.23±14.29	22.73±14.4	0.6647
Round cell (cell/HPF)	9.13±7.77	9.35±7.26	0.9059

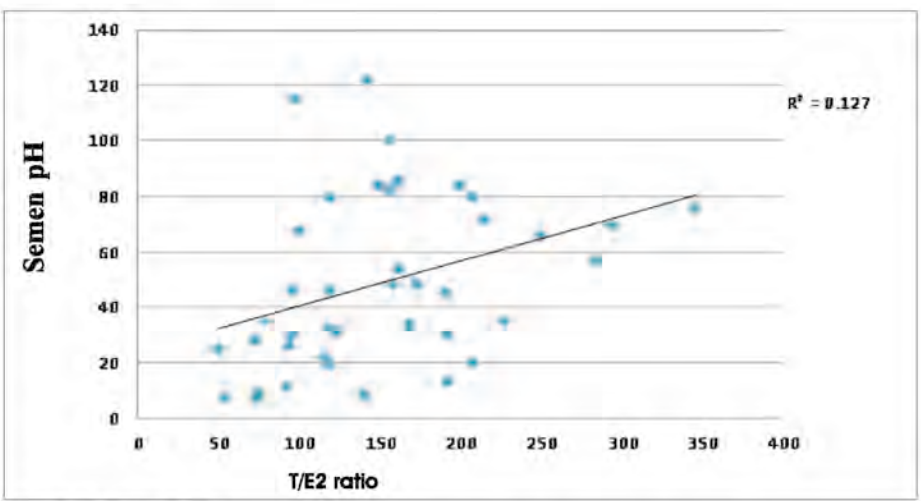
p value for student t-test

Figures (1 and 2) show significant positive correlation between T/E2 ratio with both semen pH and sperm concentration in non-smokers group (r=0.347, 0.356, p= 0.028, 0.024 respectively) but this correlation was non-significant in smokers group.



r=0.347, p=0.028

Figure (1): Correlation between testosterone/estradiol ratio and sperm pH Non-smokers group



r = 0.356, p = 0.024

Figure (2): Correlation between testosterone/ estradiol ratio and sperm concentration in non-smokers group

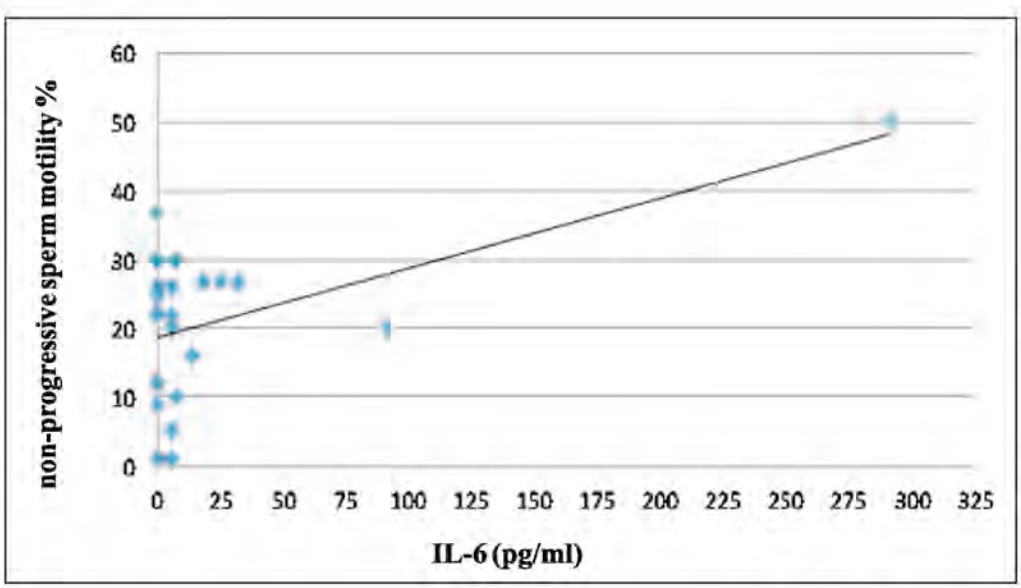
Table (4) shows positive significant ($p < 0.05$) correlation between SP IL-2 and SP IL-6 in smokers group while this correlation was non-significant in non-smokers group. On the other hand, a significant negative correlations was found between SP IL-2 with semen volume, progressive motility percentage and normal morphology percentage (r = -0.392, -0.58 6, -0.714, p = 0.024, < 0.001, < 0.001 and a significant positive correlation with immotile sperm percentage (r=0.584, p<0.001).

Seminal IL-6 was only significantly ($p < 0.01$) positively correlated with non-progressive motile sperm percentage in smokers group as shown in figure (3), while non-significantly correlated with hormonal or semen parameters.

Table (4): Correlation of SP IL-2 with different parameters in the smokers group and non-smokers group

Parameters	SP IL-2			
	Smokers		Non-Smokers	
	R	P	R	P
SP IL-6	0.471	0.031*	0.314	0.075
Semen volume	0.051	0.826	0.392	0.024*
Semen pH	0.128	0.58	-0.107	0.552
Sperm Conc.	-0.428	0.053	0.315	0.074
PR %	-0.205	0.373	-0.586	<0.001**
NP %	-0.009	0.968	0.308	0.081
IM %	0.14	0.544	0.584	<0.001**
Total sperm count /ejaculate	-0.541	0.011*	0.438	0.011*
Morphology %	-0.384	0.086	-0.714	<0.001**
Agglutination %	-0.469	0.032*	0.126	0.486
Round cells %	0.193	0.401	0.21	0.24

r: correlation coefficient, p: level of significance, PR: progressive motility, NP: non-progressive motility, IM: immotile sperms.
* p value < 0.05.
** p value < 0.001.



r = 0.551, p = 0.01

Figure (3): Correlation between seminal plasma IL-6 with non-progressive sperm motility percentage in smokers group

Discussion

The age and BMI of smokers and non-smokers were matched, and this excludes their effect on hormonal, interleukins, and semen parameters. The total testosterone level and E2 in serum were higher in non-smokers than in smokers but the difference was non-significant, while the T/E2 ratio was higher in smokers than in non-smokers but also this difference was non-significant. This agrees with Yardimci *et al* (17), who found significant decrease in total serum testosterone in smokers. The possible mechanism for that was that smoking, over time, cause degeneration of Leydig cells, which in turn reduces testosterone production. In contrast, English et al study showed a significantly higher total testosterone in smokers than in non-smokers (18). This may be explained as the study was done on healthy subjects while in our study was on

infertile men. Another assumption is that man with high testosterone level may become addict to cigarette smoking.The hormones per se in this study had no significant correlation with semen parameters or with seminal plasma interleukins. But the T/E2 ratio was positively correlated with semen pH and sperm concentration in non-smokers but not in smokers and this agrees with Cakana et al (19). This correlation was non-significant in smokers which may be due to smoking effect on the T/E2 ratio in the testis and their role in the spermatogenesis.For semen parameters, the study showed no significant difference between smokers and non-smokers, these results were in the same line with the results of (20,21,22,23), but disagree with (24) who found that cigarette smoking was associated with a significant decrease in total sperm count (-17.5%), total number of motile sperm (-16.6%), significant reduction in the percentage of normal forms and sperm vitality, but ejaculate volume were slightly but non-significantly affected. This could be due to high number of subjects involved in their study compared with the number in this study.

On the other hand,colladel *et al* (25) found that semen quality in infertile men seems not to be dramatically affected by cigarette consumption, only heavy smokers show significantly lower sperm concentration.The exact pathophysiology underlying cigarette smoking and sperm deteriorating is unclear. Possible mechanisms include the effect of cigarette smoke on function of Sertoli and Leydig cell and testicular microcirculation (23).The relationship between seminal plasma cytokine level and semen quality is still in controversy. It has been found (11,26,27,28) that there is no relationship, while the recent studies have supported such a relationship (29,30). The result of other study (31) also found that seminal plasma IL-6 had no correlation with semen parameters.

Over production of the pleiotropic cytokine IL-6 was occurred in many inflammatory conditions (32), and according to matalliotakes *et al* (33), the prostate appears to be the main site of origin of IL-6 in the semen.In this study, the seminal plasma IL-2 was significantly higher in smokers than non-smokers while IL-6 was higher but non-significant. IL-2 was positively correlated with IL-6 (as both are inflammatory markers); these findings may suggest a mild inflammatory conditio n in genital tract due to the effect of toxic substances of tobacco smoking. Gruschwitz *et al* (34) mentioned that cytokine content in the seminal plasma represents a better diagnostic parameter for urogenital infections than the presence of inflammatory cells. Moreover, politchal (35) reported that IL-2 is found only in low concentration in seminal plasma in small No. of healthy fertile subjects, while IL-6 found in small concentration in large No. of healthy fertile subjects. These findings agree with our explanation of an inflammatory process found in the genital tract of infertile patients especially the smokers. On the other hand, naz and kapiny ulcova-Gallova *et al* (15) and (36) found that seminal plasma IL-6 is significantly higher in the infertile men than fertile men. Seminal plasma IL-2 was negatively correlated with total sperm count per ejaculate and with agglutination percentage in smokers group which agrees with (37), while seminal plasma IL-6 was only positively correlated with non-progressive motility in smokers. So it is clear that cytokine affected semen quality in smokers. These findings agree with (38) and disagree with (39) who found no correlation between IL-6 with any of the semen parameters of subfertile patients except with WBC which were not measured in this study.

In the present study, these correlations in smokers group may be explained that toxic substances found in cigarette may affect cytokine level in semen and their relation with semen parameters. From this study, it can be concluded that cigarette smoking and the toxic materials in the tobacco affect cytokine levels in the seminal plasma and in turn affect motility of the sperms, thus has an effect on spermatogenesis and cross-talk between cells in the genital tract. However, the present study showed no effect of smoking on sex hormones level.

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