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Influence of Cigarette Smoking on Seminal Plasma Soluble Fas as a Marker of Germ Cell Apoptosis

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Abstract

Background Male infertility constitutes about 50% of overall causes of infertility and apoptosis is known to have an essential role in the control of germ cell number in testis. Cigarette smoking is common in males at reproductive age. Studying the influence of smoking on apoptosis in male genital tract and its influence on fertility helps in the management of infertility in smokers.

Objectives To assess the influence of cigarette smoking on seminal plasma (SP) soluble Fas (sFas) and the correlation between SP sFas and serum sex steroidal hormones and conventional semen parameters in males of infertile couples.

Methods Seventy male partners of infertile couples (30 smokers and 40 non-smokers) were enrolled in this study. A subject was considered smoker if he had history of smoking of more 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 the measurement of SP sFas and serum testosterone and estradiol (E2).

Results In smokers' group, SP sFas was significantly ($p < 0.05$) negatively correlated with age and sperm motility and positively correlated with immotile sperm and round cell number.

Conclusion Germ cell apoptosis in smoker males of infertile couples is interrelated with sperm motility.

Keywords Male infertility, Smoking, Apoptosis, Seminal plasma sFas.

Introduction

Male infertility factor is identified in almost 50% of infertile couples while it is the sole cause in 20-30% of infertile couples; and a comprehensive male infertility evaluation is the goal to optimize a man's reproductive potential while maximizing his overall health [(1)].

Cigarette smoking is a widely recognized health problem and the highest prevalence of smoking observed in young adult males during their reproductive period [(2)]. Many literatures support the hypothesis that a significant correlation exists between tobacco smoking and altered reproductive physiology [(3)].

Apoptosis is an active, gene-directed cellular self-destruction which may occur in both physiologic and pathologic conditions [(4)]. It is thought to be one of the important factors in regulating the production of spermatozoa [(5)]. One factor implicated in sperm apoptosis is the cell surface protein, fibroblast associated (Fas). The interaction between Fas (CD95/Apo-1; a type I transmembrane glycoprotein receptor) and a cellular death inducing ligand (a type II transmemberaneglycoprotein; FasL) plays an important role in triggering the apoptotic pathway. Both Fas and FasL exist as membrane bound and soluble forms [(6)].

Human sFas is a 26-35 kD glycoprotein formed from cleavage of the specific extracellular region of FasL by the Matrilysin (protease enzyme) [7]. Soluble FasL is not as efficient as membrane-bound FasL in executing apoptosis [8,9] and can be antiapoptotic in some circumstances [10,11]. Previous reports have suggested that the Fas mediated system is implicated in the elimination of defective spermatozoa from the ejaculate and shows possible irregularities that could account for certain forms of male infertility [12].

Testosterone hormone is obligatory for spermatogenesis and the proper functioning of Sertoli cells [13]. It increases germ cell attachment to Sertoli cells and permit 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 [14].

About 80% of the 17β-estradiol (E2) in the plasma of adult men is produced by extragonadal and extraadrenal aromatization of circulating testosterone and androstenedione by the enzyme aromatase particularly in the adipose tissue. The remainder (20%) comes from Leydig cells [15]. Some E2 is also produced by aromatization of androgens in testicular germ cells, sperms and Sertoli cells [16].

Estrogen is considered as an indispensable "male hormone" in the early spermatogenetic cycle as spermatogonial stem cell renewal is promoted by estradiol implantation and many 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 follicular stimulating hormone (FSH) [17]. Additionally, estradiol and testosterone also inhibit luteinizing hormone (LH) in a negative feedback loop [18].

The aim of the present study was to evaluate the influence of cigarette smoking on the SP sFas levels in infertile males, and the correlation between SP sFas with serum testosterone and E2, and conventional seminal parameters in those subjects.

Methods

This study was done on seventy male partners of infertile couples attending to the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University. The couples had no history of pregnancy or abortion for more than one year from marriage with regular unprotected sexual intercourse and had no recent history or previous medical history of chronic disease. 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.

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 World Health Organization (WHO, 2010) [19], after incubation and liquefaction period (30-60 min). From 106 subjects enrolled primarily 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 oligozoospermia (sperm concentration less than 5 million sperm/ml) were excluded. Seminal plasma was collected after centrifugation of semen for 15 min. at 3000 rpm; and then frozen at -20^{o}C till analysis of sFas was done.

Seminal plasma sFas was measured using the kit of IBL International GMBH sAPO-1/FAS ELISA Enzyme immunoassay for the quantitative determination of human sAPO-1/Fas in human cell culture supernatants, serum, plasma or other body fluids ref. BE51901 (Germany).

Two ml of venous blood were drawn from anticubital fossa for the assay of serum testosterone and estradiol using the following kits: Serum total testosterone and E2 were assayed using {VIDAS® testosterone kit (Ref. 30 418, BioMérieux® SA, France), and VIDAS® estradiol II kit (Ref. 30 431, BioMérieux® SA, France)}, which are automated quantitative tests

for use on the VIDAS instruments for the enzyme immunoassay measure of total testosterone and E2 in human serum or plasma (lithium heparinate), using the ELFA (Enzyme Linked Fluorescent Assay)technique.

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 shows that there was no significant difference of age, body mass index (BMI) and serum sex steroidal hormones between smokers and non-smokers.

Table 1. Comparison between smokers and non-smokers infertile men parameters by unpaired t-test

Parameter	Infertile men		*P* value
	Smokers N = 30	Non-Smokers N = 40	
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 (T) (ng/ml)	5.57±2.88	5.67±3.22	0.8906
Estradiol (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

This study revealed that there was no significant difference in median of SP sFas between smokers and non-smokers infertile men ($P > 0.05$), table (2).

Table 2. Comparison of seminal plasma sFas between smokers and non-smokers groups by Mann-Whitney U test

Parameter	Smokers N = 21		Non-Smokers N = 36		*P* value
	Range	median	Range	median	
sFas (pg/ml)	0.0-298.0	32.0	0.0-712.0	4.0	0.653

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

The age was significantly negatively correlated with SP sFas in smokers group (r = -0.474, $P = 0.03$) as shown in figure 1.

Figures 2 and 3 shows significant negative correlation between seminal sFas and progressive and non-progressive sperm motility percentage (r = -0.473, -0.448, P = 0.03, 0.042 respectively) and significant positive correlation with immotile sperm percentage and round cell no. in semen (r = 0.537, 0.712, P = 0.012, <0.001 respectively) in smokers group (figures 4 and 5), while these correlations were insignificant in non-smokers group.

Table 3. Comparison of semen parameters between smokers and non-smokers groups by unpaired t-test

Parameters	Infertile men		*P* value
	Smokers N = 30	Non-Smokers N = 40	
Semen Volume (ml)	2.25±0.82	2.09±0.78	0.4108
Semen pH	8.01±0.13	8.0±0.24	0.7222
Sperms Concentration (ml)	39.07±26.29	48.8±30.83	0.1595
Sperms Progressive motility (%)	33.53±17.83	31.28±16.58	0.593
Sperms Non-progressive motility (%)	21.0±12.63	20.88±9.38	0.9638
Immotile Sperms (%)	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 Sperms Morphology (%)	24.23±14.29	22.73±14.4	0.6647
Sperms Agglutination (%)	1.27±4.38	1.2±3.02	0.9432
Round cell count/HPF	9.13±7.77	9.35±7.26	0.9059

HPF= high power field

Discussion

In this study, there was no significant difference in median of SP sFas between smokers and non-smokers males of infertile couples ($P > 0.05$); however, the SP sFas was negatively correlated with age of smokers; this may be explained as cigarette smoking may enhance apoptosis process in the testis and genital tract with age [7] as sFas is considered antiapototic factor and the decrease in sFas level indicates increased apoptosis as mentioned above [10,11].

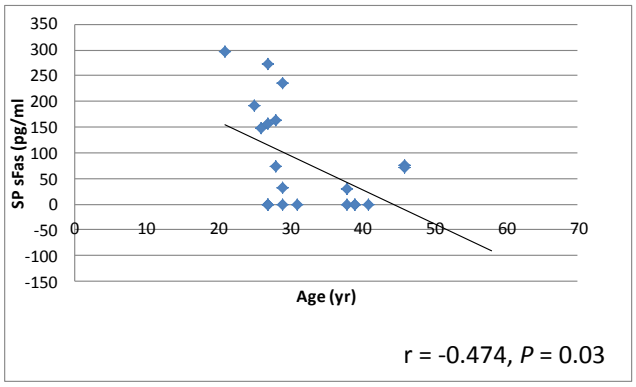

Figure 1. Correlation between age and seminal plasma sFas in smokers group

This study also showed that SP sFas was significantly negatively correlated with sperm motility and positively correlated with immotile sperms. This result disagree with Zedan et al [20], but it is go with Chen et al [21] who found inverse associations between percent apoptosis in ejaculated human semen and sperm motility, progressive motility, and morphology. It could be explained that increased antiapoptotic factor (sFas) and decrease apoptosis would lead to overcrowding of growing sperms and decrease space within seminiferous tubules and this causes increase percentage of defected sperms in the semen and increase immotile sperms and round cells (which are mainly immature germ cells) [22].

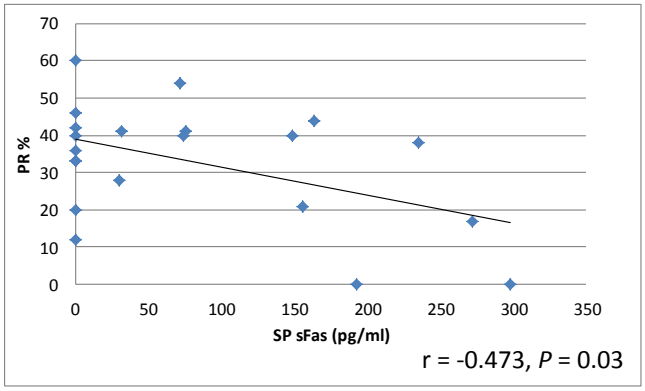

Figure 2. Correlation between seminal plasma sFas and sperm progressive motility percentage in smokers group

The hormones per se in this study had no significant correlation with semen parameters or with sFas. However, the total testosterone level and E2 in serum were higher in non-smokers than in smokers although the difference was non-significant, while the T/E2 ratio was higher

(but non-significant) in smokers than in non-smokers. These results go with Yardimci et al [23] who found significant decrease in total serum testosterone in smokers.

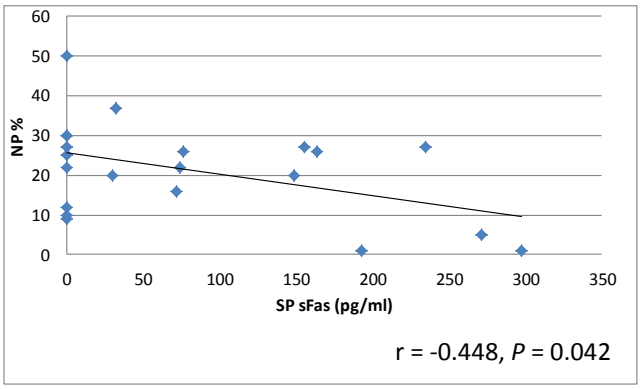

Figure 3. Correlation between seminal plasma sFas and sperms non-progressive motility percentage in smokers group

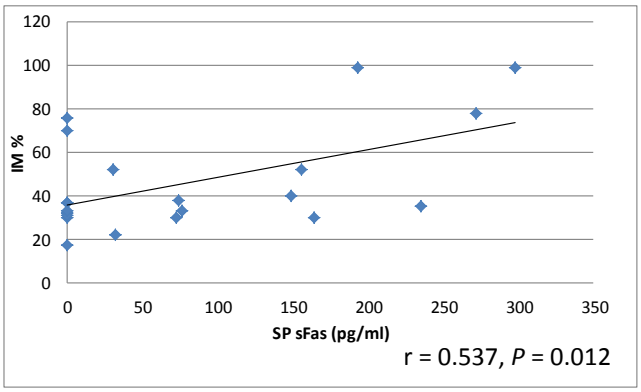

Figure 4. Correlation between seminal plasma sFas and immotile sperms percentage in smokers group

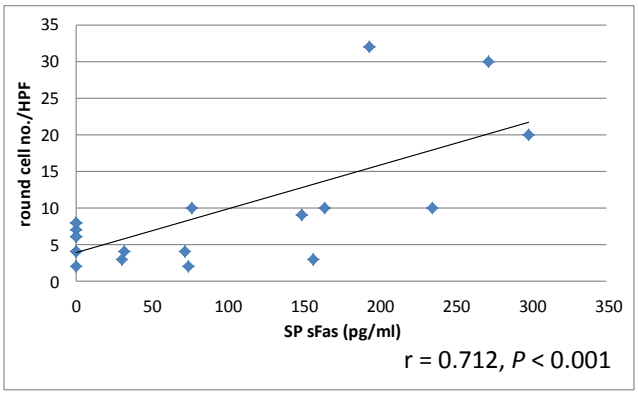

Figure 5. Correlation between seminal plasma sFas and round cell number in smokers group

The possible mechanism for these results, the smoking, over time, may cause degeneration of Leydig cells, which in turn reduce testosterone production. In contrast, English *et al* study showed a significantly higher total testosterone in smokers than non-smokers [24]. This maybe explained as the study was done on healthy subjects while in our study was on males of infertile couples. Another assumption is that man with high testosterone level may become addict to cigarette smoking. Regarding E2, Trummer et al (25) also had found no significant different of E2 level between smokers and non-smokers.

For semen parameters, the study showed no significant difference between smokers and non-smokers, these results were similar to the results of several other authors [26-29], but disagree with Künzle et al [30] 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 low number in this study. On the other hand, Collodel et al [31] found that semen quality in infertile males seems not to be dramatically affected by cigarette smoking, only heavy smokers show significantly lower sperm concentration. The exact pathophysiology underlying cigarette smoking and sperm deterioration is unclear. Possible mechanisms include the influence of cigarette smoke on the function of Sertoli and Leydig cells and testicular microcirculation [30].

In conclusion, Germ cell apoptosis in smoker males of infertile couples is interrelated with sperm motility.

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