Seminal plasma oxidant–antioxidant imbalance is a dominant feature of primary idiopathic infertile male smokers

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Abstract: ***Background:*** Although, reactive oxygen species may induce defective semen quality, some authors denied such association. ***Objectives:*** To assess seminal plasma oxidant – antioxidant status in tobacco smoking men. ***Patients and methods:*** Semen samples were obtained from: (a) 30 tobacco smoker married men and (b) 30 strict non-smoker married men. Half of each group was primary infertile. Their semen samples presented nonleuko and nonhemo-cytospermia. After liquefaction, semen samples were analyzed for: (i) convential semen parameters by a computer assisted semen analyzer. ii) Seminal plasma oxidant-antioxidant status including: the lipid peroxidation (LPO) index [malondialdehyde (MDA)], and various antioxidants [α tocopherol, vitamin C, superoxide dismutase (SOD) and reduced glutathione (GSH)]. ***Results:*** In seminal plasma, mean MDA concentration was significantly higher in fertile or infertile tobacco smokers than the corresponding of the non-smokers ($p<0.001$) but more marked in the infertile cases. Enhanced LPO of sperm plasma membranes induced decrease of sperm motility and viability %. Whereas, the antioxidants α tocopherol, ascorbic acid, SOD and GSH concentrations in seminal plasma were significantly decreased in infertile or fertile smokers than the corresponding in the nonsmokers ($P<0.01$) but more in the infertile cases. There were significant negative correlations of basic semen parameters in cigarette smokers with the seminal plasma MDA concentrations and significant positive correlations between sperm motility and viability% and different seminal plasma antioxidant levels. ***Conclusion:*** Insufficient scavenging antioxidants in seminal plasma of chronic heavy smoking men could underly the deleterious spermatozoal quality and function defects and consequently male infertility.

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Introduction:

Human semen has a complex set of antioxidants (micronutrients, vitamins and enzymes) that prevent oxidative damage of the life-saving sperm components such as plasma cell membrane and nuclear DNA. In general, oxidants play a major role in human infertility while, antioxidants either prevent reactive oxygen species (ROS) from being formed or remove them before they can damage vital components [1-3]. Although ROS are produced in small physiological amounts, they are functionally important in driving tyrosine phosphorylation cascades associated with sperm production and function. However, when ROS spermatozoal production exceeds the antioxidant defense, a state of peroxidative damage of sperm plasma membrane and breakage of DNA strand are induced. Such oxidative stress not only disrupts the fertilizing potential of human spermatozoa but also the ability of these cells to create a normal healthy embryo due to sperm deformity [2,3]. However, in many cases, it is unclear if oxidants trigger testicular tissue damage or are produced as a consequence of the disease.

Although oxidative stress is a dominant feature in seminal plasma and sperm nuclei of primary idiopathic infertile male smokers, the underlying causative mechanism (s) remains unsolved. Alkaloids (nitrosamine, nicotine, cotinine and hydroxy-cotinine) present in cigarette smoke can initiate ROS production [4]. Cigarette smoking can induce semen quality defects which compromise the chance of pregnancy[6] by lowering antioxidant protective and increasing ROS damaging effects on chromatin structure and DNA

integrity of human sperms[7,8]. Blood plasma total antioxidant capacity was significantly lower, whereas plasma total peroxides level and oxidative stress index were significantly higher in passive smokers[5]. Cigarette smoking can induce semen quality defects which compromise the chance of pregnancy [6]. Alternatively, cigarette smoking cessation was followed by a marked increase in blood antioxidant concentrations and substantially improved resistance towards oxidative challenge [9]. However, some studies denied significant association between smoking and sperm quality-function or nuclear chromatin-DNA defects [10].

The present study assessed seminal plasma oxidant-antioxidant status in tobacco smoking men with primary idiopathic infertility.

Subjects and Methods:

The investigated subjects in this study were randomly withdrawn from the Outpatient Clinics of Fertility, Andrology and Endocrinology, Mansoura University Hospitals, Egypt. They included:

(a) Thirty tobacco smoking men: All of them were married for more than five years. However, only one half of them had children (Table 1). Each man smoked from 15 to 35 cigarettes per day for > 10 years. Beside, they smoked also other forms of tobacco as goza and/or shisha but without Hashish (cannabis).

(b) Thirty strict tobacco non-smoking men: Of them, one half was primarily infertile after ≥ five years marriage with unprotected intercourse (Table 1).

All investigated men were matched in age and body mass index (BMI) (Table 1).

The participants had clinically normal epididymis and ductus deferens. None of them gave a past history of genital infection or trauma; chronic systemic disease (hepatorenal, cardiovascular and musculoskeletal, anemia or fever); long-term medications therapy (methotrexate, colchicine, cimetidine, spironolactone) or chronic exposure to chemicals. The inclusion criteria included primary idiopathic infertility since marriage (≥ 5 years) commonly associated with signs of androgen deficiency (increased body fat, decreased facial and body hair and muscle mass together with small testes). Almost all of them had pulmonary manifestations of chronic smoking. On the other hand, the included fertile men had fathered two or more children, after which their wives used suitable contraceptive measure(s).

All maternal partners of these men (60 women) had no inducing factor for infertility (pelvic, genital, endocrinal and inflammatory diseases or chronic medications intake) and had normal menstrual and secondary sex characters (no hirsutism, acne or clitoromegally) as well as normal reproductive investigations for ovulation (normal basal body temperature charting) and ovarian, tubal and uterine clinical status (transvaginal ultrasonography, endometrial biopsy and uterosalpingography).

Informed consents were taken from all male and female partners.

Semen samples were collected from all male participants by masturbation after an abstinence period of 3–4 days. After liquifaction (within 1.0 hour from collection), each semen sample was subjected for analysis [11] by a computer assisted semen analyzer (Weili Color Sperm Analysis System: ALJY-9000, China).

Then within 4 hours from collection, plasma of each semen sample was separated after whole semen centrifugation at 5000/rpm for 10 minutes and frozen at -70ºC till being subjected to the following biochemical determinations:

a) Pituitary-testicular hormones: LH and FSH (uIU/ml) [12] and testosterone (ng/dl) [13] by solid phase, 2 site chemiluminescent immunometric assay. [IMMULITE 1000: Diagnostic Products Corporation (DPC), corporate offices: 5210 pacific concourse drive, Los Angeles, CA 90045 – 6900, USA].

b) Oxidant-antioxident status which included:

- Lipid peroxidation (LPO) index [malondialdehyde (MDA) nmol/ml] [14].
- Superoxide dismutase (SOD) activity (U/l) [15].
- Reduced glutathione, GSH activity (nmol/l) [16].
- Alpha tocopherol activity (umol/l) [17].
- Ascorbic acid activity (umol/l) [18].

Statistical analysis:

The Statistical Package for Social Scientists (SPSS) for windows program Version 11 (SPSS Inc., Chicago, USA) was used for all statistical calculations. Results were expressed as mean ±SD. Comparison of two mean values were done by analysis of variance with t-test. The statistical significance between mean values of two groups (unpaired data) was calculated by Mann-Whitney u test. The statistical significance of a difference between mean values of a single group (paired data) was calculated by Wilcoxon Sigand Ranks test. Correlation between two variables was done using Spearman correlation coefficient (r). The level of significance was read at the probability value $p<0.05$.

Results:

Table (1) lists some demographic and clinical data of the whole studied men. Their mean age (±SD) was 37.5±4.0 years, while their BMI was 26.4±2.8 kg/m^2. Of

these men 36 had BMI within the normal range (< 27 kg/m^2) and 24 men were overweight (BMI ≥ 27 kg/m^2). 30 men were cigarette smokers, one half of them were fertile, and each had ≥ 2.0 children. The other half was absolutely primary infertile since marriage (more than 5 years). Twenty two of the smokers were heavy cigarette smokers (> 30 cigarettes/day) while the remaining were current smokers (≤20 cigarettes/day) besides smoking other forms of tobacco.

Significant difference between fertile and infertile (smokers or nonsmokers) in some semen variables was noted (Table 2). Significant decrease of sperm motility% and viability% was found in the two infertile subgroups as compared with the corresponding data of the fertile subgroups. However, the nonsmoker fertile men showed more favorable seminal features than the corresponding smoker subgroups.

One case (3.3%) of the fertile smokers had subnormal sperm concentration (10^6/ml) and motility (36%), the remaining 29 men had normal semen pictures. On the other hand, 70% of the whole infertile series (21 individuals) had abnormal two or more convential sperm analytical parameters according to WHO criteria [(11)]. Each of the remaining 9 semen samples had only one anomaly.

Table 3: shows pituitary-testicular hormonal (FSH, LH and total testosterone) levels in semen plasma of the different investigated subgroups. Significant decrease of total testosterone and increase of FSH and LH concentrations in semen plasma were found in the infertile smokers in comparison to the corresponding data in the fertile nonsmokers.

Seminal plasma oxidant-antioxidant imbalance (high MDA and low antioxidants concentrations) was observed in 24 (80%) of the infertile men (nonsmokers or smokers). Seminal plasma oxidant-antioxidant data of the smokers (fertile or infertile) showed significant difference in comparison to the corresponding levels of the nonsmokers.

The seminal plasma oxidation byproduct malondialdehyde mean concentration (Table 4) was significantly higher in infertile smokers than the corresponding of the infertile non-smokers (P<0.00) and fertile smokers (P<0.01) or nonsmokers (P<0.000). While the different seminal plasma antioxidants: α tocopherol, ascorbic acid, SOD and GSH concentrations were significantly decreased in infertile nonsmokers and smokers than the corresponding fertile subgroup (P<0.001) and in the smokers (fertile or infertile) than the corresponding nonsmokers (p<0.01).

There was significant positive correlation between percent decrease of sperm motility% and vitality% in cigarette smokers and seminal plasma MDA percent increase. Meanwhile, significant negative correlation between percent decrease in sperm motility% and viability% with percent decrease in seminal plasma GSH, SOD, α tocopherol and ascorbic acid was noted.

Table (1): Demographic data (No. or mean values) of the investigated 60 men.

	Variables	No. or X
i)	Age (years)	37.5±4.0
ii)	Body mass index (kg/m^2) in the whole 60 individuals.	26.4±2.8
	▪ Normal BMI (≥22-≤27 kg/m^2)	36 men
	▪ Overweight (BMI ≥27-≤30 kg/m^2)	24 men
iii)	Tobacco smoking (30 individuals: 15 fertile and 15 infertile):	
	▪ Current smokers (≤20cigarrete/day)	8 men
	▪ Heavy smokers (> 30 cigarrete/day)	22 men
iv)	Fathering children:	
	▪ Fertile men (15 nonsmokers and 15 smokers): each of them fathering ≥ 2 children.	
	▪ Infertile men (15 nonsmokers and 15 smokers): Absolute childless.	

X: Mean value

Table (2): Semen parameters (X± SD) of the different investigated subgroups within one hour after ejaculation.

Variables	Nonsmokers		Smokers	
	Fertile (n:15)	Infertile (n:15)	Fertile (n:15)	Infertile (n:15)
Volume (ml)	3.1± 0.71	2.8± 0.95	2.9± 0.63	2.1± 0.54
Viscosity	Normal	more than normal	Normal	less than normal
Count (million/ml)	65.8±16.2*	49.9± 12.9	38.0± 8.3*	32.1± 10.5▲
Progressive motility %	80.3±19.1*	41.6±10.8	62.6±19.9*	33.6±8.0▲
Normal morphology %	61.5±13.5*	31.8±9.2	53.6±14.4*	23.3±9.5▲
Viability %	67.7±16.9*	40.4±9.7	54.4±12.9*	28.6±8.1▲
Round head	17.9± 6.0*	31.5±5.8	23.3±6.3*	40.5± 9.7▲

* Significant difference between fertile smokers and nonsmokers and between infertile smokers and nonsmokers ($p<0.01$, $P<0.001$).
▲ Significant difference between infertile smokers and fertile smokers or nonsmokers ($p<0.01$, $p<0.000$, $p<0.001$).

Table (3): Seminal plasma FSH, LH and total testosterone concentrations in the investigated groups [nonsmokers (fertile or infertile) and smokers (fertile or infertile)].

Variables	Nonsmokers		Smokers	
	Fertile (n:15)	Infertile (n:15)	Fertile (n:15)	Infertile (n:15)
FSH (mIU/ml)	6.1±2.4	7.3±2.2	6.3±2.1	7.8±2.5*
LH (mIU /ml)	4.1±1.2	5.2±1.3	5.4±1.9	6.8±1.9*
Testosterone (ng/dl)	509.2±117.0	378.0±75.9	491.4±97.5	326.6±78.2*

* Significant difference from the nonsmoker fertile group ($p<0.01$).

Table (4): Seminal plasma malonedialdehyde, α tocopherol, ascorbate; suproxide dismutase and glutathione concentrations in the investigated groups.

Variables	Non smokers		Smokers	
	Fertile (n:15)	Infertile (n:15)	Fertile (n:15)	Infertile (n:15)
MDA nmol/ml	3.1± 0.8	5.0±1.1*	5.8± 1.6*	7.9± 1.2**
α tocopherol umol/L	28.7±5.5	20.9±5.2*	19.6±5.0*	13.8±4.1**
Ascorbate umol/L	69.4±10.7	51.3±9.4*	50.3±9.6*	41.6±10.0**
SOD U/L	815.2± 170.5	705.9 ± 111.6*	648.5 ± 123.4*	449.7± 81.2**
GSH nmol/L	67.8± 13.5	56.4± 12.9*	54.2± 13.0*	38.1± 10.5**

* Significant difference from the fertile nonsmoker subgroup ($P<0.001$).
** Significant difference from the nonsmoker (fertile or infertile) subgroups ($p<0.000$) and fertile smoker subgroup ($P<0.01$).

Discussion:

Infertility is the incapacity to fulfill pregnancy after reasonable duration (≥ one year) of regular intercourse without use of any contraceptive measure. The etiology of male infertility is multifactorial. Many environmental, pathophysiological and genetic factors have been implicated in sperm dysfunction and subsequent infertility. However, about 40-50% of primary infertility may not display any objective underlying cause (unexplained or idiopathic infertility). Impaired spermatogenesis and defective sperm function comprise the most prevalent cause of primary idiopathic male infertility, a condition that is difficult to treat [2,3,19,20].

In the present study, significantly abnormal sperm quality and function were found in semen of infertile smoker and nonsmoker subgroups than their corresponding's in the fertile subgroups. A high number of abnormal sperm heads was associated with decreased fertilization capacity in otherwise normal men (Table 2). These anomalies may be attributed to cigarette smoking – induced oxidative stress. A provoked oxidant-antioxidant imbalance can over-whelm the antioxidant defense system and result in tissue oxidative damage. Cigarette smoking induced oxidative stress could affect the physiology of spermatozoa and considered a major factor in the etiology of male primary infertility [21]. Both spermatogenesis [9,22] and testicular steroidogenesis[23] are vulnerable to oxidative stress.

In the present study, infertile paternal smokers had significant pituitary – testicular hormonal dysfunction. The seminal plasma contained significant lower total testosterone level and higher FSH and LH concentrations than those in the nonsmoker subgroups. The pituitary gonadotropic hyperfunction reflected a feed-back reaction to a testicular Leydig cell hypofunction most probably due to oxidative stress (Table 3). Similar hormonal reaction in response to excess ROS was reported in seminal plasma of non idiopathic infertility [24].

The production of small amounts of ROS in semen is a normal physiological process but an imbalance between ROS generation and scavenging commonly associated with male infertility is detrimental to the sperms [25]. Oxidative stress causes adverse effects on sperm plasma membranes and nuclear DNA affecting the quality of spermatozoa and impairing the capacity of fertilization [26]. Thus oxidative stress is considered an independent factor for induction of sperm apoptosis and male idiopathic infertility [2,27].

Spermatozoal membranes are rich in polyunsaturated fatty acids that are sensitive to oxygen – induced lipid peroxidation. This can result in decreased sperm motility and vitality and increased mid-piece sperm morphological defects [20 ,25].

In the present study (Table 4), chronic smoking was associated with significantly higher MDA level in seminal plasma of infertile and fertile participants than the corresponding nonsmoking value. Cigarette smoking is related to increase of oxidant production and antioxidant depletion [5,9,10,22]. Heavy smokers had higher than normal lipid peroxidation products that decreased after smoking abstinence [9,25,28]. In spermatozoa, LPO propagation will lead to accumulation of lipid peroxides in the seminal plasma inducing DNA damage and loss of sperm functions [25,26].

The results of the present study (Table 4) showed that α-tocopherol and ascorbic acid levels were significantly decreased in semen plasma of cigarette smokers whether infertile or fertile compared to the corresponding data of the nonsmokers. Vitamin E (α-tocopherol) is a powerful lipophilic antioxidant that is vital for maintenance of spermatogenesis. It suppresses lipid peroxidation in the testicular microsomes and mitochondria and reverses the detrimental effects of oxidative stress on testicular function [23,29,30]. Also, vitamin C (ascorbic acid) stimulates spermatogenesis by α-tocopherol maintenance in an active state. Deficiencies of vitamins C or E leads to oxidative stress in the testes that disrupts both spermatogenesis and steroidogenesis. Alternatively endogenous ascorbate level is decreased when oxidative stress is induced in the testes by different toxins [31,32]. However, it is not clear whether low plasma and tissue vitamins E and C were a consequence of or a mere association with cigarette smoking in primary idiopathic male infertility.

Seminal plasma SOD protects spermatozoa against O_2 toxicity and LPO through direct deactivation of many ROS [33,34] due to its high intracellular concentration and its central role in maintaining cells redox status. Also glutathione is an important intracellular antioxitdant[34,35]. Seminal plasma SOD and GSH mean concentrations (Table 4) were significantly lower in infertile and fertile smoking men than in the corresponding non-smokers ($P<0.01$).

In the conclusion, the detrimental factor in primary masculine infertility could be an oxidant – antioxidant imbalance due to idiopathic factor(s) as tobacco smoking.

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