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Effect of smoking on seminal plasma ascorbic acid in infertile and fertile males

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Summary

This work aimed to assess the relationship of seminal ascorbic acid levels with smoking in infertile males. One hundred and seventy men were divided into four groups: nonobstructive azoospermia [NOA: smokers ($n = 20$), nonsmokers ($n = 20$)]; oligoasthenozoospermia [smokers ($n = 30$), nonsmokers ($n = 20$)]; asthenozoospermia [smokers ($n = 20$), nonsmokers ($n = 20$)] and normozoospermic fertile men [smokers ($n = 20$), nonsmokers ($n = 20$)]. The patients underwent medical history, clinical examination, conventional semen analysis and estimation of ascorbic acid in the seminal plasma calorimetrically. There was a significant decrease in the mean seminal plasma ascorbic acid levels in smokers versus nonsmokers in all groups (mean ± SD; 6.03 ± 2.18 versus 6.62 ± 1.29, 7.81 ± 1.98 versus 9.44 ± 2.15, 8.09 ± 1.98 versus 9.95 ± 2.03, 11.32 ± 2.15 versus 12.98 ± 12.19 mg dl^{-1} respectively). Fertile subjects, smokers or not, demonstrated significant higher seminal ascorbic acid levels than any infertile group. Seminal plasma ascorbic acid in smokers and nonsmokers was correlated significantly with sperm concentration ($r = 0.59$, 0.60, $P < 0.001$), sperm motility ($r = 0.65$, 0.55, $P < 0.001$) and negatively with sperm abnormal forms per cent ($r = -0.53$, -0.50, $P < 0.001$). Nonsignificant correlations were elicited with semen volume ($r = 0.2$, 0.09) or liquefaction time ($r = 0.03$, 0.06). It is concluded that seminal plasma ascorbic acid decreased significantly in smokers and infertile men versus nonsmokers and fertile men, and is significantly correlated with the main sperm parameters: count, motility and normal morphology. Also, cigarette smoking is associated with reduced semen main parameters that could worsen the male fertilizing potential, especially in borderline cases.

Introduction

One of the notable peculiarities of the human seminal plasma is its high concentration of some organic substances and enzymes such as fructose, citric acid, glycerylphosphocholine, acid phoshatase, 5-nucleotidase and ascorbic acid. Ascorbic acid was shown to be an essential factor in numerous biological functions of both metabolic and clinical importance. Human seminal plasma contains about 10 mg dl^{-1} ascorbic acid, which is considerably more than that in the human blood plasma (range: 0.6–2.5 mg dl^{-1}) (Dawson *et al.*, 1992). Apparently, minimal levels of ascorbic acid are required to protect the formation, maturation and delivery of spermatozoa from endogenous reactive oxidants, being the main extracellular water-soluble antioxidant (Frei *et al.*, 1990). Necrospermic as well as azoospermic semen samples apparently contain less content of ascorbic acid than normozoospermic samples (Blumenkrantz *et al.*, 1967; Srivatava *et al.*, 1983). Also, seminal plasma ascorbic acid was shown to reduce nonspecific sperm-agglutinin and ensures the molecular coating of the sperm heads (Dawson *et al.*,

1992). Its other role is in the epithelium of the germinal layer and cauda epididymidis where sperm maturation occurs (Chinoy *et al.*, 1986).

Consumption of tobacco exerts widely adverse effects on different aspects of health (O'Dowd, 2006). Smoking was associated with a 107% increase in reactive oxygen species (ROS) levels and a 10-point decrease in ROS-total antioxidant capacity (TAC) scores (Saleh *et al.*, 2002). The addition of reactive oxidants from exogenous sources creates a matching requirement for increased ascorbic acid content (Faraga *et al.*, 1991). It was demonstrated that heavy smoking in males is associated with a 20–40% decrease in serum ascorbic acid levels associated with increased sperm abnormalities (Kul'Krauchas *et al.*, 1985). Dawson *et al.* (1992) suggested supplementation of ascorbic acid to heavy smokers with improved sperm quality. This work aimed at assessing seminal ascorbic acid levels relationship with smoking in infertile men.

Materials and methods

One hundred and thirty infertile men were selected prospectively after consent from the andrology outpatient clinic of the University Hospitals after Institutional Review Board (IRB) approval. They were divided into four groups: nonobstructive azoospermia (NOA) [smokers ($n = 20$), nonsmokers ($n = 20$)]; oligoasthenozoospermia [smokers ($n = 30$), nonsmokers ($n = 20$)]; asthenozoospermia [smokers ($n = 20$), nonsmokers ($n = 20$)]. The results were compared to those of 40 normozoospermic proven fertile volunteers [smokers ($n = 20$), nonsmokers ($n = 20$)]. Males who smoked >20 cigarettes day^{-1} were enrolled in this study. Cases with leukocytospermia or varicocele were excluded from this study because of their well-known high seminal ROS levels.

A detailed medical history was taken and physical examination was performed for the studied cases. Ejaculates were obtained in the early morning (7.00–9.30 AM) after four days of sexual abstinence. The samples were examined immediately after liquefaction according to WHO guidelines (World Health Organization, 1999) (normally, sperm count >20 × 10^6 sperm ml^{-1}, sperm motility >50%, abnormal sperm morphology <70%, vitality >75% and leukocytes <10^6 ml^{-1}). Semen samples were verified after at least two different analyses. Azoospermia was verified after three different analyses and centrifugation. Seminal plasma was separated at 1200 g after complete liquefaction. Ascorbic acid levels were estimated in the fresh seminal plasma samples calorimetrically in duplicates (Beckman Du 7400 spectrophotometer, Fullerton, CA, USA) with sensitivity 0.5 mg dl^{-1}.

Statistical analysis

Data were expressed as mean ± SD and range. Statistical analysis was performed using SPSS version 10. Student's *t*-test was used to compare parametric groups. Correlations were tested by Spearman's test. They were considered statistically significant when $P < 0.05$.

Results

Seminal plasma ascorbic acid levels were found to be significantly decreased in smokers versus nonsmokers in all studied groups except NOA. The results were (mean ± SD) NOA group 6.03 ± 2.18 versus 6.62 ± 1.29 mg dl^{-1}, $P > 0.05$, oligoasthenozoospermia 7.81 ± 1.98 versus 9.44 + 2.15 mg dl^{-1}, $P < 0.05$, asthenozoospermia 8.09 ± 1.98 versus 9.95 ± 2.03 mg dl^{-1}, $P < 0.05$, normozoospermia 11.32 ± 2.15 versus 12.98 ± 12.19 mg dl^{-1}, $P < 0.05$. Fertile normozoospermic cases demonstrated the same relation, but seminal plasma ascorbic acid levels were higher than those in the infertile cases, significantly irrespective of smoking state (Table 1). Seminal plasma ascorbic acid of either smokers or nonsmokers demonstrated significant positive correlation with sperm concentration ($r = 0.59$, 0.60, $P < 0.001$), sperm motility per cent ($r = 0.65$, 0.55, $P < 0.001$) and negatively with sperm abnormal forms per cent ($r = -0.53$, -0.50, $P < 0.001$). Nonsignificant correlations were elicited with semen volume ($r = 0.2$, *P*: 0.09) or liquefaction time ($r = 0.03$, *P*: 0.06).

Discussion

In this work, seminal plasma ascorbic acid was found significantly decreased in smokers versus nonsmokers in all studied groups, pointing to the negative effect of smoking. This could be explained by the exogenous addition of reactive oxidants to the semen that exert a matching requirement for increased ascorbic acid as an essential seminal antioxidant (Smith & Hodges, 1987). Seminal plasma ascorbic acid in azoospermic groups (smokers or nonsmokers) showed a significant decrease compared with normozoospermic fertile groups, which is in accordance with Blumenkrantz *et al.* (1967) and Srivastava *et al.* (1983). This may be attributed to the absence of sperm, which seems to be a provocative factor for maintenance of certain seminal ascorbic acid levels through potentiation of its secretion and/or consumption.

In addition, seminal ascorbic acid demonstrated positive significant correlation with sperm concentration, sperm motility per cent and a significant negative correlation with abnormal forms of sperm. Jedrzejczak *et al.* (2004) showed that smokers had significantly fewer sper-

Table 1 Comparison between different parameters in the studied groups

	NOA		Oligoasthenozoospermia		Asthenozoospermia		Normozoospermia	
	Smokers ($n = 20$)	Nonsmokers ($n = 20$)	Smokers ($n = 30$)	Nonsmokers ($n = 20$)	Smokers ($n = 20$)	Nonsmokers ($n = 20$)	Smokers ($n = 20$)	Nonsmokers ($n = 20$)
Semen volume (ml)								
Mean ± SD	2.31 ± 0.84	2.09 ± 0.53	2.28 ± 0.47	2.64 ± 0.74	2.62 ± 0.59	2.62 ± 0.74	2.38 ± 0.81	2.85 ± 0.59
Range	1.5–3.0	1.5–3.0	1.5–4.5	1.2–3.0	1.5–4.0	1.5–3.5	2.0–8.0	1.5–4.0
P-value	>0.05		<0.05*		>0.05		<0.05*	
Liquefaction (min)								
Mean ± SD	12.5 ± 3.09	12.3 ± 3.01	14.7 ± 2.78	14.5 ± 3.14	15.0 ± 2.29	15.0 ± 3.27	14.3 ± 2.94	14.1 ± 2.02
Range	10–20	10–20	10–20	10–20	10–20	10–20	10–15	10–15
P-value	>0.05		>0.05		>0.05		>0.05	
Sperm count ($10 \times {}^{6}$ ml^{-1})								
Mean ± SD	0	0	7.56 ± 3.19	8.41 ± 5.43	26.6 ± 5.82	35.27 ± 12.79	69.55 ± 7.59	71.25 ± 8.85
Range			0.1–18	2–15	20–43	22–68	55–95	60–90
P-value			>0.05		<0.05*		>0.05	
Sperm motility %								
Mean ± SD	0	0	31.0 ± 10.7	35.6 ± 12.5	37.67 ± 5.5	38.5 ± 11.6	65.5 ± 4.6	63.75 ± 6.19
Range			5–40	5–40	20–40	5–40	60–80	60–80
P-value			>0.05		>0.05		>0.05	
Sperm abnormal forms %								
Mean ± SD	0	0	46.1 ± 1.76	55.7 ± 12.3	64.0 ± 12.42	53.5 ± 11.37	14.4 ± 3.59	13.5 ± 3.28
Range			40–80	40–70	50–90	30–70	10–20	10–20
P-value			>0.05		<0.05*		>0.05	
Ascorbic acid (mg dl^{-1})								
Mean ± SD	6.03 ± 2.18	6.62 ± 1.29	7.81 ± 1.98	9.44 ± 2.15	8.09 ± 1.98	9.95 ± 2.03	11.32 ± 2.15	12.98 ± 2.19
Range	4.2–9	4.3–11.5	4.5–13.6	6.5–12.8	5.7–12.4	7.1–13.5	6.9–14	10–16
P-value	>0.05		<0.05*		<0.05*		<0.05*	

matozoa with motility grades A, B and C, and more abnormal forms in the ejaculate especially with head defects and cytoplasmic droplets. Zhang *et al.* (2000) showed that medium, heavy and long-term smoking adversely affected the semen quality and that the effect of smoking on semen parameters of infertile men was dose–effect and time–effect relationship. Kunzle *et al.* (2003) associated cigarette smoking with a significant decrease in sperm density, total sperm count, total number of motile sperm and normal sperm morphology. Mak *et al.* (2000) indicated that cigarette smoking is associated with retention of sperm cytoplasmic droplets in infertile men, a morphologic characteristic associated with impaired sperm function.

Merino *et al.* (1998) showed that defective sperm picture or function gave a motive to the surrounding biological reactive processes with the additive factors such as smoking associated with decreased sperm concentration, motility and increased sperm abnormal form per cent. Mehran (2005) and Arabi & Moshtaghi (2005) showed that exposure of spermatozoa from smokers to the seminal plasma from nonsmokers resulted in an improvement of sperm dysfunction. Taszarek *et al.* (2005) and Agarwal *et al.* (2005) demonstrated that cigarette smoking alters semen quality, which could worsen the fertilizing capability in infertile men.

On the contrary, our results are not in agreement with other issues. Pasqualotto *et al.* (2006) demonstrated nonsignificant differences in sperm concentration, sperm motility, levels of serum FSH, LH or total testosterone hormones or sperm motion characteristics. Semen volume was the only semen variable that tended to decrease according to the number of cigarettes smoked. Trummer *et al.* (2002) showed that smoking does not affect conventional semen parameters. Belcheva *et al.* (2004) showed nonsignificant differences in standard sperm parameters between smokers and nonsmokers, but demonstrated a significant increase of apoptotic sperm in the smokers' ejaculates. It is concluded that cigarette smoking is associated with reduced semen main parameters that could worsen the male fertilizing potential especially in borderline cases.

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