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ORIGINAL ARTICLE

Nitric Oxide Level and Total Antioxidant Capacity in the Seminal Plasma of Infertile Smoking Men

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Abstract

Introduction and aim: **To assess the effects of smoking on total antioxidant capacity (TAC) and nitric oxide (NO) levels in seminal plasma of patients who suffer from oligoasthenozoospermia.**

Patients and methods: **This study was conducted on 90 males with age range from 20 to 40 years. They were classified into two groups. Group A included 45 smokers with oligoasthenozoospermia, and group B included 45 nonsmokers with oligoasthenozoospermia. This research was conducted between January 2017 and May 2018. Semen samples were collected and examined. Parameters of semen were considered according to WHO (2010). Hormonal profile (FSH, LH, testosterone, and prolactin) was assessed. Seminal plasma level of nitrite was used as a surrogate for NO. Seminal plasma level of TAC was assessed.**

Results: **Statistical analysis showed a significant increase of NO in group A than group B ($P < 0.05$) and a significant decrease of total antioxidants (uuM/l) in group A than group B ($P < 0.05$) with oligoasthenozoospermia. It showed a negative correlation between NO level and TAC level.**

Conclusions: **Smoking had an obvious effect on fertility, specifically normal morphology of sperm and sperm motility. This effect was most probably owing to oxidative stress as a result of smoking.**

Keywords: Antioxidant, Nitric oxide, Oligoasthenozoospermia, Smoking

1. Introduction

Infertility means not able to have a baby after a year of regular unprotected sexual relationship. Oxidative stress is effective in the pathophysiology of male infertility. Oxidative stress is caused by an inequality between human antioxidant and free radicals.[1]

Tobacco has ~4000 harmful compounds including nitrosamines, nicotine, alkaloids, and hydroxycotinine, and they act as a source of reactive oxygen species (ROS) in addition to reactive nitrogen species.[2]

It is clear that the increased production of ROS as a result of smoking could reduce oxidant defense mechanism, leading to aerobic injury of seminal plasma.[3]

Spermatozoa are very sensitive to oxidative stress owing to small amounts of cytoplasmic enzymes, and they are not able to repair such damage. On the contrary, the plasma membrane of spermatozoa contains increased amounts of polyunsaturated fatty acids, making them easily affected by ROS through lipid peroxidation.[4]

ROS that affects the reproductive biology are hydrogen peroxide, superoxide anion (O_2), hydroxyl radicals (OH^-), and peroxyl radicals (ROO^-). The nitrogen species are peroxynitrite anion ($ONOO^-$) and nitric oxide (NO•).[5]

NO has a double effect on sperm performance, so under normal conditions, NO has a significant role in sperm motility, normal sperm production, capacitation, and zona pellucida sperm-binding

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protein. However, high NO level has a negative effect on sperm motility in humans.[6,7]

Total antioxidant capacity (TAC), including both enzymatic and nonenzymatic reactions, suppresses the free radical-induced oxidative damage.[8]

By collecting and analyzing data from Al-Hussein University Hospital in Cairo, we aimed to assess the effects of smoking on TAC and NO levels in seminal plasma of patients with oligoasthenozoospermia.

2. Patients and methods

A comparative study was conducted on 90 men with age range from 20 to 40 years. They were classified into two groups: group A included 45 smokers with oligoasthenozoospermia and group B included 45 nonsmokers with oligoasthenozoospermia. This research was performed between January 2017 and May 2018.

Inclusion criteria were as follows:

(1) Donors with age range from 20 to 40 years and patients with oligoasthenozoospermia.

Exclusion criteria were as follows:

(1) Men on any kind of antioxidants (vitamins, carotenes, etc.) during the last 2 months before sample taking, men who have any associated systemic disease, men with clinical varicocele or abnormal testicular examinations, men with cryptorchidism, vasectomy, men with endocrine disorders, men with leukocytospermia, and men who consume alcohol.

Each participant was subjected to the following: complete history taking, including age, sex, occupation, special habits, previous treatment complete medical examination, and smoking history (duration, type, number, and active or passive smoker).

Semen sample was collected and examined for parameters of semen, and they were considered according to WHO (2010). The reference values for these parameters included sperm concentration of 15 million/ml, a semen volume of 1.5 ml, sperm total motility of 40%, and sperm with a normal morphology of 4%.

2.1. Statistical methods

IBM SPSS Statistics, version 23.0, was used to gather and analyze the data (IBM Co., Madison Avenue, New York , United States).

For comparison between two groups with quantitative data, we used independent *t* test or Mann–Whitney test. For comparison between more than two groups, we used one-way analysis of variance test or Kruskal–Wallis test.

Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. The significance level was set at *P* value less than 0.05.

3. Results

Statistical analysis showed a significant increase of NO in the smoker group than the nonsmoker group ($P < 0.05$) and a significant decrease of total antioxidants (uuM/l) in the smoker group than the nonsmoker group ($P < 0.05$) (Table 1). Sperm count showed a nonsignificant decrease in the smoker group more than the nonsmoker group ($P > 0.05$), a highly significant decrease in sperm morphology in the smoker group than the nonsmoker group ($P < 0.01$), and a significant decrease in sperm motility (%) in the smoker group than the nonsmoker group ($P < 0.05$) (Table 2). Sperm morphology, sperm motility, and TAC showed a highly significant decrease ($P < 0.05$), with increased NO levels (Table 3). Sperm morphology, sperm motility, and NO levels showed a highly significant decrease with increased TAC ($P < 0.05$) (Table 4). Smoking degree and NO levels showed a positive relation and smoking degree and TAC showed a

Table 1. Comparative statistical study of nitric oxide (Um/l) and total antioxidants (uuM/l) between smoker patients with oligoasthenozoospermia and nonsmoker patients with oligoasthenozoospermia

	Nonsmoker group	Smoker group	Test value	*P* value	Significance
	$N = 45$	$N = 45$			
Nitric oxide (Um/l)					
Mean ± SD	4.70 ± 1.46	5.54 ± 1.67	−2.556•	0.012	S
Range	2.1–8.4	2.3–8.6			
Total antioxidants (uuM/l)					
Median (IQR)	1738 (1395–1918)	1482 (1234–1754)	−2.591≠	0.010	S
Range	593–2823	421–1932			

•: Independent t-test; ≠: Mann-Whitney test

Table 2. Comparative statistical study of sperm count (million/ml) and sperm motility (%) between smoker patients with oligoasthenozoospermia and nonsmoker patients with oligoasthenozoospermia

	Nonsmoker group	Smoker group	Test value	*P* value	Significance
	N = 45	*N* = 45			
Sperm count (million/ml)					
Median (IQR)	5 (3–8)	4 (2–7)	−1.200≠	0.230	NS
Range	0.2–13	0.2–11			
Sperm morphology					
Median (IQR)	3 (2–4)	2 (1–3)	−2.820≠	0.005	HS
Range	1–8	1–5			
Sperm motility (%)					
Median (IQR)	20 (10–30)	15 (10–20)	−2.418≠	0.016	S
Range	5–35	2–30			

IQR, interquartile range; ≠: Mann-Whitney test.

Table 3. Correlation between nitric oxide and total antioxidants and sperm count, motility, and morphology.

	Nitric oxide (Um/l)					
	All cases		Smoker group		Nonsmoker group	
	r	*P* value	*r*	*P* value	*r*	*P* value
Total antioxidants (uuM/l)	−0.586**	<0.001	−0.733**	<0.001	−0.426**	0.003
Sperm count (million/ml)	−0.010	0.925	−0.164	0.282	0.219	0.149
Sperm morphology	−0.541**	<0.001	−0.630**	<0.001	−0.356*	0.016
Sperm motility (%)	−0.546**	<0.001	−0.565**	<0.001	−0.455**	0.002

* Significant and ** Highly significant

Table 4. Correlation between total antioxidants and nitric oxide, sperm count, motility, and morphology.

	Total antioxidants (uuM/l)					
	All cases		Smoker group		Nonsmoker group	
	r	*P* value	*r*	*P* value	*r*	*P* value
Nitric oxide (Um/l)	−0.586**	0.000	−0.733**	0.000	−0.426**	0.003
Sperm count (million/ml)	0.020	0.853	0.112	0.463	−0.105	0.493
Sperm morphology	0.605**	0.000	0.540**	0.000	0.579**	0.000
Sperm motility (%)	0.593**	0.000	0.462**	0.001	0.630**	0.000

** Highly significant

Table 5. Correlation between smoking degree and nitric oxide and total antioxidant capacity levels.

	Smoking degree			Test value	*P* value	Significance
	Mild	Moderate	Severe			
	N = 14	*N* = 16	*N* = 15			
Nitric oxide (Um/l)						
Mean ± SD	4.75 ± 1.99	5.39 ± 1.13	6.44 ± 1.48	4.393•	0.019	S
Range	2.3–8.6	3.8–8.3	2.6–8.6			
Total antioxidants (uuM/l) (decreased with the high degree)						
Median (IQR)	1727 (1234–1871)	1531 (1304–1695)	1341 (834–1586)	6.463≠	0.040	S
Range	425–1932	639–1862	421–1862			

•: One Way ANOVA test; ≠: Kruakal-Wallis test

negative relation with NO (Table 5). Smoking degree and sperm morphology and sperm motility levels showed a positive relation, whereas smoking degree and sperm count showed a nonsignificant relation (Table 6). Duration of smoking and NO levels showed a positive relation, whereas duration of smoking and TAC showed a negative relation (Table 7). Duration of smoking (years) and sperm

Table 6. Correlation between smoking degree and sperm count, motility, and morphology.

	Smoking degree			Test value	*P* value	Significance
	Mild	Moderate	Severe			
	N = 14	*N* = 16	*N* = 15			
Sperm count (million/ml)						
Median (IQR)	3.5 (2–8)	4 (3–5.5)	3 (2–7)	0.485≠	0.785	NS
Range	1–9	0.4–11	0.2–10			
Sperm morphology (decreased with high degree)						
Median (IQR)	3 (2–4)	2 (1.5–3)	2 (1–2)	6.887≠	0.032	S
Range	1–5	1–4	1–4			
Sperm motility (%) (decreased with high degree)						
Median (IQR)	20 (20–25)	13.5 (10–15)	10 (5–10)	12.629≠	0.002	HS
Range	3–30	5–20	2–25			

≠: Kruakal-Wallis test

Table 7. Correlation between duration of smoking (years) and nitric oxide and total antioxidant capacity levels.

	Duration of smoking (years)		Test value	*P* value	Significance
	<10 years	>10 years			
	N = 25	*N* = 20			
Nitric oxide (Um/l)					
Mean ± SD	4.7 ± 1.56	6.59 ± 1.15	−4.520•	0.000	HS
Range	2.3–8.3	5.1–8.6			
Total antioxidants (uuM/l)					
Median (IQR)	1636 (1341–1825)	1352.5 (798.5–1584.5)	−2.501≠	0.012	S
Range	639–1932	421–1862			

•: Independent t-test; ≠: Mann-Whitney test

Table 8. Correlation between duration of smoking (years) and sperm count, motility, and morphology.

	Duration of smoking (years)		Test value	*P* value	Significance
	<10 years	>10 years			
	N = 25	*N* = 20			
Sperm count (million/ml)					
Median (IQR)	4 (2–6)	4 (2–7.5)	−0.403≠	0.687	NS
Range	0.4–11	0.2–11			
Sperm morphology					
Median (IQR)	3 (2–4)	2 (1–3)	−2.381≠	0.017	S
Range	1–5	1–3			
Sperm motility (%)					
Median (IQR)	15 (10–25)	10 (6–16.5)	−2.048≠	0.041	S
Range	5–30	2–20			

≠: Mann-Whitney test

morphology and sperm motility levels showed a positive relation, whereas it showed a nonsignificant relation with sperm count (Table 8).

4. Discussion

Infertility is the inability of a couple to have a baby after one year of normal, unprotected sexual relation. It affects minimum 180 million worldwide. The male is alone responsible for about 20% and is a participating factor in 30–40% of all infertility cases.[9] The causes for male fertility are many, including age, toxins, genetic problems, smoking, medications, alcohol, and other systemic diseases.[10] In this study, we assessed the relationship between smoking and TAC and NO levels of seminal plasma to illustrate the relation between the increase in oxidative damage level and infertility rate in men.

The results of the current study showed a statistically significant increase of NO in the smoker group than the nonsmoker group ($P < 0.05$) and a significant decrease of TAC in the smoker group than the nonsmoker group ($P < 0.05$). These results are in agreement with a previous study, which mentioned that the increase of NO in the smoker

group was more than the nonsmoker group and the decrease of TAC was more in the smoker group than the nonsmoker group.[6]

In our study, regarding sperm count, morphology, and motility between smoker patients with oligoasthenozoospermia and nonsmoker patients with oligoasthenozoospermia, there was a statistically nonsignificant decrease of sperm count in the smoker group more than the nonsmoker group ($P > 0.05$) and a highly significant decrease of sperm morphology in the smoker group more than the nonsmoker group ($P < 0.01$) and a significant decrease of sperm motility (%) in the smoker group more than the nonsmoker group ($P < 0.05$). These results agree with many previous studies that mentioned a negative relationship between current cigarette smoking and semen analysis. These studies demonstrated that the decreased semen parameters in smokers can be due to ROS.[11-13]

In contrary with our study, Pasqualotto et al.[13] demonstrated that there was a decrease in semen volume with increased smoking, but no differences were observed concerning sperm concentration and motility. This may be owing to the samples being collected from all of the men who were presumed to be fertile, with no infertile group in the sample.

Moreover, in this study, regarding the correlation between NO and TAC, sperm count, morphology, and motility, there was a statistically highly significant decrease of sperm morphology motility and total antioxidants ($P < 0.05$) with increased NO levels. These results agree with many previous studies that mentioned a significant negative correlation between NO levels in seminal plasma and sperm morphology, viability, and motility.[14,15]

In contrary with our study, Badade and colleagues demonstrated a statistically negative correlation only between NO levels and sperm count. They founded that the high concentration of NO may react with superoxide ion and hydrogen peroxide and forms peroxynitrite, hydroxyl radical, leading to oxidation of sperm membrane lipids and thiol-proteins, resulting in reduction of sperm count.

The results of the current study showed that regarding the correlation between TAC and NO, age, sperm count, sperm morphology, and sperm motility, there was a statistically highly significant decrease of sperm morphology, sperm motility, and NO levels with increased TAC ($P < 0.05$). These results are in agreement with many previous studies that mentioned a significant linear positive correlation between TAC levels in seminal plasma and NO, sperm motility, morphology, and count.[6,16]

Moreover, in this study, regarding the correlation between smoking degree and NO and TAC levels, statistical analysis showed a positive relation between smoking degree and NO levels and a negative relation between smoking degree and TAC. These results agree with many previous studies that mentioned a significant linear positive correlation between smoking degree and NO levels and a significant linear negative relation between smoking degree and TAC,[17] and a significant correlation only between smoking degree and NO levels.[18] In this study regarding the correlation between smoking degree and sperm count, morphology, and motility levels, there was a positive relation between smoking degree and sperm morphology and motility levels and a nonsignificant relation with sperm count. These results agree with a previous study that mentioned a significant positive correlation between smoking degree and sperm morphology and motility levels and a nonsignificant relation with sperm count.[19] In this study, regarding correlation between duration of smoking (years) and NO and TAC levels, statistical analysis showed a positive relation between duration of smoking and NO levels and a negative relation between duration of smoking and TAC. These results agree with the previous study that mentioned a positive correlation between duration of smoking and NO levels and negative correlation with TAC.[6] Regarding correlation between duration of smoking (years) and sperm count, morphology, and motility levels, statistical analysis showed a positive relation between duration of smoking (years) and sperm morphology and motility levels and a nonsignificant relation with sperm count. These results agree with a previous study that mentioned a linear positive correlation between duration of smoking (years) and sperm morphology and motility levels and a nonsignificant correlation between duration of smoking with sperm count.[19]

5. Conclusion

Smoking has a negative effect on fertility, specifically sperm motility and morphology. This effect is due to oxidative stress resulting from smoking, which has catastrophic effects on semen quality, leads to reduction of male fertility.

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Authors declare that there is no conflict of interest, no financial issues to be declared.

Conflicts of interest

None declared.

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