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Comparison of Oxidative Stress Intensity in the Seminal Plasma and Serum of Infertile Patients with Different Varicocele Grades and Subjects with No Varicocele: A Cross-Sectional Study

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Abstract

Background: Varicocele is the leading cause of male infertility throughout the world.
Objectives: This study aimed to identify the effects of varicocele on oxidative stress, total antioxidant capacity (TAC), and semen parameters.
Methods: In this cross-sectional study, serum and semen samples were collected from subjects with no varicocele (n, 35) and patients with varicocele (n, 86), who were referred to infertility and in vitro fertilization (IVF) centers of Babol Clinic Hospital, Babol, Iran during 2015 - 2016. The semen samples were analyzed according to the world health organization (WHO) guidelines. After the ultrasound and physical examinations, varicoceles were classified into three grades: G1, G2, and G3. TAC and malondialdehyde (MDA) levels were measured in the serum and seminal plasma of subjects, using the ferric reducing ability of plasma (FRAP) and thiobarbituric acid reaction (TBAR) methods, respectively. Nitric oxide (NO) was also measured using Nitric Oxide assay kit.
Results: The mean total sperm count in the G1, G2, and G3 groups were significantly lower than the control group ($P = 0.037$; $P = 0.003$, and $P = 0.044$, respectively). A trend of lower semen volume was observed in the G3 group, compared with the control group ($P = 0.06$). A significant positive correlation was observed between elevated serum MDA level and varicocele degree ($P < 0.05$). The MDA level from the highest to the lowest is as follows: G3 > G2 > G1 > controls. There was also a significant negative correlation between the serum and seminal plasma TAC levels ($P < 0.05$). Varicocele patients with G3 degree had a significantly lower mean TAC level in the serum and seminal plasma, compared with the control group ($P = 0.001$ and $P = 0.008$, respectively). No significant difference was found in the mean Nitric Oxide level between the groups. However, an increasing trend for the mean Nitric Oxide level was observed in the serum and seminal plasma of varicocele patients.
Conclusions: The reduced level of seminal plasma antioxidants, which is associated with increased lipid peroxidation, is one of the main reasons for low sperm quality in patients with varicocele. Antioxidant therapy may be useful in decreasing oxidative stress intensity and improving the condition of varicocele patients.

Keywords: Infertility, Malondialdehyde, Nitric Oxide, Oxidative Stress, Sperm, Total Antioxidant Capacity, Varicocele

1. Background

Male infertility is a major health problem in 10% to 15% of couples throughout the world (1). It is a multifactorial syndrome encompassing a wide range of disorders. Varicocele is among the most common causes of male infertility (2). This abnormality has been reported in 15% of the healthy population and over 40% of infertile men (3). The pathological effects of varicocele on male infertility are suspected, as it can increase the temperature in the scrotum and testes, leading to impaired spermatogenesis, increased apoptosis of germ cells, and decreased sperm count, motility, viability, and normal morphology (3-6).

Although different studies have shown the pathological effects of varicocele on sperm quality, the exact mechanism of its action is not well understood (7, 8). One of the probable mechanisms is oxidative stress (OS), induced by reactive oxygen species (ROS) and reactive nitrogen species

(RNS), with adverse effects on sperm quality and function (9-11). OS occurs as a result of instability between the yield of free radicals and defense system of available antioxidants (10). ROS and RNS (e.g., H_2O_2, O_2, and NO) are particularly reactive oxidizing factors, which are among free radicals reacting with macromolecules for compensation of their electron deficit (12). They can oxidize macromolecules such as polyunsaturated fatty acids (PUFA), proteins, and carbohydrates (13).

Lipid peroxidation (LPO) is the primary pathological effect of free radicals, correlated with PUFA oxidation in the sperm membranes (13). Malondialdehyde (MDA) is a steady byproduct of PUFA oxidation, which can be measured as a diagnostic index of sperm lipid peroxidation (13). Nevertheless, human body fluids include several enzymatic and non-enzymatic antioxidants, known as total antioxidant capacity (TAC), which counteract the toxic effects of ROS (12, 14). The increased level of OS induced by free radicals seems to be one of the main mechanisms of pathological effects of varicocele on poor sperm quality and male infertility.

To the best of our knowledge, there is no comprehensive study on OS intensity among patients with different varicocele grades. Therefore, the aim of this study was to determine changes in the concentrations of TAC and oxidants, such as MDA and nitric oxide (NO) in the serum and seminal plasma of infertile varicocele and subjects without varicocele. This study can help assess the role of OS in the pathophysiology of infertility in varicocele patients.

2. Methods

2.1. Patients

In this cross-sectional study, 121 serum and semen samples were collected from patients with (n = 86) and without (n = 35) varicocele, who were referred to the infertility and in vitro fertilization (IVF) centers of Babol clinic Hospital, Babol, Mazandaran Province, Iran during 2015 - 2016. The Ethics review board of Babol University of Medical Sciences approved this study, and all the patients signed an informed consent. Individuals who met the following criteria were excluded from the study: smoking, alcohol use, testicular trauma, inguinal hernia operation, cryptorchism, and sexually transmitted diseases.

In this research, all the samples were collected from infertile patients with varicocele. Ultrasound and physical examinations were performed for the diagnosis of varicocele. After examinations, varicocele was divided into three grades, including G1 (palpable on physical examination during straining), G2 (palpable on routine physical examination while standing), and G3 (visible by the eye and palpable on examination).

2.2. Semen Analysis

After sexual abstinence for 2 - 3 days, semen samples were collected after intercourse in a sterile container. The semen samples were investigated according to the world health organization (WHO) criteria (2001) and were examined for volume, appearance, viscosity, and pH. All semen samples were liquefied at 37°C for 30 minutes, and then, the percentage of sperm motility, sperm count, and sperm morphology were evaluated using microscopic examinations. The sperm count and motility were investigated according to the WHO 2001 guidelines (15). In addition, the percentage of sperm morphology was determined, based on the Kruger's strict criteria (16).

2.3. TAC Measurement

The serum and semen samples (~ 100 μL) were centrifuged at 12,000 g for 7 minutes at 5°C. The supernatants were removed from the pellets and used for TAC measurements. TAC was measured by ferric reducing of antioxidant power (FRAP) method, as previously described by Benize (1996) (16). Briefly, 1.5 mL of FRAP reagent was added to each tube and maintained in a water bath for 5 minutes at 37°C. Afterward, 50 μL of the samples was added to each tube and placed in the water bath again for ten minutes at 37°C. Finally, absorbance was read, using a spectrophotometer (UV1600, Germany) at 593 nm.

2.4. MDA Measurement

The serum and seminal MDA levels were analyzed according to the methods described by Sharma and Krishna (1968) (17). Briefly, 500 μL of supernatant was added to a separate tube, containing 1 mL of thiobarbituric acid (TBA) solution. The tubes were held on a boiling water bath for 10 minutes, and then, the tubes were cooled, and optical density (OD) was read at 535 nm.

2.5. NO Measurement

The total NO level in the serum and seminal plasma was assessed, using an Nitric Oxide Assay Kit (ZellBio GmbH, Germany). The results are expressed as μmol/L.

2.6. Statistical Analysis

Data were analyzed using SPSS version 19.0 (IBM Corp., Armonk, N.Y., USA). Independent t-test was applied to compare the mean values of parameters between and without groups. The average oxidative biomarkers and semen quality were compared between varicocele (G1, G2, and G3) and patients with no varicocele, using one-way ANOVA and Tukey's post hoc test. P value ≤ 0.05 was considered statistically significant.

3. Results

The mean levels of sperm parameters in patients with and without varicocele are depicted in Table 1. The primary study sample consisted of 121 infertile patients, of whom 28.92% were without varicocele, and 71.07% had varicocele. The sperm quality parameters in both groups are shown in Table 1. No significant difference was found in the mean age of subjects between the groups. Although there was no significant difference in the mean sperm quality parameters between the groups, the mean total sperm count in patients without varicocele was significantly higher than that of varicocele groups (P = 0.003). Furthermore, a higher sperm count was found in the semen of subjects with no varicocele, compared to the varicocele group (P = 0.07).

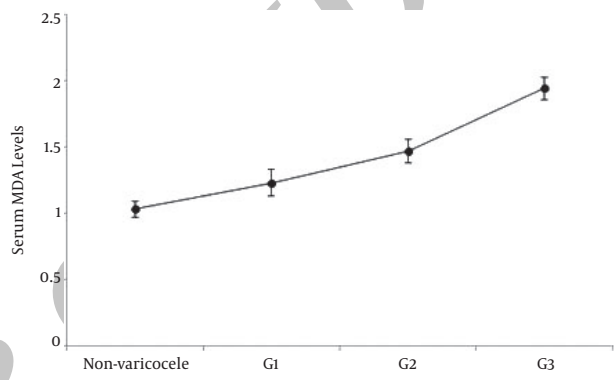
The mean levels of OS biomarkers in the serum and seminal plasma of patients with and without varicocele are demonstrated in Table 2. The mean serum concentration of MDA was significantly different between the groups (P < 0.0001), while no significant difference was detected in the mean seminal plasma MDA level (P = 0.41). Based on the findings, the mean serum MDA level was significantly higher in patients with varicocele (1.55 ± 0.57 nmol/mL), compared to others. subjects (1.03 ± 0.37 nmol/mL).

The mean TAC level in the seminal plasma and serum of patients showed significant differences (P = 0.004 and P = 0.001, respectively). The mean TAC concentrations in the serum and seminal plasma of patients with no varicocele (723.46 ± 250.09 and 846.69 ± 333.17, respectively) were higher than those of varicocele patients (583.60 ± 181.30 and 678.50 ± 263.32, respectively). On the other hand, no significant difference was found in the mean NO level between the groups (Table 2). Nevertheless, a higher mean level was reported in the serum and seminal plasma of varicocele patients, compared with the other subjects.

We also compared the mean sperm quality parameters and OS biomarkers after dividing varicocele patients into three groups of G1, G2, and G3 (Tables 3 and 4). There was no significant difference in the mean semen volume, sperm count, total sperm count, sperm motility, and normal sperm morphology between G1, G2, and G3 groups. However, a lower semen volume was observed in G3 group, compared with other subjects (P = 0.06). Based on the findings, the G2 group had a significantly lower sperm count, compared with no varicocele group (P = 0.032). The mean total sperm count in no varicocele men was significantly higher than that of G1, G2, and G3 groups (P = 0.037; P = 0.003, and P = 0.044, respectively). In addition, the mean pH in the G2 group was significantly higher than that of G3 group (P = 0.003).

A significant positive correlation was found between elevated serum MDA level and varicocele degree (P < 0.05; Figure 1). The MDA level from the highest to the lowest was as follows in the groups: G3 > G2 > G1 > control (Table 4). The varicocele G3 group had a significantly higher mean MDA level in the serum, compared other groups (P < 0.0001). An insignificant difference was observed in the mean serum MDA level between no varicocele and G1 groups (P = 0.09), while G2 group had a higher serum MDA level, compared with no varicocele (P < 0.0001) and G1 (P = 0.05) subjects.

Figure 1. The Mean Serum MDA Levels in the Groups



There is a significant positive correlation between serum MDA level and disease degree (P < 0.05).

No significant difference was found in the mean seminal plasma MDA between the groups. Nevertheless, G3 patients showed a significantly higher MDA level in their seminal plasma, compared with no varicocele (P = 0.05) and G1 (P = 0.018) groups (Table 4). There was also a significant negative correlation between the serum and seminal plasma TAC and disease degree (P < 0.05; Figure 2). Varicocele G3 patients had a significantly lower mean TAC level in their serum and seminal plasma, compared with other group (P = 0.001 and P = 0.008, respectively).

The G2 patients had also a lower mean TAC level in the serum and seminal plasma, compared with the control group (P = 0.003 and P = 0.012, respectively). There was no significant difference in the mean serum and seminal plasma levels of TAC between the G1 and other groups (P = 0.09 and P = 0.08, respectively). Moreover, there was no significant difference in the mean serum and seminal plasma NO between the groups. Nevertheless, a higher NO level was reported in the seminal plasma of G3 group, compared with no varicocele (P = 0.066) and G1 (P = 0.062) groups.

Table 1. The Sperm Quality Parameters in Patients with and Without Varicocele[a]

Parameters	Without Varicocele (N, 35)	Varicocele (N, 86)	P Value
Age, y	36.41 ± 6.49	35.71 ± 7.19	0.72
Volume, mL	3.29 ± 1.42	2.89 ± 1.23	0.12
Sperm count, $\times 10^6$/mL	61.11 ± 28.27	51.54 ± 25.25	0.07[b]
Total sperm, $\times 10^6$	202.25 ± 124.27	143.19 ± 83.77	0.003[b]
Motility, %	59.11 ± 10.05	56.63 ± 12.92	0.31
Normal morphology, %	8.46 ± 1.69	8.43 ± 1.42	0.92
pH	7.89 ± 0.20	7.91 ± 0.27	0.68

[a]The results are presented as mean ± SD.
[b]P value < 0.05 is considered significant.

Table 2. The Mean OS Biomarkers in the Serum and Seminal Plasma of Patients with and Without Varicocele[a]

Parameters	Without Varicocele (N, 35)	Varicocele (N, 86)	P Value
Serum MDA, nmol/mL	1.03 ± 0.37	1.55 ± 0.57	< 0.0001[b]
Seminal MDA, nmol/mL	2.57 ± 0.73	2.73 ± 1.08	0.41
Serum TAC, μM/L	723.46 ± 250.09	583.60 ± 181.30	0.001[b]
Seminal TAC, μM/L	846.69 ± 333.17	678.50 ± 263.32	0.004[b]
Serum NO, μM/L	14.02 ± 6.76	14.84 ± 7.81	0.59
Seminal NO, μM/L	67.65 ± 12.62	70.91 ± 15.26	0.26

[a]The results are presented as mean ± SD.
[b]P value < 0.05 is considered significant.

Table 3. The Sperm Quality Parameters in Patients with and without Varicocele[a]

Parameters	Without Varicocele (N, 35)	Varicocele			P Value
		G1 (N, 27)	G2 (N, 31)	G3 (N, 28)	
Age, y	36.41 ± 6.49	35.18 ± 6.39	36.2 ± 5.18	35.71 ± 6.21	0.48
Volume, mL	3.28 ± 1.42	3.23 ± 1.15	2.77 ± 1.25	2.67 ± 1.23	0.15
Sperm count, $\times 10^6$/mL	61.11 ± 28.27	52.03 ± 29.64	47.09 ± 21.50[a]	55.96 ± 24.59	0.17
Total sperm, $\times 10^6$	202.25 ± 124.26	149.63 ± 79.05[a]	129.64 ± 85.05[c]	151.98 ± 87.77[a]	0.02
Motility, %	59.11 ± 10.05	58.66 ± 11.77	53.93 ± 13.00	57.64 ± 13.80	0.32
Normal morphology, %	8.45 ± 1.69	8.33 ± 1.47	8.25 ± 1.41	8.71 ± 1.38	0.67
pH	7.88 ± 0.20	7.911 ± 0.24	8.003 ± 0.22[d]	7.8 ± 0.32	0.024

[a]The results are presented as mean ± SD.
[b]P < 0.05 compared with the no varicocele group.
[c]P < 0.01 compared with the no varicocele group.
[d]P < 0.01 compared with G3 group.

4. Discussion

Varicocele is a major surgically treatable cause of infertility among men, which is associated with alterations in semen parameters (18). In this study, patients with varicocele had significantly low sperm and total sperm counts, compared with other subjects. These results are consistent with previous studies, which reported abnormalities in the semen parameters of varicocele patients (19-21). Our findings also revealed that a higher grade of varicocele is associated with reduced sperm count and volume; the G3 group had a lower sperm and semen volume, compared to other groups. Similarly, Guzel et al. (2015) demonstrated that a high-grade left testicular varicocele is associated with deterioration of sperm parameters (22).

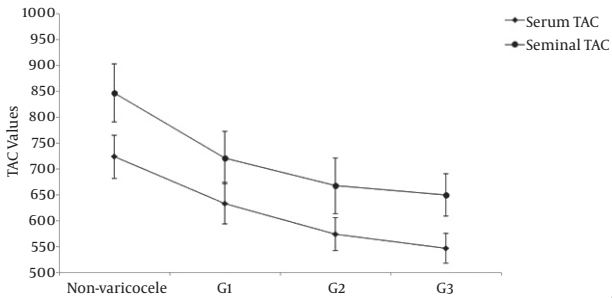
Although several studies have reported poor sperm quality and male infertility among patients with varico-

Table 4. The OS Biomarkers in the Serum and Seminal Plasma of Patients with and Without Varicocele[a]

Parameters	Without Varicocele (N, 35)	Varicocele			P Value
		G1 (N, 27)	G2 (N, 31)	G3 (N, 28)	
Serum MDA, nmol/mL	1.03 ± 0.37	1.23 ± 0.53	1.47 ± 0.51	1.94 ± 0.45	< 0.0001[b]
Seminal MDA, nmol/mL	2.57 ± 0.73	2.42 ± 1.07	2.72 ± 1.02	3.05 ± 1.10	0.1
Serum TAC, μM/L	723.46 ± 250.09	633.44 ± 207.85	573.29 ± 176.77	546.96 ± 152.16	0.003[b]
Seminal TAC, μM/L	846.69 ± 333.17	721.37 ± 265.40	667.45 ± 301.31	649.39 ± 216.16	0.027[b]
Serum NO, μM/L	14.02 ± 6.76	13.39 ± 6.68	14.88 ± 6.27	16.20 ± 10.06	0.53
Seminal NO, μM/L)	67.65 ± 12.62	67.11 ± 15.32	71.01 ± 16.75	74.46 ± 12.97	0.19

[a]The results are presented as mean ± SD.
[b]P value < 0.05 is considered significant.

Figure 2. The Mean Serum and Seminal Plasma Levels of TAC in the Groups



There Is a Significant Negative Correlation Between TAC Level and Disease Degree (P < 0.05).

cele, the mechanisms of sperm abnormalities are unclear. We speculate that increased OS, induced by free radicals, is a major cause of poor sperm quality among these patients. The present results indicated an increase in OS in the serum and seminal plasma of infertile patients with varicocele. In this study, varicocele patients demonstrated higher mean levels of serum and semen MDA, compared to patients without varicocele. On the other hand, the mean concentration of TAC in these patients was significantly lower than that of other subjects.

The present findings also revealed that a higher grade of varicocele was correlated with higher MDA and NO levels, while reduced TAC level was reported in patients with a higher grade of varicocele. The patients with G3 varicocele had higher mean MDA and NO levels, while they showed a lower mean level of TAC in their serum and semen, compared with G1, G2, and other groups. These findings suggest the increased risk of lipid peroxidation, along with antioxidant depletion in the semen and seminal plasma of these patients.

An increase in oxidative damage, induced by ROS and NO, has been reported by several investigators. In this regard, in a study by Ferramosca et al. (2015), varicocele patients were compared with men without varicocele (19). The varicocele patients showed increased OS markers (e.g., ROS and lipid peroxides) and reduced mitochondrial respiratory activity in their serum and seminal fluid, which were associated with defects in the sperm midpiece and low sperm count and motility. In addition, the results of another study indicated that NO concentration was significantly higher in the testicular vein of patients with varicocele (23).

In a previous study, an increase was reported in the mean level of NO in the serum of patients with varicocele, which was associated with low sperm production, motility, and morphology (24). In another survey by Aksoy et al. (2002), NO production could be specifically related to varicocele, since the NO level did not increase in no varicocele patients (25). Additionally, Kisa et al. (2004) considered NO as an important mediator in the pathogenesis of varicocele (26).

In another similar study, Mehraban et al. (2005) compared NO level in the seminal plasma of infertile varicocele, no varicocele, and healthy subjects (27). They suggested that increased NO level in the seminal plasma of varicocele patients could induce sperm dysfunction in the semen, compared with infertile men without varicocele. Similarly, our findings showed an increase in the mean NO level in the serum and seminal plasma of varicocele patients, which was correlated with disease degree. Varicocele has been also shown to negatively affect sperm mitochondrial respiration, resulting in increased ROS production and oxidative damage (28). Recent investigations have also demonstrated a significantly greater rate of DNA fragmentation in infertile men with varicocele, compared to fertile men (18, 29, 30).

The results of the present study and some previous research implicated that reduced level of seminal TAC and increased sperm membrane lipid peroxidation are the ma-

jor causes of low sperm quality in patients with varicocele (31, 32). One consequence of antioxidant deficiency in the semen of patients with varicocele can be related to the rise in OS damage, induced by free radicals (33). Therefore, higher production of free radicals in the serum and seminal plasma of varicocele patients can decrease antioxidant activities and increase the pathological effects of free radicals, such as lipid peroxidation on sperm cells (9, 11, 34). Lack of ROS analysis in the seminal plasma is one of the limitations of this study.

4.1. Conclusion

The decreased level of seminal plasma TAC and as a result increased sperm membrane lipid peroxidation may be the main reasons for low sperm quality in patients with varicocele. Therefore, treatment with antioxidants in varicocele patients may be helpful in protecting sperm cells against OS-induced damage.

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Footnote

Competing Interests: None declared.

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