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Association of Seminal Plasma Total Antioxidant Capacity and Malondialdehyde Levels With Sperm Parameters in Infertile Men With Varicocele

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Abstract

Background: Varicocele is one of the most common reasons for male infertility and could impair spermatogenesis through mechanisms that are not well known. Recently, oxidative stress has been introduced as a major reason for male infertility caused by varicocele.
Objectives: In the current study, we aimed to assess the TAC (total antioxidant capacity) and MDA (malondialdehyde) as stress oxidative markers in infertile men with varicocele and fertile men, and moreover, their correlation with sperm parameters.
Patients and Methods: This case control study was performed on 43 infertile men with varicocele and 46 men with proven fertility. The ferric reducing ability of plasma (FRAP) and thiobarbituric acid (TBA) reaction methods were used for seminal plasma TAC and MDA assay, respectively.
Results: Lower TAC levels (1.7 ± 0.2 vs. 1.3 ± 0.4 mmol/L, $P = 0.0004$) and higher MDA levels (2.5 ± 1.1 vs. 5.8 ± 1.9 mmol/L, $P < 0.0001$) were observed in infertile men with varicocele compared to fertile men. There was no correlation between TAC and MDA in fertile men ($r = 0.02$, $P = 0.9$), however, a negative correlation was found between TAC and MDA levels in varicocele infertile men ($r = -0.44$, $P = 0.003$). Moreover, a positive correlation was found between sperm count and sperm motility with TAC levels in varicocele infertile men ($r = 0.4$, $P = 0.02$ and $r = 0.6$, $P < 0.0001$, respectively). There was a correlation between sperm motility and TAC levels in fertile men ($r = 0.5$, $P = 0.001$), but other parameters did not correlate with TAC in this group. A negative correlation was shown between semen volume, sperm count, total sperm, sperm motility, and sperm morphology with MDA levels in varicocele infertile men ($r = 0.3$, $P = 0.045$; $r = -0.4$, $P = 0.009$; $r = -0.5$, $P = 0.002$; $r = -0.5$, $P = 0.001$ and $r = -0.4$, $P = 0.008$, respectively). There was no correlation between these parameters and MDA in fertile men.
Conclusions: Our findings indicated that oxidative stress could play an essential role in male infertility caused by varicocele and may impair spermatogenesis leading to infertility.

Keywords: Malondialdehyde, Semen, Total Antioxidant Capacity, Varicocele

1. Background

According to the world health organization (WHO), infertility is defined as the inability to achieve pregnancy within 12 months of regular sexual intercourse for couples trying to conceive. Infertility affects 10% - 20% of couples endeavoring to conceive, regardless of race or ethnicity (1). It is estimated that the male factor of couple infertility is nearly 50% (2). Male fertility disorder is attributed to environmental factors such as exposure to certain chemicals, heavy metals, pesticides, and heat, or electromagnetic radiation. Smoking, alcohol abuse, chronic stress, obesity, urogenital trauma, and inflammation in the male reproductive system are also associated with decreased male fertility. Anatomical abnormalities, such as varicocele, semen outflow tract obstruction, or neurological disorders of ejaculation may cause abnormal spermatogenesis and failure in sperm function. Varicocele is the most prevalent abnormal physical finding and most common surgically correctable risk factor for male infertility (2, 3). Varicocele is a pathologic enlargement of the pampiniform venous plexus within the spermatic cord. Fifteen percent of the normal male population has been shown to have varicocele and approximately 40% of these present with infertility (2). Although the exact pathophysiology of varicocele-induced infertility is not completely understood, there are several mechanisms that are thought to contribute to the pathogenesis of this complication such as hypoperfusion leading to hypoxia, oxidative stress, heat stress hormonal

imbalances, and exogenous toxicants (2).

Oxidative stress is created from the imbalance between the production of so-called reactive oxygen species (ROS) and the protective action of the antioxidant system that is responsible for their neutralization and removal. A pathological response to ROS leads to damage of cells and tissues. Spermatozoa are particularly susceptible to the damaging effects of ROS (4, 5). ROS are essential byproducts of aerobic life, which are detoxified by natural processes in the body (4). Oxidative stress occurs when ROS overcome our natural ability to detoxify ROS-induced damage (4, 5). It is understood that controlled levels (physiological levels) of ROS are required for sperm physiology, maturation, capacitation, acrosomal reactions, and normal fertilization. However uncontrolled production of ROS (pathological levels) leads to sperm dysfunction including, lipid peroxidation, sperm DNA damage and loss of motility (6).

The excessive amount of ROS could be produced from endogenous sources including immature or abnormal spermatozoa and leukocytes or from environmental factors such as cigarette smoking and alcohol (7).

Total antioxidant capacity (TAC) and ROS production are in balance in fertile men, however, in pathological conditions, such as chronic disease, autoimmune disorders, alcohol consumption, smoking, infections, advanced age, and stress, the oxidative stress has been increased (4). In the seminal fluid, antioxidants play a crucial role in antioxidant defense mechanisms. Therefore, relatively low amounts of scavenging enzymes or non-enzymatic antioxidants within the cytoplasm, and large amounts of polyunsaturated fatty acids in membranes make spermatozoa susceptible to ROS from lipid peroxidation (8). As a result, the most essential form of antioxidant defense available to spermatozoa is antioxidants in seminal fluid. The most important antioxidants in semen are vitamins, glutathione, thioredoxin, and superoxide dismutase (8). Several studies have indicated that high levels of ROS in seminal fluid increase the risk of male infertility including varicocele (9, 10). Elevated levels of ROS in semen samples of varicocele infertile men were observed, which were associated with an increased DNA fragmentation index (9, 11, 12). These findings suggest that the imbalance between oxidant capacity and the natural antioxidant defense system could play a role in varicocele-associated infertility.

Among various sperm parameters, sperm count and sperm motility are essential parameters that show the functional ability of spermatozoa. Asthenozoospermia (low sperm motility) is considered to be associated with male infertility. Although the factors that affect sperm motility are not clear, the oxidative stress that is produced by ROS is a key factor in this complication (13).

The antioxidant power of biological fluids, including seminal fluid, can be analyzed either with measurement of each antioxidant or with total antioxidant capacity. In addition, a malondialdehyde (MAD) assay is a useful method to display the amount of peroxidation damage of spermatozoa (14).

2. Objectives

Since the findings regarding the correlation of seminal plasma TAC and MAD with varicocele infertility and sperm parameters are controversial, the purpose of this study was to explore the total antioxidant capacity and malondialdehyde status in seminal fluid of varicocele infertile and fertile men and their correlation with sperm parameters.

3. Patients and Methods

This study was performed on 43 consecutive infertile men who presented with varicocele within the last year that were referred to the urology clinic in Ali-Ebne-Abitaleb and Khatamolanbia hospitals in Zahedan, from August 2013 to March 2014. The diagnosis of a clinically significant varicocele was made on physical examination of the scrotum and its contents. The patients were examined in the supine and standing position in a warm room that promotes relaxation of the scrotal dartos muscle and facilitates accurate evaluation for varicocele.

Forty-six men with proven fertility were considered as controls. All patients and fertile controls with specific genital infection, genital diseases, undescended testis, testicular atrophy, and systemic disease were excluded from the study.

All semen samples were collected by masturbation in sterile polypropylene containers after three to five days of abstinence. Semen specimens were liquefied at 37°C for 30 minutes. Routine semen analysis was performed according to the world health organization guidelines of 2010 (15).

A written informed consent was obtained from the patients and controls. This study was approved by the ethics committee of research of Zahedan University of Medical Sciences.

The hematoxylin-eosin (H and E) staining method was used to determine the percentage of normal morphology of spermatozoa. Morphology of the spermatozoa was assessed using Kruger's criteria, and morphology < 14% was considered abnormal (16).

3.1. TAC Assay

TAC was assessed using the ferric reducing ability of plasma (FRAP) method, as discussed by Benzie et al. (17).

In the FRAP method the ability of seminal plasma antioxidants to reduce ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}) was measured. The working FRAP reagent was prepared using 10 volumes of 300 mmol/L acetate buffer; pH 3.6 with 1 volume of 10 mmol/L 2, 4, 6,-tripyridyl-s-triazine in 40 mmol/L HCl with 1 volume of 20 mmol/L $FeCl_3.6H_2O$. Then, 1.5 mL of the working FRAP reagent was aliquoted into a glass tube and warmed to 37°C for five minutes. Subsequently, 50 μL of seminal plasma and 50 μL of distilled water (reagent-free), as well as 50 μL of each of the standard solutions ($FeSO_4.7H_2O$; 1000, 500, 250, 125 μM) were added to 1.5 mL FRAP reagent and heated to 37°C for 10 minutes. Absorbance was measured at 593 nm using a spectrophotometer (UV-visible). The final results were shown as mmol/L.

3.2. MDA Assay

Seminal MDA levels were measured according to the method that was defined by Rao et al. (18). This method was based on a thiobarbituric acid (TBA) reaction and extraction with normal

butanol. As the standard, 1, 1, 3, 3-tetramethoxypropane was used. Spectrophotometric detection of absorbance was done at 532 nm wavelength and compared with a standard curve. The TBA was purchased from Merck.

3.3. Statistical Analysis

Data was analyzed using the statistical software SPSS-18 (SPSS, Chicago, IL). Data are shown as mean ± SD. The normal distribution of data was evaluated using the Kolmogorov-Smirnov (K-S) statistical test. The independent sample t-test or Mann-Whitney U test were used for comparison between two groups, whenever appropriate. In addition, Pearson's correlation coefficient test was performed to determine the correlation among different factors. Values of $P < 0.05$ were considered statistically significant.

4. Results

The semen parameters, TAC, and MDA levels are presented in Table 1. The semen volume, sperm motility, sperm morphology, and sperm count between varicocele infertile men and fertile men were significantly different. A higher TAC level was observed in fertile men compared to varicocele infertile men (1.7 ± 0.2 vs. 1.3 ± 0.4 mmol/L, P = 0.0004). Moreover the MDA level was significantly lower in fertile men compared to varicocele infertile men (2.5 ± 1.1 vs. 5.8 ± 1.9 mmol/L, P < 0.0001).

Table 1. Semen Parameters, Total Antioxidant Capacity (TAC) and Malondialdehyde (MAD) Levels in Varicocele Infertile Men and Fertile Men

Semen Parameters	Varicocele Infertile Men (n = 43)	Fertile Men (n = 46)	P Value
Volume, mL	3.2 ± 1	3.8 ± 1.1	0.005
Sperm count, × 10^6/mL	51.4 ± 20.6	60.1 ± 9.2	0.01
Total sperm, × 10^6	159 ± 74	227 ± 68	< 0.0001
Sperm motility, %	43.5 ± 18	60 ± 5.6	< 0.0001
Sperm morphology, %	19.5 ± 4.4	24.2 ± 5	< 0.0001
TAC, mmol/L	1.3 ± 0.4	1.7 ± 0.2	0.0004
MDA, mmol/L	5.8 ± 1.9	2.5 ± 1.1	< 0.0001

Although there was no correlation between TAC and MDA in fertile men (Figure 1A; r = 0.02, P = 0.9), a negative correlation was found between TAC and MDA levels in varicocele infertile men (Figure 1B; r = -0.4, P = 0.002).

A positive correlation was observed between sperm count and sperm motility with TAC level in varicocele infertile men (Table 2). Although there was a correlation between sperm motility and TAC level in fertile men, other parameters were not correlated with TAC in this group (Table 3).

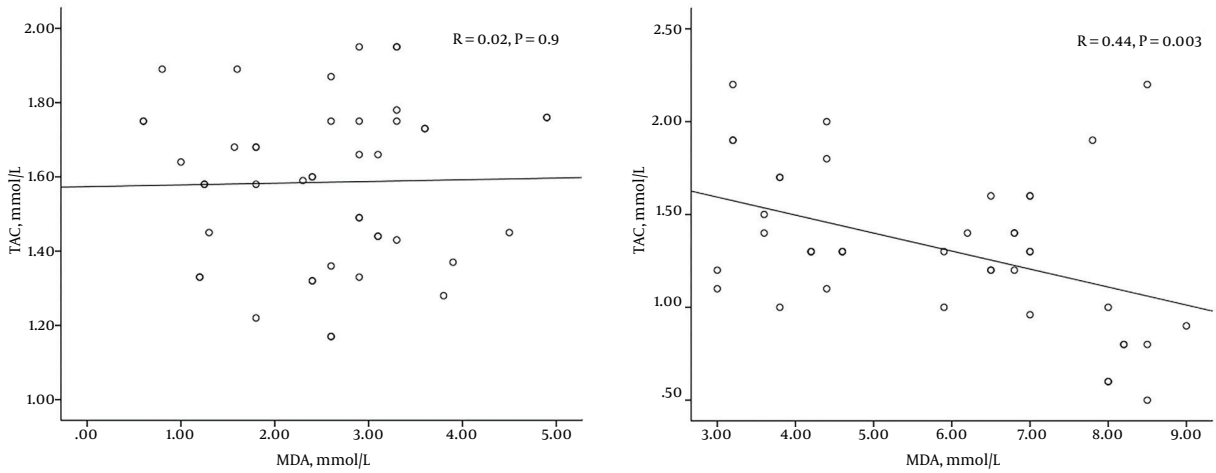
In addition, a negative correlation was shown between semen volume, sperm count, total sperm, sperm motility, and sperm morphology with MDA level in varicocele infertile men (Table 2). There was no correlation between these parameters and MDA in fertile men (Table 3).

5. Discussion

In the present study, MDA levels were significantly higher and TAC levels were significantly lower in varicocele infertile men compared to fertile men. A positive correlation was found between sperm count, total sperm, and sperm motility with TAC level in varicocele infertile men. There was a correlation between sperm motility and TAC level in fertile men; however, other parameters were not correlated with TAC in this group. Furthermore a negative correlation was shown between semen volume, sperm count, total sperm, sperm motility, and sperm morphology with MDA level in varicocele infertile men. No correlation between these parameters and MDA was observed in fertile men.

Varicocele is one of the most frequent reasons for male infertility, and despite many advances in its diagnosis and treatment the exact mechanisms by which it leads

Figure 1. Correlation of Total Antioxidant Capacity (TAC) and Malondialdehyde (MAD)



A, Fertile; B, Varicocele infertile men.

Table 2. Correlation of Sperm Parameters With Total Antioxidant Capacity (TAC) and Malondialdehyde (MAD) in Varicocele Infertile Men

Semen Parameters	TAC, mmol/L		MDA, mmol/L	
	r	P Value	r	P Value
Volume, mL	-0.05	0.7	0.3	0.045
Sperm count, $\times 10^6$/mL	0.4	0.02	-0.4	0.009
Total sperm, $\times 10^6$/mL	0.3	0.07	-0.5	0.002
Sperm motility, %	0.6	< 0.0001	-0.5	0.001
Sperm morphology, %	0.2	0.4	-0.4	0.008

Table 3. Correlation of Sperm Parameters With Total Antioxidant Capacity (TAC) and Malondialdehyde (MAD) in Fertile Men

Semen Parameters	TAC, mmol/L		MDA, mmol/L	
	r	P Value	r	P Value
Volume, mL	0.1	0.5	-0.2	0.2
Sperm count, $\times 10^6$/mL	-0.05	0.8	-0.1	0.4
Total sperm, $\times 10^6$/mL	0.1	0.5	-0.3	0.07
Sperm motility, %	0.5	0.001	0.1	0.7
Sperm morphology, %	-0.05	0.8	0.2	0.3

to changes in spermatogenesis are unknown (19). It is believed that seminal ROS may be one of the key factors in the pathogenesis of this complication (4).

Higher levels of seminal ROS have been reported in 25% of infertile men. Excessive amounts of ROS may lead to lipid peroxidation, loss of motility, and sperm DNA damage (20, 21). There are high amounts of polyunsaturated fatty acids in the plasma membranes of spermatozoa, therefore, they are predisposed to oxidative damage (5, 22). In addition, ROS could induce base alterations, DNA strand breaks, DNA cross-links, and chromosomal rearrangements (23).

Several studies have shown elevated reactive oxygen species and decreased total antioxidant capacity levels in varicocele infertile men. In 2010, Abd-Elmoaty et al. observed higher levels of oxidants and lower levels of antiox-

idants in the seminal fluid of infertile men with varicocele compared to controls (24). Increased ROS and DNA fragmentation, and decreased antioxidant levels and TAC have been observed both in infertile and varicocele individuals (25, 26). In addition, several reports have shown a significant association between ROS levels and TAC with sperm parameters in infertile and varicocele individuals, and they agreed that these abnormal levels of antioxidants and ROS are complicated by the occurrence of oligospermia, sperm motility defects, and/or abnormal sperm morphology (27, 28).

Similar to the results of the current study, Pasqualotto et al. found that the mean semen quality scores of the infertile men with varicocele were lower compared to control subjects. In addition the infertile men with varicocele had higher ROS levels but lower TAC levels compared to the healthy subjects (29). Although Giulini et al. observed no significant differences in peripheral blood TAC concentrations between controls and infertile men with varicocele, lower TAC concentrations were shown in patients with varicocele and moderate or severe oligoasthenozoospermia compared to controls and normozoospermic patients with varicocele. TAC levels were also positively correlated with sperm concentration and motility (28). In a meta-analysis, which was performed by Agarwal et al. higher ROS and lower total antioxidant capacity levels in the varicocele population compared to fertile men have been shown (30).

In a study by Chen et al. a decrease in ROS level following varicocele repair has been reported also. Therefore, varicocele repair may restore spermatogenesis, improve semen parameters, and decrease DNA damage via decreases in ROS levels (31). In addition, several studies have shown higher MDA levels as lipid peroxidation markers in infertile men. Mostafa et al. found higher levels of MDA in the internal spermatic venous blood compared to those in the peripheral fluid (32). Similarly, in a study by Altunoluk et al., MDA levels were greater in the varicocele group (33).

In conclusion, lower levels of TAC and higher levels of MDA were found in infertile men with varicocele. A positive correlation was revealed between TAC and sperm count, and total sperm and sperm motility in infertile men with varicocele and sperm motility in fertile men.

In addition a negative correlation was shown between sperm motility, sperm morphology, and sperm count and MDA level only in idiopathic infertile men, while volume, sperm count, total sperm, sperm motility, sperm morphology correlated negatively with MDA level in varicocele infertile men. No correlation between these parameters and MDA were observed in fertile men.

Therefore, the TAC and MDA assays, as oxidative stress markers, could be useful in evaluation of infertility caused by varicocele. In addition, these markers may serve as a guide in antioxidant therapy.

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Footnote

Authors' Contribution: Saeedeh Salimi: study concept and design, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis; Faramarz Fazeli: study concept and design, acquisition of data, analysis and interpretation; Paria Khosravi: study concept and design, acquisition of data; Sima Nabizadeh: drafting of the manuscript, critical revision of the manuscript for important intellectual content.

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