

Correlation of Seminal Plasma Total Antioxidant Capacity and Malondialdehyde Levels With Sperm Parameters in Men With Idiopathic Infertility

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Received 2015 May 09; Revised 2015 July 14; Accepted 2015 July 17.

Abstract

Background: Oxidative stress is the result of an imbalance between the production and scavenging of reactive oxygen species (ROS). Recently, oxidative stress has been introduced as a major cause of male infertility.

Objectives: The aim of the present study was to determine the correlation between total antioxidant capacity (TAC) and malondialdehyde (MDA) as markers of oxidative stress in relation to idiopathic male infertility and sperm parameters.

Patients and Methods: This case control study was conducted using 35 men with idiopathic infertility and 34 men with proven fertility. Seminal plasma TAC and MDA were measured by ferric reducing ability of plasma (FRAP) and thiobarbituric acid (TBA) reaction methods, respectively.

Results: Seminal TAC levels were significantly lower and seminal MDA levels were significantly higher in men with idiopathic infertility than in fertile men ($P < 0.0001$ and $P = 0.004$, respectively). A positive correlation was shown between sperm motility, sperm morphology, and TAC levels in men with idiopathic infertility ($P = 0.002$ and $P = 0.002$, respectively). In addition, there was a correlation between sperm motility and TAC levels in fertile men ($P = 0.005$). There was no correlation between sperm count and TAC levels in either men with idiopathic infertility or in fertile men. Negative correlations were observed between MDA levels and sperm motility, morphology, and sperm count only in men with idiopathic infertility ($P = 0.003$, $P = 0.001$, and $P = 0.006$, respectively).

Conclusions: Our results show that oxidative stress could play an important role in male infertility as well as in sperm motility and sperm morphology.

Keywords: Infertility, Male, Malondialdehyde, Semen, Total Antioxidant Capacity

1. Background

Infertility is a major social and medical problem worldwide. Studies in normal couples reveal that 90% of them will conceive within one year of unprotected intercourse. Thus, the classic definition of infertility became the absence of conception after one year of regular and unprotected intercourse (1). However, because a small number of normal couples will conceive between 12 and 24 months, the world health organization (WHO) has suggested that absence of conception after 24 months of unprotected intercourse is the preferred definition of infertility (2). The prevalence of infertility is increasing, and at present 10 - 15% of all couples in worldwide suffer from infertility (3). The male factor seems to be responsible for approximately 50% of the cases (4). There are several known etiologies of infertility, such as male accessory gland infection, hypogonadotropic hypogonadism, retrograde ejaculation, and positive anti-sperm antibody, but despite technical ad-

vances, the exact etiology of male infertility is not clear in 25% of all cases. Idiopathic male infertility is also known as idiopathic oligoasthenospermia, which indicates that the men have an unexplained reduction in semen quality (5).

Recently, oxidative stress has been shown to be an important cause of idiopathic male infertility (6). Oxidative stress is a result of an imbalance between the production of reactive oxygen species (ROS) and their neutralization or scavenging by the antioxidant system. ROS are inherent byproducts of aerobic life, and oxidative stress occurs when they overcome our natural ability to detoxify ROS-induced damage (7, 8). It is believed that controlled levels (physiological levels) of these ROS are necessary for sperm physiology, maturation, capacitation, acrosomal reactions, and normal fertilization. However, uncontrolled production of ROS (pathological levels) causes sperm dysfunction, including lipid peroxidation, sperm DNA damage, and loss of motility (9).

The excessive production of ROS could originate from endogenous sources, including immature/abnormal spermatozoa and leukocytes, or from environmental sources such as cigarette smoking and alcohol (10).

In fertile men, total antioxidant capacity (TAC) and ROS production remain in balance. However, pathological conditions such as autoimmune disorders, chronic disease, alcohol consumption, advanced age, smoking, infections, and stress result in oxidative stress (7). Antioxidants in the seminal fluid play a key role in antioxidant defense mechanisms. Therefore, relatively low concentrations 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 (11). As a result, the most important form of antioxidant defense available to spermatozoa is the antioxidants in seminal fluid. Antioxidants naturally found in semen include vitamins, glutathione, thioredoxin, and superoxide dismutase (11). Several studies have indicated that high levels of ROS in seminal fluid increase the risk of male infertility (12, 13).

Sperm count and sperm motility are important parameters that determine the functional ability of spermatozoa. Low sperm motility, which is called asthenozoospermia, is considered to be associated with male infertility. Although the factors that could affect sperm motility are not well known, oxidative stress, which is induced by ROS, may be an important factor in this condition (14).

The antioxidant power of biological fluids such as seminal fluid can be assessed either with measurement of each antioxidant or with total antioxidant capacity (TAC). Malondialdehyde (MAD) is used to measure the degree of peroxidation damage in spermatozoa (15).

2. Objectives

Because the findings about the correlation of seminal plasma TAC and MAD with sperm parameters are controversial, the purpose of this study was to explore the total antioxidant capacity (TAC) and malondialdehyde (MAD) levels in the seminal fluid of infertile and fertile men and their relationship with sperm parameters.

3. Patients and Methods

This study was performed using 35 consecutive infertile men with the complaint of infertility (absence of conception after 12 months of regular and unprotected intercourse) within the last year that were referred to the urology clinic in Ali-ebne-Abitaleb and Khatamolambia hospitals in Zahedan from August, 2013 to March, 2014. Thirty-four men with proven fertility were considered as controls.

All patients and healthy controls with specific genital diseases, genital infections, undescended testes, testicular atrophy, or 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 world health organization guidelines 2010 (2).

Written informed consent was obtained from the patients and controls. The project has been approved by the ethics committee of research of the Zahedan university of medical sciences.

For determination of the percent normal morphology of spermatozoa, the hematoxylin-eosin (H and E) staining method was used. Morphology of the spermatozoa was assessed using Kruger's criteria that morphology < 14% is considered abnormal (16).

3.1. TAC assay

TAC was assessed using ferric reducing ability of plasma (FRAP) according to the method of Benzie et al. (17). We measured the ability of seminal plasma antioxidants in reduction of ferric-tripyridyltriazine (Fe^{3+} -TPTZ) to a ferrous form (Fe^{2+}). The working FRAP reagent was prepared by 10 vol. 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 one volume of 20 mmol/L $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. 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 ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 1000, 500, 250, 125 μM) were added to the 1.5 mL of FRAP reagent and heated to 37°C for 10 minutes. Absorbance was measured at 593 nm using a spectrophotometer (UV-visible). The final results are shown as mmol/L.

3.2. MDA Assay

Seminal MDA levels were measured according to the method described by Rao et al. (18). This method is based on thiobarbituric acid (TBA) reaction and extraction with normal butanol. 1,1,3,3-tetramethoxypropane was used as the standard. Spectrophotometric detection of absorbance was performed at 532 wave length and compared with the standard curve. TBA was purchased from Merck.

3.3. Statistical Analysis

Data were analyzed using the statistical software SPSS-18 (SPSS, Chicago, IL). Data have been presented as mean \pm SD. The normal distribution of data was analyzed using the

Kolmogorov-Smirnov (KS) statistical test. Comparison between two groups was performed using the independent sample t-test or Mann-Whitney U test whenever appropriate. In addition, Pearson's correlation coefficient test was used 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 shown in Table 1. There were significant differences in semen volume, sperm motility, sperm morphology, and sperm count between men with idiopathic infertility and fertile men. The TAC level was higher in fertile men than in men with idiopathic infertility (1.8 ± 0.4 vs. 1.3 ± 0.2 mmol/L, $P < 0.0001$). In addition MDA level was significantly lower in fertile men than in men with idiopathic infertility (2.6 ± 1 vs. 3.4 ± 1.5 mmol/L, $P = 0.004$).

A positive correlation was observed between sperm motility, sperm morphology, and TAC levels in men with idiopathic infertility (Figure 1A and B). Although there was a correlation between sperm motility and TAC levels in fertile men (Figure 2), sperm morphology was not correlated with TAC in this group. There was no correlation between sperm count and TAC levels in either men with idiopathic infertility or fertile men.

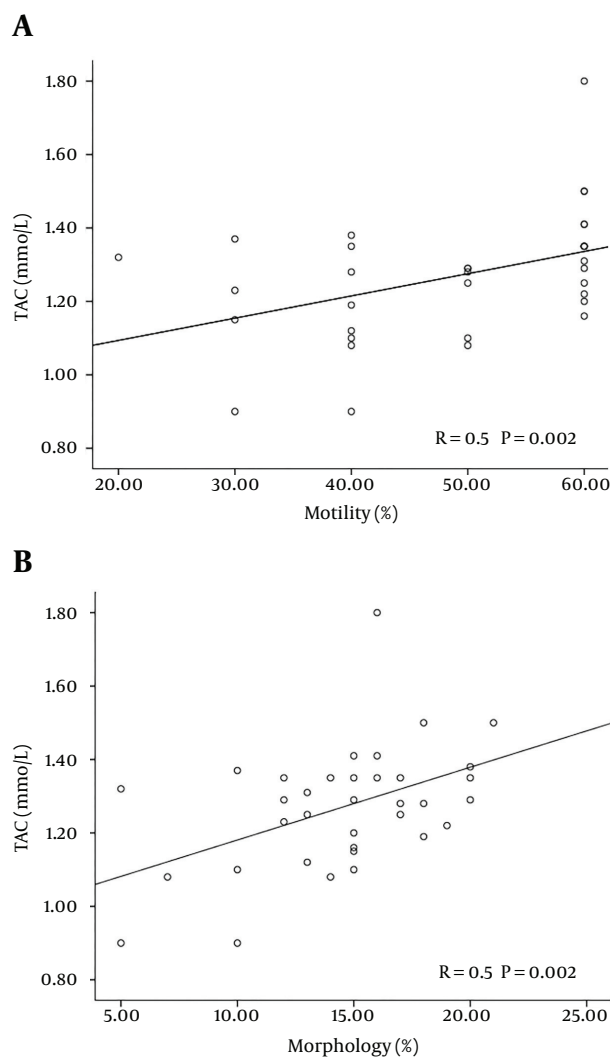
In addition, a negative correlation was identified between sperm motility, normal sperm morphology, and sperm count and MDA level in men with idiopathic infertility (Figure 3A - C). There was no correlation between these parameters and MDA in fertile men.

5. Discussion

In the current study, higher levels of MDA and lower levels of TAC were observed in men with idiopathic infertility than in fertile men. A positive correlation was shown between sperm motility, normal sperm morphology, and TAC levels in men with idiopathic infertility and between sperm motility and TAC levels in fertile men. A negative correlation was observed between sperm motility, normal sperm morphology, and sperm count and MDA levels only in men with idiopathic infertility.

Male infertility is a serious health problem and, in spite of major advances in its diagnosis and treatment, its specific etiology remains unknown. However, it is believed that seminal oxidative stress could be one of the main factors in the pathogenesis of this condition (7). In addition, it is reported that 25% of infertile men have high levels of seminal ROS, which could cause lipid peroxidation, loss of motility, and DNA sperm damage (19, 20). Because spermatozoa possess high amounts of polyunsaturated fatty acids

Figure 1. The Correlation of Seminal TAC Level



A, sperm motility; B, sperm morphology in men with idiopathic infertility.

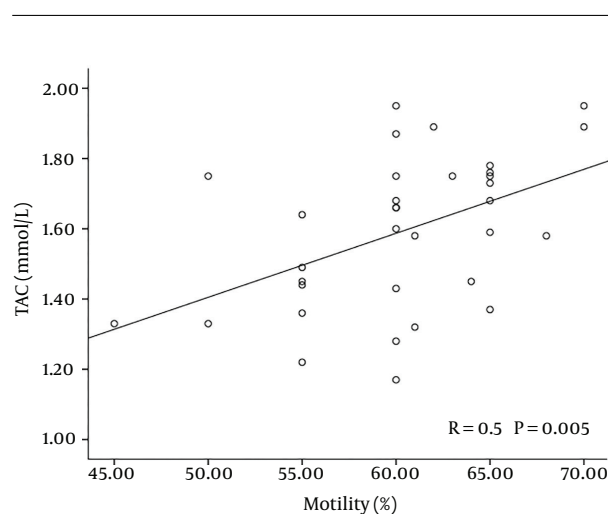
in their plasma membranes, they are predisposed to oxidative injury. Therefore, they are susceptible to radical attack and consequently to lipid peroxidation in the plasma membrane (8, 21). ROS could also induce base alterations, DNA strand breaks, DNA cross-links, and chromosomal rearrangements (22).

Different studies have revealed that seminal antioxidant capacity is lower in infertile men with high ROS levels. In 2009, Mahfouz et al. evaluated cutoff value, sensitivity, specificity, and intra- and inter-observer variability of TAC in the seminal plasma of fertile and infertile men and reported that seminal plasma TAC was higher in fertile men. Moreover, they showed that the best cutoff point

Table 1. Semen Parameters, TAC, and MDA Levels in Men With Idiopathic Infertility and Fertile Men^a

Semen Parameters	Men With Idiopathic Infertility (n = 35)	Infertile Men (n = 34)	P Value
Volume, mL	2.9 ± 0.9	3.6 ± 1	0.004
Sperm count, × 10 ⁶ /mL	36 ± 7.5	62 ± 9	< 0.0001
Total SPERM, × 10 ⁶	105 ± 40	220 ± 68	< 0.0001
Sperm motility, %	31 ± 11	60 ± 6	< 0.0001
Sperm morphology, %	14.5 ± 4	26 ± 4.3	< 0.0001
TAC, mmol/L	1.3 ± 0.2	1.6 ± 0.2	< 0.0001
MDA, mmol/L	3.4 ± 1.6	2.6 ± 1	0.008

^aValues are presented as mean ± SD.

**Figure 2.** The Correlation of Seminal TAC Level and Sperm Motility in Fertile Men

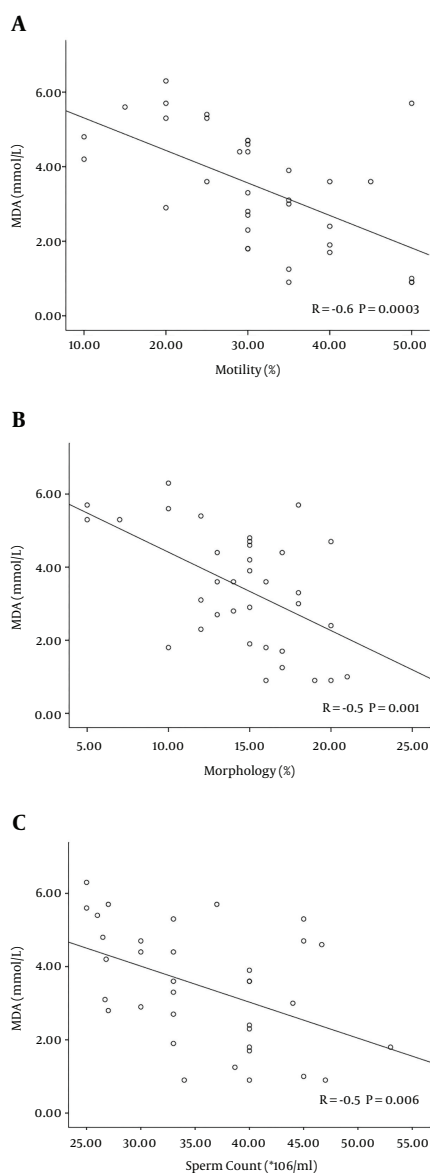
to distinguish between the two groups was 1420 microM (specificity 64% and sensitivity 76%) (23).

Hosseinzadeh Colagar et al. also demonstrated a correlation between lower levels of TAC and low sperm count, motility, and low rates of normal morphology in the seminal plasma of asthenoteratozoospermic and oligoasthenoteratozoospermic men. In addition, they documented higher MDA levels in the seminal plasma of asthenoteratozoospermic and oligoasthenoteratozoospermic men and MDA's negative correlation with sperm count, motility, and normal morphology (21). In another study, Patel et al. observed elevated levels of MDA in all infertile groups, except the azoospermic group, compared to fertile men. Indeed, they demonstrated a negative correlation between seminal MDA and normal sperm motility and morphology and suggested that free radicals could damage sperm membrane integrity (24).

Seminal MDA was significantly higher in infertile men than in controls in a study by Akbari-Asbagh et al. They observed an association between MDA and abnormal sperm morphology and seminal TAC and weak sperm motility. However, they did not find any association between smoking and sperm parameters in infertile men (25).

Similarly, Pahune et al. observed a positive correlation between seminal plasma TAC and seminogram parameters, including sperm concentration, sperm motility, and normal sperm morphology. Therefore, they suggested that decreased seminal TAC could play a key role in the etiology of impaired sperm functions (26). Koca et al. suggested that antioxidant capacity is positively related to sperm motility and that decreased antioxidant capacity could impair sperm function due to increased ROS production or insufficient antioxidant capacity (27). Appasamy et al. observed a negative correlation between antioxidant activity and sperm concentration, which suggests that oxidative stress could influence sperm concentration (28).

In a study by Omran et al. the normozoospermic samples showed lower DNA fragmentation and higher seminal plasma TAC levels than abnormal samples. In infertile subjects who had abnormal chromatin condensation, lower TAC levels were observed (29). Lower levels of TAC and catalase have been reported by Khosrowbeygi et al. in patients with abnormal seminal parameters. In addition, catalase and TAC levels were significantly lower in infertile patients and positively correlated with sperm motility and normal morphology. In addition, asthenozoospermic, asthenoteratozoospermic, and oligoasthenoteratozoospermic groups had significantly lower levels of catalase activity and TAC than normozoospermic males (30). Pasqualotto et al. showed that men with idiopathic infertility had lower sperm concentration, sperm motility, reduced rates of normal morphology, and lower semen quality scores than controls. The ROS levels were higher and TAC levels were lower in idiopathically infertile men (31).

Figure 3. The Correlation of Seminal MDA Level

A, sperm motility; B, normal sperm morphology; C, sperm count in men with idiopathic infertility.

Recently, Yousefniapasha et al. reported that sperm parameters were significantly higher in fertile men than in infertile men; however, the differences were not significant between the infertile non-smoker and the fertile non-smoker groups. Although the seminal plasma TAC was higher in fertile non-smokers than in infertile non-smokers and infertile smokers, the differences were not significant (32).

Lower total antioxidant capacity and vitamin E lev-

els were observed in the seminal plasma of infertile men in a study by Benedetti et al. Like those of the current study, Benedetti et al.'s results indicated higher MDA levels in infertile patients, which was negatively correlated with sperm motility and normal morphology. In addition, lower concentrations of TAC, carotenoids, and vitamin E were documented in blood samples of infertile men, and TAC and carotenoids were positively associated with sperm motility, normal morphology, and concentration (33).

Because different studies have demonstrated the effects of oxidative stress on male infertility, several investigations have attempted to study the effects of antioxidant supplements on these conditions. Although the effects of different doses and types of oral antioxidants in attempts to improve semen parameters have not been established, it is believed that antioxidant therapy has beneficial effects on male infertility (34, 35). However the results of antioxidant therapy alone or in combination are controversial. For example, some studies have shown that vitamin C, vitamin E, zinc, folic acid, and selenium alone or in combination could improve sperm parameters, especially sperm motility and sperm DNA integrity (36-39). In contrast, several reports refute the effects of these antioxidants on sperm parameters (40, 41).

In conclusion, higher levels of MDA and lower levels of TAC were observed in men with idiopathic infertility. A positive correlation was revealed between sperm motility, normal sperm morphology, and TAC levels in men with idiopathic infertility and between sperm motility and TAC levels in fertile men. In addition, a negative correlation was observed between sperm motility, normal sperm morphology, and sperm count and MDA levels only in idiopathically infertile men. Therefore, TAC and MDA could be used as oxidative stress markers, and their assays could be useful in the evaluation of idiopathic infertility. In addition, these markers may guide us in antioxidant therapy.

Acknowledgments

This article is an extract from an MD thesis (registered number 1340) at the Zahedan university of medical sciences. The authors thank the Zahedan deputy of research affairs for funding this project.

Footnotes

Authors Contribution: Faramarz Fazeli: study concept and design, acquisition of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, administrative, technical, and material support; Saeedeh Salimi: study concept and design, acquisition of data, analysis and interpretation of

data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, administrative, technical, and material support, study supervision.

Funding/Support: This article was supported by a grant (registered number 1340) from the Zahedan university of medical sciences.

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