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Relation of Serum and Semen Malondialdehyde and Total Anti-Oxidants with Sperm Parameters in Infertile Men

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Abstract: Problem statement: Oxidative Stress (OS) has been recognized as one of the most important cause of male infertility. We studied the relation of serum and Semen Malondialdehyde (MDA) and Total Antioxidant Capacity (TAC) with sperm parameters in infertile men with sperm count within the normal range. **Approach:** The prospective case- control study performed on infertile men presenting to the infertility clinics of Mirzakochak khan hospital, Tehran university of Medical Sciences from June 2007 to June 2009. The samples were collected consecutively and the total of 40 infertile men was enrolled in the study. Also, 40 healthy men were matched as control group. MDA and TAC in serum and seminal plasma were measured and relation between them and semen analysis parameters were evaluated. The MDA was measured as nmol mL^{-1} and the TAC was expressed as g dL^{-1}. **Results:** Analysis showed that the amount of semen MDA was statistically different in infertile and healthy control groups. We did not find any significant relation between smoking and sperm parameters in infertile men. The relation between semen MDA and abnormal sperm abnormal morphology ($p = 0.003$, $r = -0.468$) and semen TAC and weak sperm motility ($p = 0.037$, $r = -0.359$) was significant. **Conclusion:** Immediate attention should be directed at simplifying and validating the evaluation of reactive oxygen species and OS status so that it can be performed routinely.

Key words: Malondialdehyde (MDA), Total Antioxidant Capacity (TAC), semen analysis, parameters, morphology, Oxidative Stress (OS), validating, seminal plasma, spermatozoa, epididymis

INTRODUCTION

Infertility is a major clinical problem, affecting people medically and psychosocially. Statistics indicate that 15% of all couples in the United States are infertile and the male factor is responsible for 25 of these cases (Makker *et al*., 2009; Sharlip *et al*., 2002; Han *et al*., 2010). Infertility is associated with Oxidative Stress (OS), normally counterbalanced by different antioxidant systems (Mancini *et al*., 2009; Golbidi and Laher, 2010I). OS has been recognized as one of the most important cause of male infertility (Lanzafame *et al*., 2009; Desai *et al*., 2009; Pons-Rejraji *et al*., 2009; Kefer *et al*., 2009; Varghese *et al*., 2008; Tempest *et al*., 2008; Gallardo, 2007).

Despite the antioxidant activity of seminal plasma, epididymis and spermatozoa, OS damages sperm function and DNA integrity (Lanzafame *et al*., 2009; Desai *et al*., 2009). OS is a common pathology seen in approximately half of all infertile men. Peroxides causing infertility are generated by sperm and seminal leukocytes. Oxidative stress occurs when production of potentially destructive reactive oxygen species exceeds

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natural antioxidant defenses resulting in cellular damage (Bozhedomov *et al*., 2009; Tremellen, 2008).

Malondialdehyde (MDA) is one of the final products of lipid peroxidation in seminal plasma (Shang *et al*., 2004). Toxic lipid peroxides are known to cause various impairments of sperm cells and may play a major role in the etiology of male infertility. Malondialdehyde (MDA) is an index of lipid peroxidation which may be a diagnostic tool for the analysis of infertility (Tavilani *et al*., 2005; 2008). Kumar *et al*. (2009) found excess ROS (Reactive oxygen species) and low antioxidant levels in the semen of infertile Oligoasthenozoospermic (OA) men. In their study, semen MDA and ROS levels of infertile group were significantly higher than their level in control group. However, antioxidants levels were significantly lower in infertile group, compared to controls.

We studied the relation of serum and semen Malondialdehyde (MDA) and Total Antioxidant Capacity (TAC) with sperm parameters in infertile men with sperm count within the normal range.

MATERIALS AND METHODS

This is a prospective case- control study performed on infertile men presenting to the infertility clinics of Mirzakochak khan hospital, Tehran University of medical science from June 2007-June 2009. The samples were collected consecutively and the total of 40 infertile men was enrolled in the study.

Inclusion criteria were the males aged >22 years which married for more than one year, having normal sperm count (sperm count of $\geq 20\times10^6$ mL^{-1}) (Engel *et al*., 1999; Mansi *et al.*, 2007), none were taking an oral antioxidant supplement for three months prior to the study and their wives have not been pregnant from their marriage. Exclusion criteria were intercourse problems, chronic disease including diabetes mellitus, substance, alcohol or drug abuse, semen samples with more than 1×10^6 mL^{-1} neutrophils using peroxidase staining (World Health Organization WHO), 1999; Marjani *et al.*, 2008) or other round cells and use of contraception methods within last year before presentation. Also, 40 healthy men were matched about age and were selected as control group. Written informed consent was obtained from all enrollees, according to the criteria of the Ethical Committee of Tehran University of Medical Sciences.

All participants were evaluated preoperatively and the baseline information was obtained including age, weight, smoking, drinking, duration of infertility and medication. Also, clinical findings including abnormal symptoms and signs were recorded. The spermatozoid motility was expressed as grade a (rapid progressive), b (slow progressive), c (non-progressive), d (immotile) were determined using WHO standard procedures (1999) which was classified as weak (c or d) and progressive (a or b).

All semen samples were collected by masturbation following 3 days of abstinence. The blood and semen sampling was followed by their plasma isolation. The semen and blood plasma were stored in -20°C. Then, Malondialdehyde (MDA) and Total Anti-oxidant Capacity (TAC) of them were measured every 6 months. The MDA was measured as nmol mL^{-1} and the TAC measured as g dL^{-1}.

Measurement of TAC levels: The total antioxidant status of the plasma was measured using a novel automated direct measurement method using a new generation, more stable ABTS (2,2-azinobis 3-ethylbenzothiazoline-6-sulfate) radical action (Erel, 2004; Xiaoming *et al.*, 2007)). In novel methods, a type of radical is generated in the assay and the antioxidant activity of the sample against the radical is measured. The most common method is ABTS-based method in which a colorless molecule, reduced ABTS, is oxidized to a blue-green ABTS. When the colored ABTS is mixed with any substrate that can be oxidized, it is reduced to its original colorless form again (Erel, 2004; Mansi *et al.*, 2007).

Measurement of MDA levels: Lipid peroxidation in spermatozoa and seminal plasma was measured by reaction of Thiobarbiuric Acid (TBA) with Malondialdehyde (MDA) (Tavilani *et al*., 2008). Content of MDA was measured spectrofluorometrically using a Jasco (FP-6200) spectrofluorometer (excitation 515 nm, emission 553 nm). The MDA fluorescence intensity of spermatozoa and seminal plasma was determined using various concentrations of tetraethoxypropane as standards. The results were expressed as nmoL MDA/10×10^6 cells, nmoL MDA mL^{-1} seminal plasma and nmoL MDA/total seminal plasma.

Statistical analysis: The collected data were analyzed by SPSS-15 statistical software. The collected data were expressed as percentage and mean±SD. Continuous (quantitative) variables were compared by Student T-test (Independent samples) or ANOVA one way. Categorical (qualitative) variables were compared by contingency tables and Chi-square test or Fisher's exact test. P-value ≤ 0.05 was considered statistically significant.

RESULTS

The average age was 33.7 year (range: 32.5-35y) in infertile (case group) and 34.7 years (range: 32.5-37y) in healthy men (control group) ($p>0.05$). As showed in Table 1, the patients' job was not significantly different in both groups.

Table 1: Comparison of the patients' job in both groups

	Occupation					
Group	Free	Worker	Employee	Farmer	Driver	Industrial
Infertile (Case)	4 (12.1%)	10 (30.3%)	14 (42.4%)	1 (3.0%)	2 (6.1%)	2 (6.1%)
Fertile (Control)	0 (0%)	9 (33.3%)	9 (33.3%)	0 (0%)	1 (3.7%)	8 (29.6%)

Table 2: The mean values of serum and plasma MDA and TAC

	Group	Mean	SD	p-value
Serum MDA	Infertile	3.4551	1.16502	<0.05
	Fertile	3.2318	0.94052	
Serum TAC	Infertile	3.0259	0.69407	<0.05
	Fertile	3.1755	0.61584	
Semen MDA	Infertile	3.0946	0.84501	0.013
	Fertile	2.6068	0.86426	
Semen TAC	Infertile	4.5521	1.04654	<0.05
	Fertile	4.8110	0.75968	

Table 3: The effect of cigarette smoking on serum and semen variables

	Smoking	Mean	SD	p-value
Serum MDA	No	3.3430	1.15580	<0.05
	Yes	3.6864	1.28447	
Serum TAC	No	2.9804	0.66664	<0.05
	Yes	3.1791	0.79113	
Semen MDA	No	3.2487	0.89758	<0.05
	Yes	2.9345	0.85535	
Semen TAC	No	4.4683	1.16991	<0.05
	Yes	4.8455	0.98257	

The mean values of serum and plasma MDA and TAC are showed in Table 2. Analysis showed that only the amount of semen MDA was statistically different in both groups (Table 2).

Table 3 shows the effect of cigarette smoking on serum and semen variables in infertile men. Analysis did not show any significant relation between smoking and studied variables in infertile men (Table 3).

We studied the relation of serum and semen MDA and TAC level with variables including sperm morphology, motility grade, duration of infertility and patient occupation. As showed in Table 4, only the relation between semen MDA and abnormal sperm morphology ($p = 0.003$, $r = -0.468$) and semen TAC and weak motility ($p = 0.037$, $r = -0.359$) was significant.

DISCUSSION

The male factor is considered a major contributory factor to infertility. Apart from the conventional causes for male infertility such as varicocele, cryptorchidism, infections, obstructive lesions, cystic fibrosis, trauma and tumors, a new and important cause has been identified: oxidative stress ((Makker *et al*., 2009). Oxidative Stress (OS) is a result of the imbalance between Reactive Oxygen Species (ROS) and antioxidants in the body. It is a major etiological cause of sperm damage, deformity and eventually, male infertility (Makker *et al*., 2009; Agarwal *et al*., 2009). OS is a common pathology seen in approximately half of all infertile men. Increased Radical Oxidative Species (ROS) generation and reduced antioxidant capacity is negatively correlated with sperm concentration and motility in infertile men (El-Taieb *et al*., 2009).

OS is induced by Reactive Oxygen Species (ROS), or free radicals and although ROS are required for critical aspects of sperm function, excessive levels of ROS can negatively impact sperm quality (Kefer *et al*., 2009). ROS have an important effect on sperm quality and quantity. Oxidative Stress (OS) occurs when production of potentially destructive ROS exceeds the body's own natural antioxidant defenses, resulting in cellular damage (El-Taieb *et al*., 2009). Aydemir *et al*. suggest that increased oxidative damage might be a factor for hyperviscosity of seminal plasma in infertile males (Aydemir *et al*., 2008).

Lipid peroxidation has a deleterious effect on the semen quality and may play a major role in the etiology of male infertility (Tavilani *et al*., 2005; 2008). Malondialdehyde can be used as a marker of oxidative stress and a potential marker in predicting assisted reproductive techniques outcome (Oral *et al*., 2006; Jedrzejczak *et al*., 2005). One can determine the level of lipid peroxidation as indicated by in the seminal plasma (Tavilani *et al*., 2005; 2008). The deleterious effect of free radicals on spermatozoa may be assessed by estimating malondialdehyde in seminal plasma and their relation with different sperm parameters (Patel *et al*., 2009). Patel *et al*. (2009) analyzed 22 fertile controls with 74 primary infertile males. Seminal malondialdehyde level was observed to be raised in all infertile groups except azoospermic cases in comparison to control. Significant positive correlation between malondialdehyde with total sperm count indicates its contribution towards free radical generation. The negative association of semen malondialdehyde with normal sperm motility and morphology suggests damaging effect of free radicals on sperm membrane integrity (Patel *et al*., 2009). In agreement with these studies, our findings show the relation between semen MDA and abnormal sperm abnormal morphology ($p = 0.003$, $r = -0.468$) and semen TAC and weak sperm motility ($p = 0.037$, $r = -0.359$) was significant.

Table 4: Assessment of relationships in infertile group

	Serum MDA		Serum TAC		Semen MDA		Semen TAC	
	r	p-value	r	p-value	r	p-value	r	p-value
Abnormal Morphology	0.049	0.768	0.075	0.652	-0.468	0.003	-0.287	0.076
Weak motility Grade d	-0.161	0.228	-0.221	0.095	0.075	0.574	-0.124	0.352
Weak motility Grade c	0.098	0.581	-0.083	0.642	0.129	0.466	-0.359	0.037
Progressive Motility (Grade b)	0.447	0.082	-0.017	0.951	0.396	0.129	0.054	0.843
Progressive Motility (Grade a)	?	?	?	?	?	?	?	?
Duration of infertility	0.067	0.694	-0.178	0.291	-0.114	0.503	-0.025	0.884
Patient Occupation	?	?	?	?	?	?	?	?

Giulini *et al*. (2009) found no significant differences in serum TAC (Total antioxidant capacity) between controls and groups (varicocele cases). In patients with varicocele and moderate or severe oligoasthenozoospermia, seminal TAC was significantly lower than controls and normozoospermic patients with varicocele. Moreover, in patients with severe oligosthenozoospermia, seminal TAC was also significantly lower than in asthenozoozpermic patients with varicocele. In all subjects, seminal TAC showed a positive correlation with sperm concentration and motility (Giulini *et al*., 2009). One study showed that TAC was negatively related to sperm concentration. However, there was no significant relationship between hormone concentrations, sperm DNA damage and total antioxidant capacity, suggesting other mechanisms for sperm dysfunction (Appasamy *et al*., 2007).

Taking into account the pros and the cons of antioxidant treatment of male infertility, the potential advantages that it offers cannot be ignored. Therefore, antioxidant therapy should remain in the forefront of preventive medicine, including human reproductive medicine (Lanzafame *et al*., 2009). Because oxidative stress is a major cause of sperm DNA damage then antioxidants should have an important therapeutic role to play in the clinical management of male infertility (World Health Organization (WHO), 1999; Ismail *et al.*, 2009). Study of aspects of oxidative stress help us to design better antioxidant trials in future, with emphasis on identifying (Makker *et al*., 2009) appropriate doses (Sharlip *et al*., 2002; Han *et al.*, 2010) selecting the right populations ((Mancini *et al*., 2009) treating for optimal durations and (Golbidi and Laher, 2010) specific intracellular targeting mechanisms (Golbidi and Laher, 2010). Understanding the physiologic and pathologic effects of free radicals on sperm function will help in designing new and effective treatment strategies in male infertility (Jedrzejczak *et al*., 2005).

Reactive Oxygen Species (ROS) levels in semen are believed to play both physiological and pathological roles in male fertility (Venkatesh *et al*., 2009). Increased ROS levels also have been correlated with decreased sperm motility. However, the exact mechanism through which ROS causes decreased motility is not understood (Makker *et al*., 2009). Not only do antioxidants prevent reduction in sperm motility, these also increase sperm motility (Makker *et al*., 2009). A randomized double-blind controlled trial has shown that vitamin E administered orally (300 mg day^{-1}) results in a decrease in malondialdehyde (a) marker for lipid peroxidation) concentration in spermatozoa and improved sperm motility (Suleiman *et al*., 1996; Ismail *et al.*, 2009). Elevated ROS levels in the idiopathic Indian infertile men is one of the underlying reasons for impaired fertility. Therefore measurement of seminal oxidative status may be used in infertile men for better understanding of the etiology and selection of antioxidant regimen in the treatment of male infertility (Venkatesh *et al*., 2009).

Tobacco smoke consists of approximately 4,000 compounds such as alkaloids, nitrosamines and inorganic molecules and many of these substances are reactive oxygen or nitrogen species. Significant positive association has been reported between active smoking and sperm DNA fragmentation, as well as axonemal damage and decreased sperm count. Levels of DNA strand breaks (Makker *et al*., 2009). In a study carried out on 655 smokers and 1131 non smokers, cigarette smoking was associated with a significant decrease in sperm density (-15.3%), total sperm count (-17.5%) and total number of motile sperm (-16.6%) (Künzle *et al*., 2003; Ismail *et al.*, 2009). Thus, smoking can affect the quality and quantity of sperm present within a male (Makker *et al*., 2009). However, we did not find any significant relation between smoking and sperm parameters in infertile men.

Oxidative stress is now recognized as a common pathology that affects up to half of all infertile men. One of the principal mechanisms by which oxidative stress produces infertility is by damage to sperm DNA, either through direct oxidation of the DNA by ROS or by the initiation of apoptosis. Antioxidant therapy resulted in significant improvements in sperm DNA integrity, accompanied by a reduction in seminal ROS production and apoptosis (Tunc *et al*., 2009). OS,

characterized by increased free radicals, may lead to oxidation of nucleotides of sperm genome. Shamsi *et al*. (2009) study, increased malondialdehyde (a product of lipidperoxides) levels, abnormal sperm morphology and higher DNA damage were observed in infertile men. The antioxidants superoxide dismutase, catalase and glutathione had a positive association with sperm count and motility while a negative association with the percentage of dead sperms and abnormal morphology was observed (Shamsi *et al*., 2009).

Mostafa *et al*. (2009) compared two ROS parameters (malondialdehyde, hydrogen peroxide) and five antioxidants (superoxide dismutase, catalase, glutathione peroxidase, vitamin E and vitamin C) in the seminal plasma of fertile and infertile men. Compared with fertile healthy men, in all other studied groups, estimated seminal ROS were significantly higher and estimated antioxidants were significantly lower. Sperm concentration, total sperm motility as well as sperm normal forms were negatively correlated with seminal malondialdehyde and were positively correlated with vitamin C (Mostafa *et al*., 2009).

Pasqualotto *et al*. (2008) used ROS-TAC score to compare the semen quality score and the seminal oxidative stress Reactive Oxygen Species (ROS) and total antioxidant capacity in men with idiopathic infertility. They concluded that patients with idiopathic infertility have lower scores of semen quality and ROS-TAC (Pasqualotto *et al*., 2008).

Mahfouz *et al*. suggested that Total Antioxidant Capacity (TAC) of the seminal plasma as measured by the colorimetric assay is a reliable and simple test for the diagnosis and management of male infertility. They showed that the best cutoff to distinguish between fertile controls and infertile men is 1420 μM of TAC in the seminal plasma (Mahfouz *et al*., 2009). Microscopically abnormal semen showed significantly higher levels of MDA in seminal plasma as compared with normal semen. The percentage of non-motile spermatozoa showed significant positive correlation with MDA. Sperm counts showed a significant negative correlation with MDA level of seminal plasma. MDA of 3.15μmol L^{-1} is an optimum cut-off limits to discriminate abnormal semen from normal (Saraniya *et al*., 2008).

Tavilani *et al*. (2008) found positive correlations between TAC with total content of MDA in seminal plasma from normozoospermic samples. However, in asthenozoospermic samples, there were no such significant correlations. These findings indicate a protective role for antioxidant enzymes of seminal plasma against lipid peroxidation of spermatozoa in normozoospermic samples (Tavilani *et al*., 2008). Abdallah *et al*. (2009) found that seminal MDA was significantly higher in oligoasthenozoospermic and asthenozoospermic groups compared with normozoospermic group. Seminal MDA were positively correlated with mobility grade b, acrosome anomalies and residual cytoplasmic droplets. In contrast, seminal MDA was negatively correlated with sperm concentrations (Abdallah *et al*., 2009).

Studies suggested that detection of MDA concentrations in seminal plasma has an indicative value on the diagnosis of male infertility induced by overproduction of reactive oxygen species in male reproductive system (Shang *et al*., 2004). The evaluation of oxidative status and antioxidant defenses may be taken as an important tool for diagnosis and treatment of male infertility (Abdallah *et al*., 2009). The measurement of the antioxidative and oxidative agents could serve to evaluate human infertility in those cases where the result of the spematobioscopy appears normal (Gallardo, 2007). Although consensus is growing about the clinical utility of seminal oxidative stress testing in infertility clinics, standardization of protocols to measure ROS is crucial before introducing these tests into routine clinical practice (Deepinder *et al*., 2008).

CONCLUSION

Evaluation of OS status and the use of antioxidants is not a routine in clinical practice. Immediate attention should be directed at simplifying and validating the evaluation of reactive oxygen species and OS status so that it can be performed routinely. The dose and duration of therapeutic antioxidants should also be determined and standardized.

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