

Studying the Levels of Malondialdehyde and Antioxidant Parameters in Normal and Abnormal Human Seminal Plasma

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Abstract: Objective: To measure the seminal levels of malondialdehyde and antioxidants in men with both normal and abnormal seminogram. Setting: Andrology Clinic, Dr. Ahmad Fikry Medical Centre, Abu Dhabi, United Arab Emirates. Patients: 34 male subjects with normal and abnormal semen analysis were recruited during the period from November 2007-June 2008. Intervention: Seminal levels of malondialdehyde, glutathione, ascorbic acid and total antioxidant status were measured for all enrolled men. Results: Malondialdehyde level was significantly elevated in oligozoospermic and azoospermic men while glutathione, ascorbic acid and total antioxidant status were significantly reduced in the previous groups compared to normozoospermic group. Conclusion: Lipid peroxidation plays a significant role in disrupting sperm functions and semen quality especially sperm motility and morphology and may account for some cases of male infertility.

Key words: Lipid peroxidation, seminal plasma, male infertility

INTRODUCTION

Oxidative damage to sperm can reduce sperm motility, interfere with sperm-oocyte binding and fusion and it has been implicated as a cause of male infertility (Agarwal A., S.A. Prabakaran, 2005).

Peroxidation damage to the plasma membrane of sperms have been suggested as an important mechanism of male infertility (Aitken R.J., D. Buckingham, *et al.* 1992). The human sperm cell membrane is particularly susceptible to oxidation due to the existence of high concentration of polyunsaturated fatty acids (PUFA) in these membranes (Tavilani H., D. Mahmoud, 2005).

Physiologically, the high concentration of PUFA in sperm are integral for maintaining membrane fluidity and flexibility during fertilization process. Lipid peroxidation is known to cause impairments such as membrane damage, decrease in a chromosomal function (Aziz N., R.A. Saleh, 2004), damage to sperm chromatin and reduced sperm-oocyte fusion (Oyawoye O., G.A. Abdel, 2003; Pasqualotto E.B., A. Agarwal, 2004).

Seminal plasma malondialdehyde which is the stable lipid peroxidation product, is a simple method to evaluate the effect of lipid peroxidation on sperm (Geva E., J.B. Lessing, 1998). The presence of considerable amounts of antioxidants, e.g. vitamin C (ascorbic acid), vitamin E (tocopherol) as well as the enzymes superoxide dismutase, glutathione peroxidase and catalase have been described (Therond P., J. Auger, *et al.* 1996).

Also, an important endogenous antioxidant in humans is the tripeptide glutathione (L δ glutamyl-L cysteinyl-glycine, GSH), which plays a central role in the defense against oxidative damage and toxins. GSH and GSH-related enzymes might play a role in sperm quality (Maarten T.M., M.J. Hennie, 2003).

Ascorbic acid, a major water soluble antioxidant, acts as a major scavenger for a wide range of ROS. It is present at approximately 10 fold higher concentrations in seminal plasma compared with blood plasma, suggesting a physiological role in seminal plasma (Lewis S.E., E.S. Sterling, *et al.* 1997).

However, it is difficult to measure the effectiveness of one antioxidant in isolation of another because there appears to be cooperation between various antioxidants. Therefore, it has been focused on the measurement of the total antioxidant status in seminal fluid.

The aim of the study is to evaluate the levels of malondialdehyde, glutathione, ascorbic acid as well as total antioxidant status in seminal plasma of fertile and infertile males and the significance of any difference, if any, detected between both groups.

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MATERIAL AND METHODS

34 male subjects with average age of 36.2 ys were selected from Andrology Clinic, Ahmad Fikry Medical Centre, Abu Dhabi, United Arab Emirates prospectively during the period from November 2007-June 2008.

A written consent was given by all subjects, they were divided into 3 main categories: 15 oligozoospermic patients, 9 azoospermic patients and 10 normozoospermic males with proven fertility as controls. Cases with leucocytospermia or varicocele were excluded from the study because of their well-known high seminal ROS levels. A detailed medical history and andrological examination was performed for all studied cases. Semen samples were obtained by masturbation technique after 3 days of abstinence. Samples were examined immediately after liquefaction according to WHO guidelines (World Health Organization, 1999). Semen samples were verified after at least two different analyses with centrifugation.

Seminal plasma was separated at 5000 rpm for 15 minutes at room temperature after complete liquefaction.

Lipid peroxidation in seminal plasma was measured by the reaction of thiobarbituric acid with malondialdehyde according to (Yagi, 1984, and Storey, 1997).

For determination of GSH concentration, a precipitating solution was added to seminal plasma to precipitate all proteins in the sample. Glutathione content of seminal plasma was assayed by the Beutler method (Ochsendorf F.R., R. Buhl, *et al*, 1998).

Fresh ascorbic acid was estimated in seminal plasma samples colorimetrically (Srivastava A., S.K. Chopra, 1983). The total antioxidant status were assayed colorimetrically by a commercially available kits (Can Ag Diagnostic AB, Gothenburg, Sweden).

Statistical analysis was performed using Windows SPSS version 10. P-values of ≤ 0.05 were considered to indicate statistical significance. Variables presented were summarized as mean values \pm standard deviation. Data collected were processed with ANOVA and Pearson correlation coefficient.

RESULTS AND DISCUSSION

Results:

The mean levels of seminal plasma malondialdehyde (nmol/ml), glutathione ($\mu\text{mol/L}$), ascorbic acid (mg/dL) and total antioxidant status ($\mu\text{mol/L}$) of all studied groups were shown in Table (1).

Table 1:

	No.	Malondi-aldehyde	Glutathione	Ascorbic acid	Total antioxidant
Normozoo-spermic	10	1.13 \pm 0.19	7.09 \pm 0.96	14.52 \pm 2.6	413.9 \pm 68.9
Oligozoo-spermic	15	2.04 \pm 0.32	4.9 \pm 0.62	-	195.5 \pm 51.06
Azoospermic	9	2.8 \pm 0.41	3. 21 \pm 0.71	-	89.1 \pm 20.79
F-test		66.48	64.33	87.27	99.7
P-value		<0.001*	<0.001*	<0.001*	<0.001*

P < 0.05 =significant; P < 0.001 = highly significant*.

A highly significant elevation of mean malondialdehyde level was observed among azoospermic and oligozoospermic groups compared to normozoospermic one. F test = 66.48, P<0.001.

Meanwhile, a highly significant decrease of mean glutathione level was found in both azoospermic and oligozoospermic groups compared to normozoospermic one. F test = 64.33, P<0.001. Also, a highly significant decrease of mean ascorbic acid level and total antioxidant status was found in both azoospermic and oligozoospermic groups compared to normozoospermic one. F = 87.27 and F = 99.7 respectively as shown in Table (1). P<0.001

Correlations

A significant negative correlation was detected in normozoospermic cases between malondialdehyde and ascorbic acid levels were $r = -0.67$ and $P < 0.05$ as shown in Fig. (1). Meanwhile, a significant positive correlation was detected between glutathione levels and total antioxidant status levels in normozoo-spermic cases were $r = 0.74$ and $P < 0.05$ as shown in Fig. (2).

A significant negative correlation was found in oligozoo-spermic males between malondialdehyde and total antioxidant status where $r = -0.58$ and $P < 0.05$ as shown in Fig. (3).

In the azoospermic group, a significant negative correlations were detected between malondialdehyde versus ascorbic acid, glutathione and total antioxidant status wehre $r = -0.84$ -0.86 and -0.88 respectively and $P < 0.001$. Meanwhile in the same group a positive significant correlations were detected between ascorbic acid versus glutathione and total antioxidant status where $r = 0.82$, $r = 0.87$ respectively and $P < 0.001$.

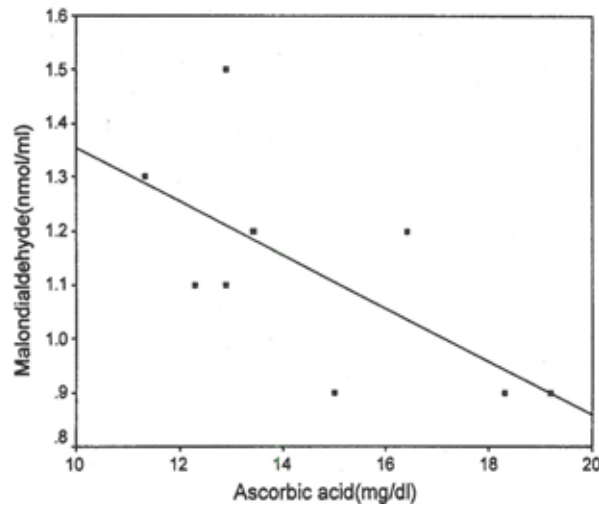


Fig. 1: Correlation between malondialdehyde and ascorbic acid levels in normozoospermic cases.

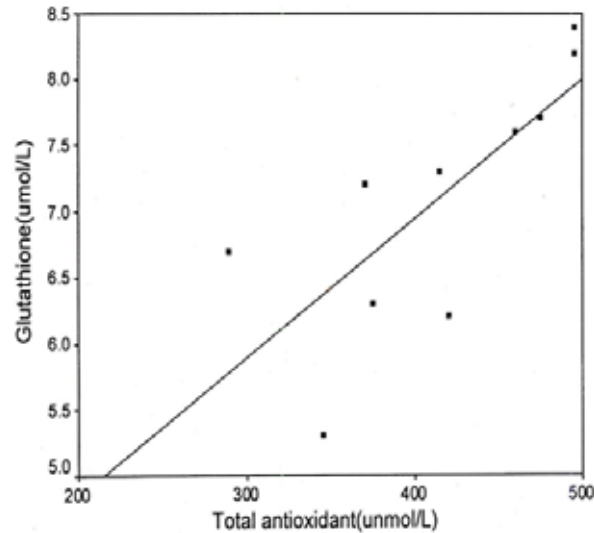


Fig. 2: Correlation between glutathione levels and total antioxidant status levels in normozoospermic cases.

Discussion

The Pathophysiology of male infertility could be explained by a cascade of molecular and biochemical events which represents itself in most of cases by abnormal semen parameters. Growing evidence indicates that imbalance between peroxidative and antioxidative substances in semen leads to metabolic and functional disorders of male germ cells and may be a primary cause of some types of infertility (Fraczek M., M. Kurpisz, 2007). It was reported that increased ROS in semen of infertile males may cause abnormal and immature spermatozoal morphology, motility and concentration (Gyun J.S., P. Edward, *et al*, 2006).

Extensive study on the peroxidation of phospholipids in mammalian sperm had demonstrated that peroxidation reaction causes membrane damage which leads to loss of motility and membrane integrity (Engel S., Th. Schreiner, 1999).

However, due to high density of mitochondria which may leak oxygen radicals in cytoplasm, the ability of spermatozoa to scavenge oxidants is limited. Therefore, the antioxidant capacity has to be present in seminal fluid as well. That is why protection against ROS and prevention of other damage are of critical importance and can be provided by both enzymatic and non-enzymatic antioxidants (Geva E., J.B. Lessing, *et al*, 1998).

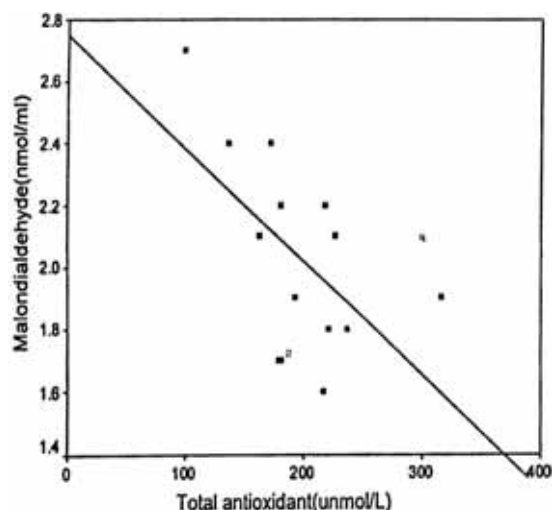


Fig. 3: Correlation between malondialdehyde and total antioxidant status in oligozoospermic males.

ROS causes a decrease in sperm motility as a result of lipid peroxidation and loss of membrane PUFA particularly docosahexanoic acid (Connor W.E., D.S. Lin, *et al*, 1998). Moreover, levels of ROS production in semen was found to be negatively correlated with the percentage of normal spermatozoal form (Ollero M., E. Gil-Guzman, *et al*. 2001).

Lipid peroxidation degradation of sperm membrane integrity may thus be held responsible for abnormal sperm form, this oxidative damage is a probable cause of idiopathic male infertility involving disruption of spermatogenesis (Shi Y.C., X.J. Shang, *et al*, 2006).

In this study, a highly significant decrease of mean levels of antioxidants in seminal plasma (glutathione, ascorbic acid and total antioxidant status) were found in oligozoospermic and azospermic cases compared to normozoospermic controls. These results were in agreement with Maarten *et al*. (2003), Mostafa *et al*. (2006), and Song *et al*. (2006).

As regards glutathione (GSH), Ochsendorf *et al*. (1998) found out moderate reduction of GSH in oligozoospermic compared to normozoospermic men, while other studies found GSH levels below the limit of detection ($<2.5\mu\text{M}$) in seminal plasma of oligozoospermics, or GSH levels to be significantly reduced in seminal plasma of subfertile males compared to those of fertile ones (Maarten T.M., M.J. Hennie, 2003).

Even glutathione therapy was found to improve the semen quality (Lenzi A., F. Culasso, 1993).

Our results provide evidence that the levels of GSH in seminal plasma seem to play a role in male fertility because GSH protects against oxidative damage of sperm cell membrane. Furthermore, higher levels of GSH seem to improve or protect the quality of sperm motility and morphology.

While stating that, other studies could not observe any difference in GSH concentration between fertile and subfertile men (Ebisch I.M.W., W.H.M. Peters, 2006).

According to their explanation, this may be due to the contribution of ROS produced by spermatozoa leading to upregulation of thiol synthesis in order to protect sperm from oxidative damage.

As regard ascorbic acid, seminal plasma ascorbic acid levels in oligozoospermic and azospermic men showed a significant decrease compared to normozoospermic fertile men, which is accepted by other studies (Srivastava A., S.K. Chopra, 1983; Mostafa T., G. Tawadrous, *et al*, 2006). This may be attributed to the absence or deficient sperm, which seems to be a provocative factor for maintenance of certain seminal ascorbic acid levels through potentiation of its secretion and/or consumption.

It was found that a defect of ascorbic acid is a possible mechanism of sperm DNA damage in infertile men, leading to ROS overproduction and increased consumption of ascorbic acid in seminal plasma (Song G.J., P. Edward, *et al*, 2006). Moreover, oral treatment with vitamin C significantly reduce sperm DNA damage (Greco E., M. Iacobelli, *et al*, 2005).

Seminal plasma total antioxidant status in this study were significantly lowered in oligozoospermics and azospermics compared to normozoospermics, this was in agreement with the results of other researchers (Lewis S.E., P.M. Boyle, 1995; Lewis S.E., E.S. Sterling, *et al*, 1997).

They concluded that the presence of ROS activity in sperm of infertile groups also associated with lower levels of chain-breaking antioxidants in seminal plasma, such antioxidants trap ROS directly to prevent amplification of radical formation and subsequent oxidative damage to sperm, reduced capacity to recycle

antioxidants in sperm membranes. Therefore, these sperm will be more susceptible to peroxidative damage.

Moreover, oxidative stress has been considered as a potential mechanism of sperm DNA damage in infertile men (Lopes S., A. Jurisicova, *et al*, 1998). It was found that sperm DNA integrity was correlated with seminal total antioxidant capacity (Song G.J., P. Edward, *et al*, 2006). The combined index from ROS generation and total antioxidant status score is reported to be a better marker of oxidative stress (Saleh R.A., A. Agarwal, 2003). Other studies stated that the total antioxidant levels in patients with idiopathic infertility were significantly less compared to fertile group (Pasqualotto F.F., R.K. Sharma, *et al*, 2000), they concluded that total antioxidant capacity might contribute to the Pathophysiology of male infertility irrespectively of clinical diagnosis.

Our study revealed a significant negative correlation between malondialdehyde versus ascorbic acid in normozoospermic group and versus total antioxidant status in oligozoospermics, while in azoospermics such negative correlation were detected between malondialdehyde versus GSH, ascorbic acid and total antioxidant status. Such results were in accord with that of several researchers (Aziz N., R.A. Saleh, 2004; Maarten T.M., M.J. Hennie, 2003; Zalata A., T. Hafez, 1995; Smith R., D. Vantman, *et al*, 1993).

Meanwhile, a significant positive correlation was detected between GSH and total antioxidant status in normozoospermics and also a positive correlation was detected in azoospermics between ascorbic acid versus GSH and total antioxidant status, such results came in accordance with other studies (Maarten T.M., M.J. Hennie, 2003). Several studies suggested that decreased levels of antioxidants in seminal plasma might be a potential cause of infertility. However, it is difficult to measure the effectiveness of one antioxidant in isolation of another because there appears to be cooperation between various antioxidants (Agarwal A., R.K. Sharma, *et al*, 2006).

Conclusion:

It is now well-accepted that ROS-induced lipid peroxidation induces a significant sperm membrane damage and markedly influences sperm motility and morphology, it is quite probable that such deleterious effect may account for some cases of male infertility and evaluation of ROS and peroxidation parameters may be a part of infertile male workup in the near future.

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