Iranian Journal of Reproductive Medicine Vol.6. No.1. pp: 1-5, Winter 2008

Vitamins C, E and lipid peroxidation levels in sperm and seminal plasma of asthenoteratozoospermic and normozoospermic men

Mohammad Nouri[1] Ph.D., Aliyeh Ghasemzadeh[2] M.D., Laya Farzadi[2] M.D., Vahideh Shahnazi[2] M.Sc., Marefat Ghaffari Novin [2,3]M.D., Ph.D.

1 Biochemistry Department, Tabriz University of Medical Sciences, Tabriz, Iran.
2 Infertility Center, Tabriz University of Medical Sciences, Tabriz, Iran.
3 Cellular and Molecular Biology Research Center, Shaheed Beheshti University (M.C.), Tehran, Iran.

Received: 22 October 2007; accepted: 9 April 2008

Abstract

Background: It has been shown that reactive oxygen species (ROS) can lead to deleterious effects on a range of sperm parameters. Vitamins E and C are capable of reducing ROS levels and lipid peroxidation.

Objective: The aim of study was to determine the level of lipid peroxidation as indicated by Malondialdehyde (MDA) and vitamins E and C in sperm and seminal plasma of asthenoteratozoospermic and normozoospermic men and their relationships with semen parameters.

Materials and Methods: Forty men with normozoospermic and 60 infertile men with asthenoteratozoospermic semen profiles were randomly selected. Semen analysis was done according to the WHO standard. Sex hormonal profiles were measured by ELISA methods. The level of vitamins (C and E) and MDA were measured by HPLC and tiobarbiotic acid, respectively.

Results: MDA concentration in the spermatozoa (0.1±0.06 nmol/ml) and seminal plasma (1.9±0.35 nmol/ml) of asthenoteratozoospermic were significantly higher than in normozoospermic males ($p<0.001$).The level of vitamins E and C in seminal plasma of normozoospermic were significantly higher than in asthenoteratozoospermic males ($p<0.01$). However, the amount of vitamin C in the spermatozoa of normozoospermic was significantly less than in asthenoteratozoospermic males ($p<0.01$). Sensitivity, specificity, positive and negative prognostic value of MDA of seminal plasma and spermatozoa were more than vitamins C and E. The level of vitamin C of spermatozoa had more diagnostic value when compare to vitamins C and E in seminal plasma.

Conclusion: The level of MDA in seminal plasma and spermatozoa and vitamin C in spermatozoa may be a diagnostic tool for etiology of infertility in the asthenoteratozoospermic patients.

Key words: *Asthenoteratozoospermia, MDA, Vitamin E, Vitamin C, Lipid peroxidation, Antioxidants activity, Infertility.*

Introduction

Plasma membrane of the mammalian sperm, which is rich in unsaturated fatty acids , is vulnerable to reactive oxygen species (ROS) - related lipid peroxidation. The cellular structure, motility, survival, and metabolic functions of the sperm can be impaired as a result of lipid peroxidation caused by ROS (1-3).

The level of lipid peroxidation can be determined by measuring the level of Malondialdehyde (MDA) which is a stable lipid peroxidation product (4).

Correspondence Author:
Dr. Mohammad Nouri, Biochemistry Department, Tabriz University of Medical Sciences, Tabriz, Iran.
E-mail: Nourimd@yahoo.com

[Downloaded from ijrm.ir on 2026-11-08]

To counteract the effects of ROS, semen is believed to possess a number of antioxidant systems including chain-breaking antioxidants capable of reducing oxidant radical levels that would otherwise initiate and propagate free radical chain reactions. There are two forms of antioxidant in seminal plasma and sperm; enzymatic and non-enzymatic antioxidant.

There is a relationship between activity of these antioxidant and function of sperm (5). Vitamins E and C which are belong to non-enzymatic antioxidant are used as a supplemented drug to improve sperm quality in male infertility (6).

It was demonstrated that MDA concentration in the spermatozoa of asthenozoospermic was significantly higher than in normozoospermic males (7).

Koca et al showed that the mean antioxidant capacity of fertile controls was significantly higher than that of the infertile patients group. Furthermore, asthenozoospermic and asthenoteratozoospermic groups had significantly lower mean antioxidant values when compared to fertile control group (8).

Song et al reported that patients with low levels of seminal ascorbic acid had increased sperm DNA damage (9).

Although there are some studies about the level of MDA, Vitamin E and C in seminal plasma and spermatozoa of fertile and infertile men, there is no report about their relationship (specificity, sensitivity and prognostic value) of these factors with semen parameters (7-9).

The aim of the present study was to determine the level of MDA and Vitamins E and C in sperm and seminal plasma of asthenozoospermic and normozoospermic men and evaluate diagnostic tool of these factors in infertility.

Materials and methods

A total of 100 men (40 normozoospermic and 60 asthenoteratozoospermic men) attending the Majidi Infertility Centre in Tabriz University of Medical Sciences were randomly selected and included in the study. Semen was collected by masturbation after 3 days of sexual abstinence. Patients fulfilling the inclusion criteria were asked to participate in a research project which was duly explained to them.

Written informed consents were obtained from all volunteers, according to the criteria of the Ethical Committee of Tabriz University of Medical Sciences. In all patients, a standard semen analysis was performed, assessing semen parameters, such as sperm count, concentration, and motility according to WHO criteria (10).

Semen samples were classified in two groups:
1) Normozoospermic fertile: $>20 \times 10^6$ sperm/ml, >50% progressive motility and >30% normal morphology from men whose wives had an ongoing pregnancy resulting from IVF.
2) Asthenoteratozoospermic infertile: $>20 \times I0^6$ sperm/ml, <50% progressive motility and <30% normal morphology.

Patients who had infertility secondary to infection, were taking medication, or had a congenital defect and more than 10^6 leukocytes/ml in semen analysis were excluded from the study. In addition, patients with abnormal testosterone, LH and estradiol serum levels were excluded form this study.

After liquefaction of semen, spermatozoa were separated from seminal plasma by centrifugation (1000×g for 10 min at room temperature).

Supernatant was removed immediately and kept in -20° C. The spermatozoa were washed two times by adding 0.2 ml phosphate buffer solution (PBS) and centrifuged (3000×g for 5 min at room temperature). The final sperm concentration was measured and sperm samples were then aliquoted into Eppendorf tubes (10×10^6 sperm concentration) and subjected to rapid freeze-thawing three times to lyse the cells.

Lipid peroxidation

Lipid peroxidation in spermatozoa and seminal plasma was measured by reaction of thiobarbituric acid (TBA) with MDA according to Yagi (11). Content of MDA was measured spectro-fluorometrically using a Jasco (FP-6200) spectro-fluorometer (excitation 515 nm, emission 553 nm). The MDA fluorescence intensity of spermatozoa and seminal plasma was determined using various concentrations of tetraethoxy-propane as standards. The results are expressed as nmol MDA/10×10^6 cells and nmol MDA/ml seminal plasma.

Measurement of vitamins E and C concentrations in seminal plasma and spermatozoa

For the assessment of the vitamins E and C concentrations in seminal plasma and spermatozoa, a high performance liquid chromatography (HPLC) method was used. Measurement of vitamin E was done according to Lee et al method (12). Briefly, an aliquot of the seminal plasma was placed into a sample preparation vial, which was filled with a reagent (Sodium Sulfate) for precipitation. A stabilizing reagent (Butanol Ethyl

[Downloaded from ijrm.ir on 2026-11-08]

Acetate: 1-1) was added. The solid component of seminal plasma being precipitated was removed by centrifugation. Finally, 20 µl of the supernatant was injected into the HPLC system. Detection was done by fluorescence at 286 nm.

Seminal and spermatozoa ascorbic acid was determined using conventional methodology. Briefly, ascorbic acid in the sample was converted to dehydroascorbate in the presence of thiourea and copper sulphate. 0.5 ml samples were mixed with 0.4 ml DTCs (containing thiourea, copper sulphate and DNPT) and incubated for 3 hours at 37°C. Dehydroascorbate was then coupled with 2, 4-dinitrophenylhydrazine forming its bis derivative.

Upon treatment with 2 ml sulphuric acid (12 mol), the derivative yields a stable red colour, which was measured spectrophoto-metrically at 520 nm (13). The serum levels of LH, testosterone and oestradiol were measured by radioimmunoassay.

Statistical analysis

Student's t-test was used to compare between two groups. All analyses were calculated with the SPSS statistical software package 10.1. Specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) were measured by standard methods after determining cut off point.

A probability value of $p<0.05$ was considered significant. Values are given as means and standard deviation.

Results

The mean values of semen parameters and sex hormonal level in normozoospermic and asthenoteratozoospermic men are shown in Table I. There was a significant difference in semen parameters between two groups. However, there was no significant difference in sex hormonal level in both groups. The mean± S.D. of lipid peroxidation, as determined with MDA assay, in seminal plasma of normozoospermic and asthenoteratozoospermic groups were 1.4±0.2 and 1.9±0.35 nmol/ml respectively. In addition, the level of MDA in spermatozoa of normozoospermic and asthenoteratozoospermic groups were 0.1 ±0.03 and 0.17±0.06 nmol per 10×10^6 spermatozoa respectively. The difference was significant between two groups (Table II).

The level of vitamins E and C in seminal plasma of normozoospermic were significantly higher than this in asthenoteratozoospermic males ($p<0.01$). However, the amount of these vitamins in the spermatozoa of normozoospermic was significantly less than this in asthenoterato-zoospermic males ($p<0.01$). For example, the level of vitamin C in the spermatozoa of asthenoteratozoospermic samples was 2 times greater than normozoospermic samples (Table II).

Sensitivity, specificity, positive and negative prognostic value of MDA of seminal plasma and spermatozoa were more than vitamins C and E. The level of vitamin C of spermatozoa had more diagnostic value when compare to vitamins C and E in seminal plasma (Table III).

Table I. Semen parameters and sex hormonal level in normozoospermic and asthenoteratozoospermic men.

	Normozoospermic (n=40)	**Asthenoteratozoospermic (n=60)**
Age (year)	37.1±4	34.2±3.4
Sperm concentration (10^6/ml)	82±20.5	30.5±15.2 **
Motility grade a (%)	25.4±6.8	5.2±4.1**
Motility grade a + b (%)	60.8±10.6	31.4±11.2**
Normal morphology	65.5±20.7	19.8±8.6**
Testosterone (nmol/L)	16.8±3.4	17.1±4.8
LH (nmol/L)	4.0±1.8	4.2±1.8
Estradiol (Pmol/L)	60.0±16.7	69.8±19.0

Results are presented as mean ± SD
Grade a= rapid progressive
Grade b= slow progressive
** $p<0.001$

[Downloaded from ijrm.ir on 2026-11-08]

Table II. MDA, vitamins E and C concentrations in sperm and seminal plasma of normozoospermic and asthenoteratozoospermic men.

	Normozoospermic (n=40)		Asthenoteratozoospermic (n=60)	
	Sperm	Seminal plasma	Sperm	Seminal plasm
MDA (nmol/ml)	0.1±0.03	1.4±0.2	0.17±0.06*	1.9±0.35*
Vitamin E (µmol/l)	#	0.5±0.20	#	0.30±0.15*
Vitamin C (µmol/l)	1.8±0.7	380±115	4.1±1.9*	250±120*

not measurable

Sperm MDA are expressed as nmol/10×10^6 spermatozoa.

* statistically significant between two groups ($p < 0.01$).

Table III. Diagnostic value of MDA and vitamins E, C in the seminal plasma and sperm in normozoospermic and asthenoteratozoospermic men.

	Cut off	Sensitivity (%)	Specificity (100%)	PPV (100%)	NPV (100%)
MDA in seminal plasma	1.6	95	86	91	93
MDA in spermatozoa	0.13	90	83	90	87
Vitamin C in seminal plasma	265	50	56	66	48
Vitamin C in sperm	2.5	90	80	90	84
Vitamin E in seminal plasma	0.35	50	63	60	50

PPV: Positive Predictive Value

NPV: Negative Predictive Value

Discussion

Reactive oxygen species play a functional role as second messengers in many cell types (1). However, if their production becomes uncontrolled, it also becomes an important factor in the etiology of pathologic conditions in general and of male infertility in particular (5). In sperm, the production of ROS is associated with loss of motility and a decreased capacity for sperm-oocyte fusion (1).

Plasma membrane of the mammalian sperm, which is rich in unsaturated fatty acids, is vulnerable to ROS-related lipid peroxidation. The cellular structure, motility, survival, and metabolic functions of the sperm can be impaired as a result of lipid peroxidation caused by ROS (1). In the present study, MDA, which is an index of lipid peroxidation, were increased in semen and sperm of infertile men when compared to fertile men. This is in agreement with the findings of Landat et al (14) and Tavilani et al (7).

Landat et al showed that MDA of sperm was higher in infertile men when compared to fertile men (14). In addition, Tavilani et al reported that MDA concentration in the spermatozoa of asthenozoo-spermic males was significantly higher than this in normozoospermic males (7). In the present study, sensitivity (95%), specificity (86%) and positive prognostic value (PPV) of this index for diagnose between fertile and infertile groups were high.

Therefore, MDA may be a diagnosis tool for the analysis of infertility in the asthenoteratozoo-spermic patients. Our results are also similar to those reported by Shang et al (15) and Mostafa et al (16). Shang et al showed that the concentration of MDA in seminal plasma differed significantly between the control group and the infertile groups (15). Mostafa et al reported that mean level of MDA was significantly higher in the internal spermatic vein blood compared to this in the peripheral venous blood in infertile men with varicocele (16). However, they did not study the sensitivity and PPV of MDA.

Ascorbic acid, a major water-soluble antioxidant, acts as a scavenger for a wide range of ROS. Ascorbic acid is a powerful electron donor that reacts with superoxide, peroxide and hydroxyl radicals to form dehydro-ascorbic acid. Results of the present study showed that level of ascorbic acid in the seminal plasma of normozoospermic men were 380 µM. Since ascorbic acid is present at a high concentration in seminal plasma compare to blood plasma (380 versus 40 µM), or other antioxidants (Table II), this indicates an important physiological role for it in seminal plasma (17, 18). Song et al reported that patients with low levels of seminal ascorbic acid had increase sperm DNA damage (9). It is also present in detectable amounts in the sperms themselves (1.8 µM), reflecting the need for its continued presence after the sperm swim out of the seminal fluid. It has been shown that ascorbic acid concentrations in seminal plasma

[Downloaded from ijrm.ir on 2026-11-08]

are positively related to the percentage of morphologically normal spermatozoa (6).

However, there was no report about sensitivity, specificity, PPV and negative prognostic value (NPV) of vitamin C. In the present study, we have shown diagnostic value of vitamin C in semen and seminal plasma of fertile and infertile men.

Tocopherol (vitamin E) is the primary lipid-soluble small molecule antioxidant in biologic systems. As such, tocopherol was present, as expected, in only small amounts in seminal plasma (0.3 to 0.5 µmol/l). Ascorbate and tocopherol cooperate to protect lipid structures against peroxidation. Ascorbate recycles tocopherol by repairing its tocopheroxyl radical, thereby permitting it to function again as a free radical chain-breaking antioxidant (19). Therefore, although tocopherol is present in minimal amounts, the availability of ascorbate may facilitate its optimal recycling, thus making higher concentrations unnecessary. The presence of tocopherol in the aqueous phase of seminal plasma rather than in the sperm membranes where it is negligible, may make it more accessible to ascorbate. Our results showed that vitamin E of seminal plasma had less diagnostic value when compared to other factors.

In conclusion, measuring MDA in seminal plasma and sperm and vitamin C in sperm provides a sensitive assay for diagnosis of etiology of infertility. It is suggested that supplementary intake of non enzymatic antioxidants vitamins E and C could improve semen quality and fertility in these patients.

Acknowledgement

This work was supported by Deputy of Research in Tabriz University of Medical Sciences.

References

1. Aitken RJ, Fisher H. Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. *Bioassays* 1994; 16: 259-267.
2. Sanocka D, kurpisz M. Reactive oxygen species and sperm cells. Reprod Biol Endocrinol 2004; 2: 12-19.
3. Agarwal A, saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil and Steril* 2003; 79: 829-843.
4. Girotti MJ, khao N, Melellen BA. Early measurement of systemic lipid peroxidation products in plasma of major blunt trauma patients. *J Trauma* 1991; 31: 32-35.
5. Zini A, de Lamirande E, Gagnon C. Reactive oxygen species in semen of infertile patients: levels of superoxide dismutase and catalase-like activities in seminal plasma and spermatozoa. *Int J Andro* 1993; 16: 183-188.
6. Thiele JJ, Friesleben HJ, Fuchs J, Ochsendorf FR. Ascorbic acid and urate in human seminal plasma: determination and interrelationships with chemiluminescence in washed semen. *Human Reproduction* 1995; 10: 110-115.
7. Tavilani H, Doosti M, Saeidi H. Malondialdehyde levels in sperm and seminal plasma of asthenozoospermic and its relationship with semen parameters. *Clinica Chem Acta* 2005; 365: 199-203.
8. Koca Y, O¨zdal O¨ L, C¸elik M, U¨nal S, Balaban N. Antioxidant activity of seminal plasma in fertile and infertile men. Archives of Andrology 2003; 49: 355–359.
9. Song GJ, Norkus EP, Lewis V. Relationship between seminal ascorbic acid and sperm DNA integrity in infertile men. *International Journal of Andrology* 2006; 29; 569-575.
10. World Health Organization WHO: Laboratory Manual for the examination of Human semen and semen- cervical Mucus interaction. 4th ed. Cambridge, UK, Cambridge University press, 1999.
11. Yagi K. Assay for blood plasma or serum. *Methods Enzymol* 1984; 105: 328-331.
12. Lee Bl, Chua SC, Ong HY, Ong CN. HPLC Method for routine determination of vitamins A and B- carotene in plasma. *Journal of Chromato* 1992; 581: 41- 47.
13. Natelson S. Determination of ascorbic acid using 2,4 dinitrophneyl hydrazine. In, "Technique of clinical chemistry." C C. Thomas, Spring field, Illinois, 1971.
14. Landat A, Karine L, Guechot J, Palluet AM. Values of sperm thiobarbituric acid- reactive substances in fertile men. *Clin Chem Acta* 2002; 325: 113-115.
15. Shang XJ, Lik Y, Chen YG, Huang YF: Analysis of lipid peroxidative levels in seminal plasma of in fertile men by HPLC. *Arch Androl* 2004; 50: 411-416.
16. Mostafa T, Anis TH, Ghazi S, El-Nashar AR, Imam H, Osman IA. Reactive oxygen species and antioxidants relationship in the internal spermatic vein blood of infertile men with varicocele. *Asian J Androl* 2006; 8: 451-454.
17. Fraga CG, Motchnik PA, Shigenaga MK, Helbock HJ, Jacob RA, Ames BN. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proceedings of the National Academy of Sciences of the United States of America* 1991; 88: 11003–11006.
18. Lewis SE, Sterling ES, Young IS, Thompson W. Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men. *Fertil and Steril* 1997; 67: 142–147.
19. Buettner GR. The pecking order of fl'ee radicals and antioxidants, lipid peroxidation, alpha-tocopherol and ascorbate. *Arch Biochem Biophys* 1993; 300: 535-543.

[Downloaded from ijrm.ir on 2026-11-08]

[Downloaded from ijrm.ir on 2026-11-08]