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Activity of Antioxidant Enzymes in Seminal Plasma and their Relationship with Lipid Peroxidation of Spermatozoa

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ABSTRACT

Purpose: To determine the activity of seminal plasma catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) and their relationship with malondialdehyde (MDA), as a marker of lipid peroxidation, content of spermatozoa and seminal plasma in normozoospermic and asthenozoospermic males.
Materials and Methods: Semen samples were obtained from 15 normozoospermic and 30 asthenozoospermic men.
Results: We observed inverse correlations between activities of CAT (k/mL) and SOD (U/mL) in seminal plasma with MDA content of spermatozoa from normozoospermic samples ($r = -0.43$, $p < 0.05$ and $r = -0.5$, $p < 0.05$, respectively). Significant correlations were observed between total activity CAT (k/total seminal plasma) with total SOD (U/total seminal plasma) and GPX activity (mU/total seminal plasma) in seminal plasma from normozoospermic samples ($r = 0.67$, $p = 0.008$ and $r = 0.455$, $p = 0.047$, respectively). Furthermore, we found positive correlations between total activities of CAT, SOD and GPX with total content of MDA in seminal plasma (nmoL/total seminal plasma) from normozoospermic samples ($r = 0.67$, $p = 0.003$; $r = 0.73$, $p = 0.003$; $r = 0.74$, $p = 0.004$, respectively). In asthenozoospermic samples, there were no significant correlations observed between activities of CAT (k/mL), SOD (U/mL) and GPX (mU/mL) of seminal plasma with MDA content of spermatozoa. However, we found significant correlations between total activities of CAT (k/total seminal plasma) and SOD (U/total seminal plasma) with total content of MDA in seminal plasma ($r = 0.4$, $p = 0.018$ and $r = 0.34$, $p = 0.03$, respectively).
Conclusion: These findings indicate a protective role for antioxidant enzymes of seminal plasma against lipid peroxidation of spermatozoa in normozoospermic samples.

Key words: *catalase; superoxide dismutase; glutathione peroxidase; semen; malondialdehyde*
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INTRODUCTION

Aerobic metabolism of human sperm produces various reactive oxygen species (ROS), which are potentially harmful to the sperm plasma membrane with its high content of polyunsaturated fatty acids (1-3). There is growing evidence that lipid peroxidation damage to the plasma membrane of spermatozoa plays an important role in the mechanism of male infertility (4-6). The toxic lipid peroxides are known to cause various impairments of the sperm cell, such as membrane damage and decrease in motility (7,8). Control of lipid peroxidation in the male reproductive tract is exerted by antioxidant molecules and protec-

tive enzymes within the spermatozoa and seminal plasma (9). Seminal plasma contains enzymatic ROS scavengers such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). These enzymes act as an antioxidant and inhibitor of lipid peroxidation. Thus, peroxidative damage in spermatozoa not only depends on ROS production, but also on sperm and seminal plasma antioxidant defenses (10).

The question of whether seminal plasma SOD, GPX and CAT can act coordinately to protect human spermatozoa from lipid peroxidation has not to date been systematically addressed, although the presence of SOD, GPX and CAT activity in seminal plasma from fertile and infertile men has been reported (11-15). Since lipid peroxidation leads to loss of motility in human spermatozoa, the possibility exists that asthenozoospermic sperm suffers from the lack of protection against lipid peroxidation due to lack of adequate or non-coordination between SOD, GPX and CAT activity in seminal plasma. Whether the protective role of seminal antioxidant enzymes, against peroxidation, can affect products of spermatozoa or seminal plasma by lipid peroxidation remains unknown? The main objective of this study was to determine the activity of seminal plasma GPX, SOD and CAT and their relationship to MDA, as a marker of lipid peroxidation, content of spermatozoa and seminal plasma in normozoospermic and asthenozoospermic males.

MATERIALS AND METHODS

Semen Samples

Semen specimens were obtained in 30 asthenozoospermic patients who attended the Omid Fertility Clinic for infertility evaluation. In addition, 15 healthy men with normal semen parameters according to World Health Organization (WHO) criteria were enrolled as controls (16). The two groups were similar as regards mean age (20-40 years of age). Patients had no systemic diseases were non smokers and had no alcohol dependence, and none were taking an oral antioxidant supplement for three months prior tot the study. Patients fulfilling the inclusion criteria were asked to participate in this research project, which was duly explained to them. Written informed consent was obtained from all enrollees, according to the criteria of the Ethical Committee of Tehran University of Medical Sciences. All semen samples were collected by masturbation following 3 days of abstinence. After liquefaction, semen volume, sperm concentration (hemocytometer), total sperm count, morphology (Pap smear), motility grades: a (rapid progressive), b (slow progressive), c (non-progressive), d (immotile) were determined using WHO standard procedures (16). All major determinations were carried out in duplicate. Semen samples with more than 1×10^6/mL neutrophils using peroxidase staining (16) or other round cells were excluded. Asthenozoospermia was indicated by a sperm concentration of $\geq 20\times10^6$/mL and motility (grade a+b) of < 50%, irrespective of the morphology results. Normozoospermia was indicated by a sperm concentration of $\geq 20\times10^6$/mL and a motility (grade a+b) $\geq$ 50% and a normal morphology of $\geq$ 14%. Following semen analysis, a volume of semen containing at least 50 million sperm (or more) was transferred into a conical centrifuge tube and was centrifuged at 1000 × g for 10 min at room temperature. Immediately after the centrifugation, the supernatant was collected and stored at -80º C and the pellet from each sample was resuspended in 0.2 mL phosphate buffer saline (17).

Measurement of Antioxidant Enzymes Activity

Seminal plasma SOD activity was measured using a Ransod kit (Randox Laboratories, Crumlin, U.K.) with xanthine and xanthine oxidase to generate superoxide radicals which react with 2- (4 - iodophenyl) - 3 - (4- nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. Seminal plasma was diluted 31-fold with l0 mM phosphate buffer, pH 7. One unit of SOD was the amount that caused a 50% inhibition in the rate of I.N.T. reduction. The SOD activity was expressed as specific activity (U/mL seminal plasma) and total activity (U/total seminal plasma).

Seminal plasma GPX was measured by a Ransel kit (Randox Laboratories Ltd., London, U.K.). GPX catalyses the oxidation of glutathione by cumene

hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione was immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured. The GPX activity was expressed as specific activity (mU/mL seminal plasma) and total activity (mU/total seminal plasma).

Catalase activity was measured according to Abei (18) by monitoring the initial rate of disappearance of hydrogen peroxide (initial concentration 10 mM) at 240 nm. The catalase activity was expressed as specific activity (k/mL seminal plasma) and total activity (k/total seminal plasma).

Measurement of Malondialdehyde Levels

Lipid peroxidation in spermatozoa and seminal plasma was measured by reaction of thiobarbiuric acid (TBA) with malondialdehyde (MDA) according to Yagi (19). 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 seminal plasma and nmoL MDA/total seminal plasma.

Statistical Analysis

Due to the fact that sperm concentration, motility, morphology, MDA and various other determined semen parameters were not normally distributed, the Mann-Whitney U test was applied to compare the asthenozoospermic and normozoospermic groups. To assess seminal plasma CAT, SOD, GPX activities and sperm count, one tailed two-independent sample t-test was used. Correlation between variables was assessed using non-parametric Spearman's coefficient (r). Data were expressed M ± Standard Error.

RESULTS

The semen profiles of normozoospermic and asthenozoospermic samples are shown in Table-1. Percent of motility grade a+b and spermatozoa with normal morphology was higher in normozoospermic compared to asthenozoospermic samples ($p < 0.001$). Results of seminal plasma CAT, SOD and GPX activities in normozoospermic and asthenozoospermic groups are shown in Table-2. Mean seminal plasma specific and total activity of SOD, GPX and CAT were not significantly different in two groups. MDA content in the spermatozoa of asthenozoospermic was significantly higher than in normozoospermic samples (0.14 ± 0.004 and 0.09 ± 0.004 nmoL/10^7 spermatozoa, respectively). The mean ± SE value of MDA in the seminal plasma of asthenozoospermic and normozoospermic were not significantly different (Table-2).

Correlations between CAT, SOD and GPX activities with MDA content of spermatozoa and seminal plasma from normozoospermic samples are shown in Table-3. There were negative and significant correlations between activities of CAT and SOD in seminal plasma with MDA content of spermatozoa from normozoospermic samples. In addition, we observed high positive correlations between total activities of CAT, SOD and GPX with total content of MDA from seminal plasma.

In asthenozoospermic samples, there were no significant correlations between specific activities of CAT, SOD and GPX of seminal plasma with MDA content of spermatozoa (Table-4). However, we found positive and significant correlations between total activities of CAT and SOD with total content of MDA in seminal plasma (Table-4).

Significant correlations were found between total activity CAT with total activity SOD and total activity GPX in seminal plasma from normozoospermic samples ($r = 0.67$, $p = 0.008$ and $r = 0.455$, $p < 0.05$, respectively). In addition, there was a significant correlation between specific activity CAT and specific activity SOD in normozoospermic samples ($r = 0.58$, $p = 0.022$). Moreover, we observed a significant correlation between total CAT and SOD activity in seminal plasma of asthenozoospermic samples ($r = 0.33$, $p < 0.05$).

COMMENTS

In the present study, we were able to determine the SOD, GPX and CAT activity in the seminal

Table 1 – *Basic semen parameters (mean ± SE) from normozoospermic and asthenozoospermic subjects.*

Semen Parameters	**Normozoospermic** (n = 15)	**Asthenozoospermic** (n = 30)
Volume (mL)	3.68 ± 0.34	3.45 ± 0.22
Sperm concentration (10^6/mL)	112 ± 19	83.9 ± 5*
Total sperm count (10^6 sperm /ejaculate)	383 ± 63	275 ± 26
Normal sperm form (%)	22 ± 1.7	8.1 ± 0.6**
White Blood Cell (10^6/mL)	0.68 ± 0.09	0.71 ± 0.08
Motility grade a+b (1) (%)	58.7 ± 2.9	27.5 ± 2**

*(1) = Grade of sperm movement according to World Health Organization criteria (16). a = rapid progressive; b = slow progressive, * p < 0.05; ** p < 0.001.*

plasma and the MDA content of the spermatozoa and seminal plasma in normozoospermic and asthenozoospermic samples. Jones et al. showed that the mechanism by which oxidative stress induced motility loss in mammalian spermatozoa involved the induction of peroxidative damage to the sperm plasma membrane (1). Human spermatozoa are particularly vulnerable to lipid peroxidation because their plasma membranes are enriched with polyunsaturated fatty acids, particularly docosahexaenoic acid with six double bonds (6,20). These polyunsaturated fatty acids are essential to produce plasma membrane fluidity that is required to participate in the membrane fusion events associated with fertilization (1).

Table 2 – *Specific and total activity of catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD) of seminal plasma and malondialdehyde (MDA) content of spermatozoa and seminal plasma from normozoospermic and asthenozoospermic samples.*

Parameters	**Normozoospermic** (n = 15)	**Asthenozoospermic** (n = 30)
Specific activity of CAT (k/mL)	$0.3 \pm 0.1 \times 10^{-3}$	$0.18 \pm 0.04 \times 10^{-3}$
Total activity of CAT (k/total seminal plasma)	$1.14 \pm 0.39 \times 10^{-3}$	$0.6 \pm 0.11 \times 10^{-3}$
Specific activity of SOD (U/mL)	6.88 ± 0.66	7.79 ± 0.64
Total activity of SOD (U/ total seminal plasma)	24.6 ± 3.6	25 ± 1.8
Specific activity of GPX (mU/mL seminal plasma)	352 ± 58	366 ± 33
Total activity of GPX (mU/ total seminal plasma)	1318 ± 251	1243 ± 111
Sperm MDA content (nmoL/10^7 spermatozoa	0.09 ± 0.004	0.14 ± 0.004*
Seminal plasma MDA content (nmoL/ mL seminal plasma)	1.2 ± 0.077	1.35 ± 0.076
Total seminal plasma MDA content (nmoL/ total seminal plasma)	4.1 ± 0.36	4.6 ± 0.31

**p < 0.05*

Table 3 – *Correlations between catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities with malondialdehyde (MDA) content of spermatozoa and seminal plasma from normozoospermic (n = 15) samples.*

Enzyme Activity	**MDA of Spermatozoa** (nmoL/10^7 spermatozoa)		**MDA in Total Seminal Plasma** (nmoL/total seminal plasma)	
	r	**p Value**	**r**	**p Value**
CAT (k/mL)	-0.43	0.048	0.36	0.1
SOD (U/mL)	-0.5	0.046	0.27	0.19
GPX (mU/mL)	-0.161	0.3	0.3	0.17
CAT (k/total seminal plasma)	-0.38	0.10	0.67	0.003
SOD (U/total seminal plasma)	-0.19	0.2	0.73	0.003
GPX (mU/total seminal plasma)	-0.02	0.4	0.74	0.004

Table 4 – *Correlations between catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities with malondialdehyde (MDA) content of spermatozoa and seminal plasma from asthenozoospermic (n = 30) samples.*

Enzyme Activity	**MDA of Spermatozoa** (nmoL/10^7 spermatozoa)		**MDA in Total Seminal Plasma** (nmoL/total seminal plasma)	
	r	**p Value**	**r**	**p Value**
CAT (k/mL)	-0.23	0.12	0.07	0.3
SOD (U/mL)	-0.2	0.14	-0.28	0.06
GPX (mU/mL)	0.041	0.4	-0.18	0.18
CAT (k/total seminal plasma)	-0.004	0.49	0.4	0.018
SOD (U/total seminal plasma)	0.04	0.4	0.34	0.03
GPX (mU/total seminal plasma)	0.27	0.09	0.14	0.24

Our study showed that asthenozoospermic men compared with normozoospermic do not have deficient seminal plasma SOD, GPX and CAT activities. In contrast, there were no significant differences between specific and total activity of SOD, GPX and CAT in seminal plasma of two groups. Different studies have investigated antioxidant enzymes of seminal plasma in asthenozoospermic samples or other altered semen parameters but their results remain controversial (11-15). Results based on this study showed a negative correlation between specific activity of CAT and SOD with MDA content of spermatozoa from normozoospermic samples. This observation suggests that CAT and SOD of seminal plasma may play a role in the protection against lipid peroxidation in the normozoospermic samples. In our study, we observed higher content of lipid peroxidation product malondialdehyde (MDA) in spermatozoa of asthenozoospermic compared with normozoospermic samples ($p < 0.05$). Although, the difference between MDA of seminal plasma was not significant between two groups. In addition, we did not find any significant correlation between spermatozoa MDA and activity of antioxidant enzymes of seminal plasma. Moreover,

the activity of seminal antioxidant enzymes could not have protected spermatozoa from asthenozoospermic samples against lipid peroxidation. Our results are in agreement with Jones et al. who reported that the addition of SOD, GPX and CAT to the medium of spermatozoa (which contain generating system of oxygen free radicals; sodium ascorbate and $FeSO_4$) did not inhibit MDA formation (21). There is evidence for transferring of various proteins to the spermatozoa, and the role of post testicular maturation of the sperm cells have been well documented (22). We suggest that in the normozoospermic samples, the membrane structure of spermatozoa is influenced to allow adsorption of seminal plasma CAT and SOD onto the membrane, thereby providing the protective action of CAT and SOD against lipid peroxidation. However, in asthenozoospermic samples, seminal antioxidant enzymes cannot be adsorbed to the plasma membrane of spermatozoa. Sperm membrane has been reported to be adversely affected by peroxidation of polyunsaturated fatty acids and accumulation of organic hydroperoides (21). Since, we found higher content of lipid peroxidation product (MDA) in asthenozoospermic samples, we suggest that membrane of spermatozoa was affected by lipid peroxidation and thereby could not have adsorbed antioxidant enzymes of seminal plasma. While there may be many reasons for increased lipid peroxidation product in spermatozoa from asthenozoospermic males, one reason may be partly due to non adsorption of seminal antioxidant enzymes to spermatozoa membrane and subsequent reduction in lipid protection.

In this study, we found a positive correlation between total activity of CAT, SOD and GPX with total content of MDA in seminal plasma (nmoL/total seminal plasma) from normozoospermic samples. In addition, our data showed the significant correlation between total activity of CAT with total activity of SOD and GPX in normozoospermic samples. These findings may indicate a cooperation and coordination between function of antioxidant enzymes in normozoospermic samples. We suggest that the activity of seminal antioxidant enzymes may be regulated by MDA content of seminal plasma. Thus, further studies are needed to clarify the role of MDA on activity of antioxidant enzymes of seminal plasma from normozoospermic and asthenozoospermic samples.

In conclusion, these findings indicate a protective role for antioxidant enzyme of seminal plasma against lipid peroxidation of spermatozoa in normozoospermic samples. We suspect that under pathological conditions (e.g. asthenozoospermia) the activity of seminal antioxidant enzymes can not protect spermatozoa and may cause an increase of lipid peroxidation from spermatozoa.

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CONFLICT OF INTEREST

None declared.

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