



IMPACT OF ANTIOXIDANTS ACTIVITY ON ENZYMES OF SEMINAL PLASMA IN ASSOCIATION WITH MENTAL STRESS SUBJECTS

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ABSTRACT

In the present study we investigated the impact of mental stress in medical students due to final examination on free radical activity and three important antioxidant enzymes of seminal plasma, Superoxide Dismutase(SOD), Glutathione Peroxidase(GSH) and Catalase in normal medical students.

For carrying out morphological and biochemical analysis, semen samples were collected thrice from 50 healthy male volunteers, who were third semester students of a medical college, just before and after stress period 12.00±0.50 week final examination and 12.00±0.50 week gap after final examination. Venous blood samples were also withdrawn and serum was separated for assessment of cortisol levels. Psychological stress of participants and non-stress subjects were assessed on the basis of questionnaire and elevated serum cortisol level.

The results demonstrated spermatozoa concentrations, motility index and percentage of rapid progressive motility decreased under stress. Moreover, serum cortisol and seminal plasma Lipid Peroxide (LPO) levels were found elevated along with decreased seminal plasma GSH and reduced SOD.

KEY WORDS: Male infertility, Lipid peroxide, Antioxidant enzymes, Stress score, Motility index



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INTRODUCTION

Rapid industrialization, environmental pollution, changing living conditions and unfavorable working conditions cause stress to the people which may play an important role in developing several disorders.

It is reported that 38 percent problems of infertility occur due to the reasons related to women, 20 percent men, 27 percent to both men and women and rest 15 percent due to unknown reasons (idiopathic)¹⁻³. It is claimed that the mental stress constitutes the measure part of unknown reasons leading to problems of infertility^{4,5}. It is known that academic anxieties and examinations cause mental stress. Supe et. al reported that medical students had high degree of stress⁶. It is thought that especially during the final year examination level of stress in medical students increased much more.

In the previous studies stress has been suggested to have a negative effect on sperm parameters related with quality of the semen such as density, motility and morphology of the sperm cells⁷⁻¹⁰. Since morphologically normal, forward motile and adequate count of sperm can maintain fertilization; a decrease in the quality of sperm of semen will cause decrease in the fertility as well.

Under normal circumstances the adequate levels of enzymatic, non enzymatic antioxidants, vitamins and minerals maintain Reactive Oxygen Species (ROS) scavenging potential of the male reproductive tract and seminal fluid^{11, 12}. On the other hand, when the production of ROS is high it may lead to oxidative damage of spermatozoa. The sperm plasma membrane is very sensitive to ROS since it contains high levels of unsaturated fatty acids. The latter provide fluidity, which is necessary for sperm motility and acrosome reaction. ROS level as found prolonged psychological stress, may lead to an imbalance between oxidant / anti-oxidant ratio, LPO resulting in sperm membrane damage and its subsequent dysfunction¹³ (Fig.1).

Till date appropriate treatment of idiopathic male infertility has not been found. However in the ancient Indian system of medicine the Ayurveda and Unani and also the Chinese system, traditional remedies were used for the improvement of endurance against infections, retardation of the giving process and improving male sexual disorders like psychogenic importance and unexplained infertility¹⁴⁻¹⁶.

Most of the earlier studies on relationship between stress and seminal quality were done with infertile men^{7,8,17}. Further most studies investigating the association between psychological stress and semen quality lacked information on Biochemical parameters. We therefore undertook this study to evaluate the effect of mental stress due to final examinations on important antioxidant enzymes in the seminal plasma, such as SOD, catalase, GSH and Malondialdehyde (MDA) in medical students, who were under psychological stress.

MATERIALS AND METHODS

Subject and Sample collection

Of the 50 male students in the IInd MBBS IIIrd semester of the faculty of Medicine, 70 students (Age 20.00 ± 0.67 years) were included in the study. All subjects were non smokers and were not currently taking any medication. Subjects with diabetes mellitus, renal and hepatic diseases, hormonal dysfunction and those suffering from any acute infection were excluded.

Oligospermic subjects (spermatozoa, density < 20 ×10⁶ ml) were also excluded from the study in order to eliminate the possible pathologists and to undertake the study only in healthy individuals. Students have no other stress factors for the last three months before stress period i.e. final examinations. During the final examinations in month of November (stress) and after the summer vacation period (11.00 ± 0.73 week later; non stress) semen sample (about 4 ml) of all the 60 students were collected on the

same day in the clinical facility by masturbation into a sterile glass container, following 48 hours of sexual abstinence. Ethical approval was approved obtained from the institutional Ethical committee and subject gave return informed consent. The same procedure is followed for the 30 subjects who were found in stress even after completion of their exams.

Evaluation of level of stress

For determining the stress level of the participants, in the periods when the semen sample were taken, the State Trait Anxiety Inventory (STAI) was performed simultaneously for evaluating chronic or acute anxiety. STAI includes 20 questions depending on the feeling of a person. The scores ranged from 22 to 80, higher scores indicating greater anxiety. This measure has been shown to have high reliability and high construct validity¹⁸.

Biochemical Analysis

Preparation of Seminal Plasma

Semen samples were collected by masturbation after 3–4 days of abstinence into sterile glass containers for analysis. The semen volume was recorded and an aliquot was taken to assess sperm motility after at least 30 min given for liquefaction. The motility characteristics of the sperm cells were classified into four groups as a rapid progressive motility, borderline progressive motility, non progressive motility and immotility, and were expressed as percent of the total. Total progressive motility was defined as the percentage of rapid

progressive motile plus borderline progressive motile spermatozoa. Motile index as motility quality indicator was derived by the formula:

Percent total progressive motility/100 × sperm density.

Morphology was measured by recoding the percentage of abnormal forms in the sample. Diff - Quick stain was used for the examination of morphological features¹⁸.

Thereafter, the semen samples were centrifuged at 1200 × g in cold (4⁰C) for 20 min for the separation of seminal plasma. The supernatant (seminal plasma) was again centrifuged at 10000 × g in cold (4⁰C) for 30 min to eliminate all possible contaminating cells and stored at –20⁰C until analyzed. All biochemical estimations were carried out in seminal plasma.

Estimation of Lipid Peroxide Levels by TBA reaction¹⁹.

Assay of Superoxide Dismutase Activity by Marklund and Marklund method²⁰.

Assay of Catalase Activity by Spectrophotometric method²¹.

Estimation of Reduced Glutathione(GSH) by Spectrophotometric method²².

Estimation of Serum Cortisol Levels by RIA²³.

Statistical Analysis

Comparisons of data between the stress and non-stress periods were made by Wilcoxon Signed-Ranks test. Also, correlations between parameters in each stage were examined by Pearson correlation test²⁴. A value of P<0.05 was considered statistically significant.

Table I
Semen quality parameters at stress and non-stress periods

Parameters	Non-stress period (n-50)	Stress period (n-50)
Sperm density ($\times 10^6$ /ml)	91.82 \pm 23.90	50.96 \pm 12.79 ***
Motility characteristics of spermatozoa at 2 nd hr		
Rapid progressive motility (%)	19.80 \pm 5.51	7.69 \pm 4.98 **
Borderline progressive motility (%)	30.10 \pm 6.26	36.14 \pm 10.50 **
Non-progressive motility (%)	13.00 \pm 5.92	16.00 \pm 5.31**
Immotility (%)	33.84 \pm 6.80	36.83 \pm 11.23 ^{ns}
Motility index ($\times 10^6$ motile sperm/ml)	41.90 \pm 17.62	19.24 \pm 8.20 ***

Values are mean \pm SD (n = 50)

** P<0.01; *** P<0.001; ns-non significant. Non stress period compared to stress period

Table 2
State anxiety score and serum cortisol levels in non stress and mental stress subjects.

Stress parameters stress	Non stress (n-50)	Psychological (n-50)
Stress anxiety score	41.07 \pm 6.10	50.77 \pm 7.26 **
Serum cortisol (μ g/dl) (08:00 hr)	9.95 \pm 0.47	14.43 \pm 1.51***
Serum cortisol (μ g/dl) (16:00 hr)	4.93 \pm 0.43	9.76 \pm 1.57 ***

Values are mean \pm SD (n = 50)

** P<0.01; *** P<0.001; ns-non significant. Non stress period compared to stress period

Table 3
Biochemical parameters of under stress and non stress subjects

Stress Parameters	Non stress (n-50)	Psychological Stress (n- 50)
LPO (nmol MDA ml ⁻¹)	2.16 \pm 0.40	3.45 \pm 0.34 ***
SOD (Unit mg ⁻¹ protein)	8.38 \pm 0.47	6.34 \pm 0.33 ***
Catalase (Unit mg ⁻¹ protein)	9.19 \pm 0.74	8.27 \pm 0.82 **
GSH (mg/dl)	1.62 \pm 0.57	1.07 \pm 0.30 **

Values are mean \pm SD (n = 50)

** P<0.01; *** P<0.001; ns-non significant. Non stress period compared to stress period

RESULTS

In our study, we observed that during stress period, there was about 50 per cent decrease in the semen quality markers such as density

(p<0.001), percent rapid progressive motility (p<0.01), borderline progressive motility and motility index (p<0.001, Table I). The percentage of immotile spermatozoa at stress period was not significantly higher than those

found at non-stress period (Table I). Stress scores at stress period were significantly higher than those found at the non-stress period ($P < 0.01$, Table II). Other stress factors such as death in family can cause deleterious effect on sperm quality²⁴. Subjects of the present study had no other stress factors for the last three months before stress period. The interval between the two periods was approximately 12 wks. Our findings showed that semen quality was adversely affected by the mental stress resulting from the final year examination. A decrease in the percentage of rapid motile spermatozoa might be a risk for fertility within the mental stress periods, since high proportions of rapid progressive motile sperm have been shown to be associated with fertilization.

Stress scores, elaborated on the basis of the questionnaire, were found significantly high in stress (infertile) subjects (Table 2). Similarly, the morning serum cortisol levels were found elevated in stress ($P < 0.001$), as compared with (control) non stress subjects.

In Table 3 the lipid peroxide level in seminal plasma of non stress (control) was 2.16 ± 0.40 nmol MDA ml⁻¹. On the other hand, it was found increased in 'under stress' ($P < 0.001$), we also observed that SOD activity in seminal plasma of non stress (control) subjects was 8.38 ± 0.47 unit mg⁻¹ protein. However, this enzyme was found significantly suppressed in stress (infertile) subjects ($p < 0.001$). Similarly, catalase activity in seminal plasma of 'under stress' subjects was found significantly reduced ($P < 0.01$), as compared with non stress (control) subjects. Similarly, GSH content in seminal plasma of subjects under stress ($P < 0.01$) was found decreased as compared with non stress (control) subjects.

DISCUSSION

In our study lipid peroxide level was significantly high in the seminal plasma of subjects who were under stress, which may be due to increased oxidative stress. Psychological stress is known to be

associated with increased oxidant production of polyunsaturated fatty acids (PUFA) of sperm membrane, resulting in unfavorable alterations in sperm structure and function^{12,13}. H₂O₂ has been shown to be the most effective toxic agent on the sperm cells of humans²⁶⁻²⁹.

Moreover, we observed that in subjects who were under psychological stress, there were low seminal plasma SOD and catalase activities and reduced level of glutathione³⁰.

Male fertility is known to be affected by various kinds of stressful conditions, including psychological stress³⁰. The autonomic nervous system and adrenal hormones participate in stress response, which also affects steroidogenesis and spermatogenesis³¹. We report elevated serum cortisol level in subjects, who were under psychological stress. The latter causes stimulation of hypothalamic-pituitary-adrenal axis (HPA) leading to the release of the corticotropin releasing hormone (CRH), adrenocorticotropin hormone (ACTH) and cortisol. Increased cortisol level, as seen during prolonged stress, may reduce the functional activity of leuteinizing hormone—release hormone (LHRH) pulse generator, which may lead to decrease in gonadotropin and testosterone levels^{30,32}. Moreover, long-term psychological stress may also decrease the concentration of catecholamines, like dopamine, noradrenalin, 5,6-dihydroxy phenyl acetic acid (DOPAC) and homovanillic acid (HVA) in brain. Decrease in the activity of dopaminergic neurons is also known to affect the fertility and motility.³²

Our findings suggested well with existing scientific knowledge that increased lipid peroxidation adversely affected on semen quality due to mental stress. Mechanism of such damage required to be explored. The role of medicinal herbs like M. Prurines, Tinospora Cardifolia, Asparagus Racemosus etc were used for the improvement of endurance against infections and improving male sex disorders required to be studied further.

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