Association between mental stress & some antioxidant enzymes of seminal plasma

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Background & objectives: Mental stress, which is responsible for various disorders, is one of the most important medical and social problems. It is reported that mental stress causes abnormality in sperm quality. Most of the previous investigations done to study the association between mental stress and infertility were carried out with infertile men. Infertility itself and/or its therapy may lead to stress. Further, most studies investigating the association between psychological stress and semen quality have lacked information on biochemical parameters. In the present study, we investigated the effect of mental stress due to final exams on two important antioxidant enzymes of the seminal plasma, superoxide dismutase (SOD) and catalase in normal healthy medical students.

Methods: Semen samples were collected from 27 healthy male volunteers, who were third semester students of a medical school, just before (stress period) and 10.19±0.83 wk after (non-stress period) the final examinations. Psychological stress of participants was measured by the State Trait Anxiety Inventory. After standard semen analysis, semen samples were centrifuged at 10,000 x g for 15 min. Superoxide dismutase (SOD) and catalase activities were measured in the seminal plasma.

Results: During stress period, stress scores and SOD activities increased significantly compared to the non-stress period. Catalase activities showed no change. Spermatozoa concentrations, motility index and percentage of rapid progressive motility decreased under stress.

Interpretation & conclusion: Our results indicated that mental stress negatively affected semen quality. Increase in SOD activities led to poor quality of semen parameters.

Key words Catalase - mental stress - seminal plasma - semen quality - superoxide dismutase

Rapid industrialization, environmental pollution, changing living conditions and unfavourable working conditions cause stress to the people which may play an important role in developing several disorders. It is reported that 38 per cent of the problems of infertility occur due to the reasons related to women, 20 per cent men, 27 per cent to both men and women, and the rest 15 per cent due to unknown reasons.
It is claimed that the mental stress constitutes the major part of unknown reasons leading to problems of infertility. It is known that academic anxieties and exams cause mental stress. Supe et al. reported that the medical students had high degree of stress. Ahaneku et al. found that during final exams, the serum high density lipoprotein cholesterol (HDL-C) levels and the ratio of HDL-C/total cholesterol of the students at the faculty of medicine were altered as a risk for coronary artery disease. It is thought that especially during the final exams, 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 cells. Since morphologically normal, forward motile and adequate count of sperms can maintain fertilization, a decrease in the quality of semen will cause a decrease in the fertility as well.

It is suggested that the reactive oxygen species (ROS) have a negative effect on the sperm parameters. The sperm plasma membrane is very sensitive to the effect of ROS since it contains abundant unsaturated fatty acids. These unsaturated fatty acids create fluidity which is necessary for sperm motility and membrane fusion events such as the acrosome reaction and sperm-egg interaction. However, the unsaturated nature of these molecules predisposes them to ROS attack and ongoing lipid peroxidation throughout the sperm plasma membrane. An increase in the seminal ROS level has been reported in 40 per cent of the infertile men. Though antioxidant defense system is active in the semen, its activity is limited as the amount of cytoplasm of the sperm cell is low. The presence of high ROS levels in semen implies an imbalance between ROS production and antioxidant system. Increased ROS levels can lead to damage with subsequent sperm dysfunction or cell death.

Most of the earlier studies on relationship between stress and seminal quality were done with infertile men. Infertility itself and/or its therapy may be the reasons for stress. 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 exams on important antioxidant enzymes in the seminal plasma such as superoxide dismutase (SOD) and catalase and the parameters of the quality of semen in medical students.

Material & Methods

Subjects and sample collection: Of the 43 male students in the third semester of the Faculty of Medicine, Trakya University, School of Medicine, Edirne, 27 (age 19.96 ± 0.98 yr) were included in the study. All subjects were non smokers, and were not currently taking any medication. Subjects with diabetes mellitus, renal and hepatic disease, hormonal dysfunction and those suffering from any acute infection were excluded. Oligospermic subjects (spermatozoa density <20 x 10⁶/ml) were also excluded from the study in order to eliminate the possible negative effects of unknown additional pathologies and to undertake the study only in healthy individuals. The students had no other stress factors for the last three months before stress period i.e., final exams. During the final exams in January (stress) and after the vacation in March (10.19 ± 0.83 wk later; non-stress) semen samples (about 4 ml) of all the 27 students were collected on the same day in the clinic facility by masturbation into a sterile glass container, following 48 h of sexual abstinence. Ethical approval was obtained from the University of Trakya, Ethical Committee, and the subjects gave written informed consent.

Semen analysis: The standard semen analysis was carried out by the same observer according to the WHO guidelines after liquefaction of semen at 37°C for one hour. Semen volume (ml), pH, sperm density (millions per ml), motility characteristics (%) and abnormal morphology of the sperm cells were
determined. A phase-contrast microscope (Olympus, Japan) was used for semen analysis. Sperm density was measured by Neubauer counting chamber manufactured in Germany. The motility characteristics of the sperm cells were classified into four groups as rapid progressive motility, borderline progressive motility, non-progressive motility and immotility, and were expressed as per cent of the total. Total progressive motility was defined as the percentage of rapid progressive motile plus borderline progressive motile spermatozoa. Motility index as motility quality indicator was derived by the formula: per cent total progressive motility/100 x sperm density. Morphology was measured by recording the percentage of abnormal forms in the sample. Diff-Quick stain was used for the examination of morphological features14.

**Evaluation of the level of stress:** For determining the stress level of the participants, in the periods when the semen samples were taken, the State Trait Anxiety Inventory (STAI) was performed simultaneously for evaluating the chronic or acute anxiety. STAI includes 20 questions depending on the feeling of a person. The answers were in the form of quadripartite Licker scale. Total scores ranged from 20 to 80, higher scores indicating greater anxiety. This measure has been shown to have high reliability and high construct validity15.

Biochemical analysis: Semen samples were centrifuged at 10,000 x g for 15 min at +4°C and seminal plasma was used for analysis.

SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to the superoxide anion generated by the combination xanthine + xanthine oxidase. One unit of SOD activity was defined as the quantity of plasma capable of decreasing the reduction of NBT by 50 per cent16.

Catalase activity was measured spectrophotometrically according to the method of Luck17 as modified by Aebi18 by measuring the decrease in the $H_2O_2$ concentration at the 240 nm.
High purity grade reagents from Sigma (St. Louis, USA) and Merck (Darmstadt, Germany) were used for biochemical analyses.

Analysis data: 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 test. A value of $P<0.05$ was considered statistically significant.

Results & Discussion

In our study, we observed that during stress period, there was about 50 per cent decrease in the semen quality markers such as density, per cent rapid progressive motility and motility index ($P<0.001$, Table I). The percentage of immotile spermatozoa at stress period was significantly higher than those found at non-stress period ($P<0.05$, 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 quality\textsuperscript{11}. 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 10 wk. Our findings showed that semen quality was adversely affected by the mental stress resulting from the final exams. 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.

Zini et al\textsuperscript{20} observed no decrease in the seminal SOD and catalase activity of the infertile men. In our study, seminal plasma SOD activity at stress condition was higher than that found at non-stress condition ($P<0.001$, Table II). High level of SOD activity could be due to an increase in the production of seminal superoxide anion radical under mental stress condition. Cells are capable of increasing synthesis of SOD in response to hyperoxidant stress\textsuperscript{21}. SOD catalyzes the enzymatic dismutation of superoxide anion radical to hydrogen peroxide ($\text{H}_2\text{O}_2$). At high concentrations, $\text{H}_2\text{O}_2$ is removed by catalase\textsuperscript{22}. However, in our study catalase activity was unaltered. These data suggested that excessive production of $\text{H}_2\text{O}_2$ as a result of high SOD activity was not removed by catalase. Seminal plasma catalase activity at the stress period was positively correlated with density, rapid progressive motility of spermatozoa and motility index ($r= 0.544$, $P<0.01$; $r= 0.426$, $P<0.05$ and $r=0.495$, $P<0.01$ respectively), while SOD activity was negatively correlated with seminal parameters (Fig.). At non-stress period no correlation was determined between enzyme levels and seminal quality parameters. These data suggested that increasing SOD activity as a result of defense mechanism was not sufficient to protect spermatozoa from damage. $\text{H}_2\text{O}_2$ has been shown to be the most effective toxic agent on the sperm cells of human\textsuperscript{23-26}. It was found that catalase protected sperm movement and ATP level of spermatozoa against ROS attacks\textsuperscript{26}. These findings support our investigation.

In conclusion, our findings suggested that semen quality was adversely affected by mental stress and that ineffective removal of the free radicals might play an important role in this process.

References


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