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RESEARCH ARTICLE

Study of effect of examination stress on lipid peroxidation and superoxide dismutase level

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ABSTRACT

Background: In day–to-day life, stress comes in various forms, and for medical students, academic stress poses one of the many challenges that they have to contend with during their graduation years. A review of the literature reveals the paucity of information about the effect of academic stress as a brief naturalist stressor on oxidative markers. Thus, this study was undertaken to determine the effect of examination stress on lipid peroxidation and superoxide dismutase (SOD) level in medical students. **Aims and Objective**: The objective of this study was to compare the effect of examination stress on lipid peroxidation and SOD level. **Materials and Methods**: This study was conducted in 80 normal healthy subjects of both sexes (40 males and 40 females) in the age ranging from 18 to 25 years in the Department of Physiology of MLN Medical College, Allahabad. The plasma malondialdehyde (MDA) activity was determined according to the method given by Utley et al. and SOD activity was determined by using the method of Marklund and Marklund. **Results**: During the high-stress period, the participants showed significantly higher levels ($P < 0.01$) of the MDA activity, a measure of lipid peroxidation as compared to low-stress period, while a marked ($P < 0.001$) decrease in the antioxidant SOD activity was observed in both sexes. **Conclusion**: The finding of this study shows that examination stress apparently shifts the delicate pro- and anti-oxidation balance to a more pro-oxidative state. This may lead to increased allostatic load and risk of chronic reactive oxygen species-related diseases. These findings may also lend support to the anecdotal practice of some individuals consuming antioxidant-rich supplementation during an examination period.

KEY WORDS: Examination Stress; Free Radical; Lipid Peroxidation; Malondialdehyde; Superoxide Dismutase

INTRODUCTION

The ability to utilize oxygen has provided humans with the benefit of metabolizing fat, protein, and carbohydrate for energy; however, it does not come without cost. The great bulk of oxygen (>95%) is used in mitochondria, but a small percentage of oxygen we breathe goes to make reactive oxygen species (ROS). It is a term which encompasses all highly reactive oxygen-containing molecules including free radicals. Type of ROS includes hydroxyl radical, superoxide anion radical, nitric oxide radical, hypochlorite radical, and lipid peroxidases. All ROSs are capable of reacting with membrane lipids, nucleic acid, proteins, enzyme, and other small molecules resulting in cellular damage.

In the human body, high levels of constantly formed free radicals and other ROS such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\cdot}OH$) are

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involved in the generation of cascade reactions that attack cell membrane phospholipids and induce membrane lipid peroxidation. Accumulation of damaged macromolecules is a major contributor of aging and other degenerative diseases.

Lipid peroxidation is an important biological consequence of oxidative damage. The end product of lipid peroxidation and malondialdehyde (MDA) can cause damage to proteins and DNA resulting in cellular apoptosis.[1,2] This leads to increase in membrane fluidity, rigidity, and loss of integrity. Hence, protection against and prevention of the consequences of the deleterious effects of ROS are of critical importance, and they could possibly be achieved by non-enzymatic (e.g.,glutathione, uric acid, bilirubin, and Vitamin C and E) and enzymatic antioxidants. Enzymatic defense against ROS-induced tissue damage in humans includes the enzymes superoxide dismutase (SOD). SOD, present in both mitochondria and cytosol, disputes superoxide free radical and anions to form H_2O_2 and oxygen (O_2).[3] Under normal conditions, a delicate balance between the generation of ROS and heightened antioxidant defense appears to be in place.[4] However, this balance can be readily upset by various factors and this imbalance, the so-called "oxidative stress," appears to play a pivotal in the pathogenesis of several diseases.[4-6]

In day-to-day life, all organisms face various types of stresses. Some people cope well with the stresses, while in other people, repeated stresses lead to derangement of the coping mechanisms and balance between pro- and anti-oxidants. This derangement leads to detrimental effects on physiological and psychological homeostasis. Stress comes in various forms. The undergraduate medical training is very stressful period. For medical students, academic stress poses one of the many challenges that they have to contend with during their graduation years.[7]

A review of the literature reveals that many studies done on oxidative stress and antioxidant status are confined to disease or age-related degenerative disorders. Lesser attention has been paid to younger age groups like students who are facing stressful conditions related to their carrier, examinations, or settlement in their life. There is a paucity of information regarding this aspect of stress and in particular that of the effect of examination stress as a brief naturalist stressor on oxidative markers. Thus, this study was undertaken to determine the effect of stress-related conditions on undergraduate medical students by measuring lipid peroxidation and SOD level.

MATERIALS AND METHODS

This study was conducted in 80 normal healthy subjects of both sexes (40 male and 40 female) in the age ranging from 18 to 25 years. The study was conducted in the Department of Physiology, MLN Medical College, Allahabad, after getting permission from the Institutional Ethical Committee. The subjects chosen were students of the first professional of the college. They were divided into two groups: Group 1 male and Group 2 female. Each group comprises of 40 subjects. Informed consent was taken from the subjects. A detailed history including the history of diet and lifestyle was taken, and general physical and systemic examination was done. All the selected subjects were healthy and were not suffering from any disease such as hypertension, diabetes mellitus, rheumatoid arthritis, malignancy, collagen disorders, or any other disease. Their biological parameters (MDA and SOD enzyme level) were measured during 1 day before the final written examination and during the low-stress period, i.e., within 1 week after the examination.

For assessment of biological parameters, 5 mL of fasting venous blood samples was taken under all aseptic conditions by venipuncture in the antecubital vein in heparinized tubes. The blood sample was collected between 8 and 10 am.

Blood samples from the heparinized tubes were centrifuged at 3000 rpm for 15 min, after which plasma from each tube meant for lipid peroxidation was separated out to be followed by the removal of the buffy coat layer. The red cell pellet left behind was hemolyzed and used for subsequent analyses of antioxidant enzyme activities. The total hemoglobin content was also measured using Sahli's method.[8]

Lipid Peroxidation Assay

The plasma MDA activity was determined according to the method given by Utley *et al*.[9] and used as an index for lipid peroxidation.[10,11] This method is based on the principle that the heat-induced reaction of malondialdehyde (MDA) with two molecules of thiobarbituric acid (TBA) in plasma forms a trimethine colored substance which is measured calorimetrically.

Antioxidant Enzyme Activity

Determination of antioxidant status was obtained by the activities of SOD that was estimated from the erythrocyte hemolysates. Total SOD activity was determined by using the method of Marklund and Marklund[12] in which reduction of the substrate, pyrogallol is used to indicate $O_2^{\cdot}-$ production. One unit (U) of the SOD activity inhibits the rate of reduction of pyrogallol by catalyzing the breakdown of superoxide by 50%. The inhibition of pyrogallol oxidation of SOD is monitored at 420 nm.

Statistical Analysis

Statistical analysis was carried out by Student's paired *t*-test. The data were expressed as mean ± SD and the $P < 0.05$ was taken as statistically significant.

RESULTS

The subjects were divided into two groups Group I and II on the basis of sex. MDA levels in males were more than the females. During estimation of biological parameters in male and female students, we found that the level of lipid peroxidation (MDA) was higher in males than females. This difference was statistically insignificant. SOD level was also more in males as compared to females in both conditions. The difference was statistically significant. During the high-stress period, the participants showed significantly higher levels of the MDA activity, a measure of lipid peroxidation. The MDA activity was significantly ($P < 0.01$) higher during high-stress period as compared to low-stress period, while a marked ($P < 0.001$) decrease in the antioxidant SOD activity was observed at the same time in both the sexes [Tables 1-4].

Table 1: Comparison of lipid peroxidation (μmol/L of MDA) in males and females

Stress period	(mean±SD)		*P* value
	Male	Female	
Low-stress period	0.92±0.24	0.86±0.21	<0.1
High-stress period	1.28±0.38	1.02±0.32	<0.1

MDA: Malondialdehyde, SD: Standard deviation

Table 2: Comparison of SOD activity (U/g of Hb) in male and female

Stress period	(mean±SD)		*P* value
	Male	Female	
Low-stress period	1859.70±204.65	1680.50±201.3	<0.001
High-stress period	1699.44±207.52	1377.56±272.09	<0.001

SOD: Superoxide dismutase, SD: Standard deviation

Table 3: Comparison of lipid peroxidation (μmol/L of MDA) in low- and high-stress periods

Groups	(mean±SD)		*P* value
	Low-stress period	High-stress period	
Group I	0.92±0.24	1.28±0.38	<0.01
Group II	0.86±0.21	1.02±0.32	<0.01

MDA: Malondialdehyde, SD: Standard deviation

Table 4: Comparison of SOD activity (U/g of Hb) in low- and high-stress periods

Groups	(mean±SD)		*P* value
	Low-stress period	High-stress period	
Group I	1859.70±204.65	1699.44±207.52	<0.001
Group II	1680.50±201.30	1377.56±272.09	<0.001

SOD: Superoxide dismutase, SD: Standard deviation

DISCUSSION

During estimation of biological parameters in male and female students, we found that the level of lipid peroxidation (MDA) was higher in males than females in both conditions. This difference was statistically insignificant while SOD level was significantly higher in males as compared to females in both conditions. During the high-stress period, the participants showed significantly ($P < 0.01$) higher levels of the MDA activity, a measure of lipid peroxidation, while a marked ($P < 0.001$) decrease in the antioxidant SOD activity was observed in both sexes.

Oxidative stress describes the injury caused to cells by the oxidizing of macromolecules resulting from increased formation of ROS and/or decreased antioxidant reserve. MDA, a secondary product of lipid peroxidation, used as an indicator of tissue damage by a series of chain reactions.[13] In our study, we found that the value of lipid peroxidation is lower in females than males. Our study is in concordance with the finding of another study who also found that women have lower lipid peroxidation levels than men.[14,15] There are compelling evidences that SOD enzyme is essential for biological defense against superoxide anions. SOD is thus a defensive enzyme that ameliorates the toxicity of oxygen free radicals by disputing superoxide anions. We found decrease levels of SOD during high-stress period in both sexes. An academic examination can significantly increase the pro-oxidant MDA while decreasing the antioxidant SOD levels. These findings are similar to those reported by others.[16-18]

This study has limitation. First, only two oxidative stress markers, i.e., MDA and SOD activities are measured in this study. In addition to these stress markers, cortisol, CAT, and GPX measurement may revealed additional information. Further studies are needed to be carried out to address these limitations.

CONCLUSION

The result of the study confirms that the brief naturalistic stressors such as examination stress apparently shift the delicate pro- and anti-oxidation balance to a more pro-oxidative state by impairing lipid oxidation and enzymatic antioxidant defense. These findings may also lend support to the anecdotal practice of some individuals consuming antioxidant-rich supplementation during an examination period.

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