**ORIGINAL ARTICLE**

# Assessment of Oxidative Stress Markers in medical students in response to examination stress

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## ABSTRACT

**Background**: Exam stress is a factor that affects many organ systems of the body. There is a relation between stress and biochemical and physiological markers.
**Aim**: To assess the level of oxidative stress markers in medical students during and after examinations.
**Methods**: This cross sectional study was conducted at physiology department of university of Lahore from January 2013 – February 2013. Sixty healthy medical students planning to take their professional exam were recruited. Students with health behaviors or health problems that may alter immune oxidative stress measures were excluded from the study. Study participants arrived at the lab in morning hours (7-11 am) in the week preceding annual exam. The second visit was scheduled a month or more (4-7 weeks) after the exam.
**Results:** out of the sixty subjects, 23 were male and 37 were female. Age ranged from19 to 21 years. Significant difference was observed in the value of malondialdehyde (MDA) and glutathione peroxidase (GPx) during stressed and relaxed phase. While the levels of superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) were raised during the stress state, but the difference between the stressed and relaxed state was non-significant
**Conclusio**n: Examination stress affects the levels of antioxidants. Examination stress has a transient effect on deranging the biochemical markers.
**Keywords**: Reactive oxygen species (ROS); Antioxidants; Oxidative Stress

## INTRODUCTION

All organisms are encountered with different types of stresses in their daily life. In medical profession, stress is common and it can lead to physical tiredness and mental fatigue[1]. Especially the examination stress is one of the well-known etiological event that can disturb the human chemistry and affect the balance between pro-oxidants and anti-oxidants, producing a wide range of detrimental effects on physiological and psychological homeostasis[2].

In human body, radicals & reactive oxygen species (ROS) are continuously being formed in high amounts. Reactive oxygen species (ROS) include peroxides, superoxide anion, hydroxyl radicals, hydrogen peroxide, nitric oxide and singlet oxygen. They lead to a series of reactions that attack cell membrane phospholipids and induce lipid peroxidation. One of the well-characterized product of lipid peroxidation is Malondialdehyde (MDA) that can cause damage to DNA and proteins resulting cellular death[3].

Antioxidants are of critical importance in providing protection against the harmful effects of ROS. Antioxidants are molecules that inhibit or delay cellular damage and inhibit free radical reactions. They exist in two forms, enzymatic and non-enzymatic forms in the body. Non-enzymatic antioxidants like vitamin C, vitamin E and glutathione, uric acid & bilirubin work by interrupting free radical chain reactions.

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Enzymatic antioxidants in humans include the enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) & catalase (CAT)[4]. Superoxide dismutase (SOD), dismutases singlet oxygen to form hydrogen peroxide and oxygen[5]. while Glutathione peroxidase helps in removing hydrogen peroxide from body fluids by converting reduced glutathione to oxidized glutathione and is also able to terminate the chain reaction of lipid peroxidation by removing hydroperoxides from cell membranes[6]. While catalase located within peroxisomes and in the cytosol of cells is responsible for the decomposition of hydrogen peroxide to water and oxygen. Increased glutathione peroxidase (GSH-Px) and/or catalase activity is accompanied by increased hydrogen peroxide production to limit damage.

Under normal conditions, a delicate balance exists between the generation of ROS and antioxidant[7]. Various factors can be readily upset this delicate balance resulting in a state known as oxidative stress. Oxidative stress has been associated with the pathogenesis of several diseases[8].

A literature review reveals scarcity of information regarding the effect of academic stress on the oxidative markers. Thus, this study was undertaken to determine the relationship between increased level of stress and oxidative stress markers and their responses.

## MATERIALS & METHODS

This cross sectional study was conducted at physiology department of university of Lahore from January 2013 – February 2013. A total of sixty healthy medical students, both male and female who were planning to take their professional exam were recruited from the medical college. Students with health behaviors or health problems that may alter immune oxidative stress measures were excluded from the study like regular use of tobacco, excess

consumption of alcohol, illegal drug use, or diagnosis of severe asthma, arthritis, diabetes, cancer, heart disease, hypertension, autoimmune disorders, major depression/ anxiety disorder or major psychiatric disorders. In addition, participants were screened on the day of arrival for the presence of an infectious illness.

Study participants arrived at the lab in morning hours (7-11 am) in the week preceding annual exam. Upon arrival at lab, written informed consent was taken from each study participant. Demographic details were noted and parameters like height, weight and blood pressure were recorded. Blood samples were obtained using aseptic technique to be used in evaluation of anti oxidative stress assays

The second visit was scheduled a month or more (4-7 weeks) after the exam, when anxiety about the exam was expected to have dissipated. Blood samples were obtained again to be used in evaluation of antioxidant assay.

**Oxidative stress marker assays:** For fifteen minutes heparinized blood samples were centrifuged at 3000 rpm. So by this way plasma was obtained for lipid peroxidation. Then buffy coat was removed. For the investigation of antioxidant activities the hemolysed red cell pellet was used.

Assessment for Lipid peroxidation: Using the method of Akku[9] the level of MDA in plasmawas observed. This MDA level is a scale of lipid peroxidation[10,11]. In this method of Akku2-thiobarbituric acid (TBA) react with plasma MDA at a temperature of boiling point which will give a supernatant of pink color. Spectrophotometrically at 532 & 600 nm, the optimal density is recorded; here 532 nm is the maximum absorbance of TBA-MDA complex and 600 nm is the correction for non-specific turbidity. The numerical quantity **for** absorbance at 600 nm was deducted from those at 532 nm in order to give the true MDA quantity that was written as micromoles per gram of hemoglobin (µmol/g Hb).

Activity of Antioxidant enzyme: Antioxidant status was gained by the activities of SOD, CAT and GPx that were calculated from the erythrocyte haemolysates. Total SOD activity was determined by the method of Kakkar[12] in which reduction of the substrate, nitrobluetetrazolium (NBT), is used to indicate $O2^-$ production. NBT is reduced to 50% by one unit of SOD.At 240nm[13]. Catalase activity was calculated by breakdown of $H_2O_2$. In one minute One unit of CAT break up one µmole of $H_2O_2$ with CAT activity is written as U/g Hb. The GPx level was estimatedby oxidation of glutathione by $H_2O_2$. This chemical change is coupled to the reduction of oxidized glutathione by glutathione reductase, which at the same time oxidizes NADPH to NADP+[14].. The level of decrease in absorbance at 340 nm is written in term of units per gram of Hemoglobin (U/g Hb).

Results were expressed as mean±SD. Statistical significance was determined by Wilcoxon test. One way analysis of variance and spearman correlation (two-tailed) was used to correlate the different variables. P-value of <0.05 was considered as statistically significant

## RESULTS

Out of the 60 subjects, 23 were male & 37 were female. Age ranged from 19 to 21years. Levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) & glutathione peroxidase (GPx) were checked.

The levels of malondialdehyde (MDA) were increased during stress than the relax state. The value of MDA at phase-1 (stress state) was 2.05 nmol/ml and at phase-II was 1.46nmol/ml. The difference of these values was significant.

The values of SOD were raised during the stress phase as compared to the relaxed phase during the stress phase, the mean value of SOD was 0.78µg/ml and during the relaxed phase was 0.50µg/ml showing non-significant difference among then.

Phase I showed higher value of glutathione as compare to phase-II which were 9.75µg/ml and 9.50µg/ml respectively. Catalase concentration determined during phase-I was 4.57µg/ml which was raised and during phase-II was 3.99µg/ml depicting a significant difference between the two.

The values of glutathione peroxidase during the stress state and relax state were 0.45µg/ml and 0.59µg/ml respectively showing a significance difference depicting higher lipid peroxidation state

Table 2 shows the variation of above discussed parameters with respect to gender. The result show non significance indicating that gender has no significant role in stressful conditions

Table-1: levels of stress markers in medical students during examinations (phase-I) and after examinations (phase-II)

| Variables | PHASE-I±SD (n=60) | PHASE-II±SD (n=60) | Sig (P≤0.05) |
|---|---|---|---|
| MDA | 2.05±0.63 | 1.46±0.50 | 0.001 |
| SOD | 0.78±0.95 | 0.50±0.19 | 0.141 |
| GSH | 9.75±1.35 | 9.50±1.22 | 0.327 |
| CAT | 4.57±1.07 | 3.99±0.86 | 0.068 |
| GPx | 0.45±0.13 | 0.59±0.28 | 0.010 |

Table-2: levels of stress markers in male and female medical students during examinations (phase-I) and after examinations (phase-II)

| Variables | males±SD (n=23) | females±SD (n=37) | Sig (p≤0.05) |
|---|---|---|---|
| MDA | 1.80±0.63 | 1.71±0.65 | 0.604 |
| SOD | 0.69±0.92 | 0.57±0.25 | 0.490 |
| GSH | 9.68±1.51 | 9.54±0.97 | 0.686 |
| CAT | 4.21±1.01 | 4.40±1.01 | 0.453 |
| GPx | 0.50±0.19 | 0.54±0.25 | 0.458 |

## DISCUSSION

Measurement of antioxidant enzyme activity, antioxidant concentrations in blood are used to quantify levels of oxidative stress. In this study, the level of antioxidant enzymes, SOD & catalase increased during the period of stress as compared to the relaxed state. This finding is concordant with the results of another study which showed a significant increase in the SOD activity during stress period as compared to the non-stress period in the semen samples collected from 27 healthy male volunteers while

the catalase showed no change[15]. In a study performed by Rostami et al. in 2008 also reported a higher level of SOD &glutathione peroxidase during stressful conditions and a decline in these levels after the stress was over[16]. But in our study, the level of glutathione showed a decline during the stress period.

Premier enzyme engaged in the metabolism of superoxide anion radicals is SOD, the decreased activity of SOD would initiate the production of increased quantity of Reactive Oxygen Species, and afterwards, increment in the membrane lipid peroxidation.

In our study, the level of MDA showed a significant increase during stressful condition and a decline in these levels after the stress was over. This finding is supported by the study performed by Nakhee et al. in 2013, who also reported significantly high levels of MDA during exams than those in post exam period. A significant positive correlation between the changes in MDA levels and anxiety scores was found during exam period[17]. A study performed in turkey in 2009, reported that in stress condition oxidative damage to DNA and sensitivity to lipid oxidation were significantly increased when compared with the same parameters in non-stress conditions[18]. But the level of glutathione peroxidase was found to be decreased in the stress period.

## CONCLUSION

Our findings suggest that examination stress affects the levels of antioxidants. Examination stress has a transient effect on deranging the biochemical markers.

## REFERENCES


1. Supe AN. A study of stress in medical students at Seth G.S. Medical College. J Postgrad Med 1998; 44: 1-6.
2. Malarkey WB, Pearl DK, Demers LM, Kiecolt-Glaser JK, Glaser R. Influence of academic stress and season on 24-hour mean concentration of ACTH, cortisol, and β-endorphin. Psychoneuroendocrinol 1995; 20: 499-508.
3. Kannan K, Jain SK. Oxidative stress and apoptosis. Pathophysiology 2000; 7: 153-163.
4. Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev 1994; 74: 139-162.
5. McCord JM, Edeas MA. SOD, oxidative stress and human pathologies: a brief history and a future vision. Biomed Pharmacother 2005; 59: 139-162.
6. McCray PB, Gibson DD, Fong KL, Hornbrook KR. Effect of glutathione peroxidase activity on lipid peroxidation in biological membranes. BiochemBiophysActa 1976; 431: 459-468.
7. McCord JM. Human disease, free radicals, and the oxidant/antioxidant balance. ClinBiochem 1993; 26: 351-57.
8. Aruoma OI. free radicals, oxidative stress, and antioxidants in human health and disease. J Am Oil Chem Soc 1998; 75: 199-212.
9. Akkus I, Saglam NI, Çaglayan O, Vural H, Kalak S, Saglam M. Investigation of erythrocyte membrane lipid peroxidation and antioxidant defense systems of patients with coronary artery disease (CAD) documented by angiography. ClinicaChimicaActa 1996; 244: 173-180.
10. Draper HH, Hadley M. Malondialdehydedetermination asindex of lipid peroxidation. Methods Enzymol 1990; 186:421-431.
11. Lazzarino G, Tavazzi B, Di Pierro D, Vagnozzi R, PencoM, Giardina B. The relevance of malondialdehyde as abiochemical index of lipid peroxidation of post ischaemic tissues in the rat and human beings. Biol Trace Elem Res1995; 47: 165-170.
12. KakkarP, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984; 21: 130-132.
13. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972; 47: 389-394.
14. RotruckJT, Pope AL, GantherHE, Swanson AB, HafemanDG. Selenium: biochemical role as a component ofglutathione peroxidase. Science 1973; 179: 588-590.
15. Eskiocak S, Gozen AS, Kilic AS and Molla S. Association between mental stress and some antioxidant enzymes of seminal plasma. Indian J Med Res 2005; 122: 491-496.
16. Rostami A, Boojar MM, Adibi P, Changiz T. Level ofoxidative stress markers among physicians in a medicalresidency program. Arch Environ Occup Health 2008; 63:154-158.
17. Nakhaee A, Shahabizadeh F and Erfani M. Protein and lipid oxidative damage in healthy students during and after exam stress.PhysiolBehav 2013; 118: 118-21.
18. Sivonová M, Zitnanová I, Hlincíková L, Skodácek I, Trebatická J. Oxidative stress in university students during examinations. Stress 2004; 7: 183-188.