

# Evaluation of oxidative stress in response to examination stress in dental students

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**Abstract:** Oxidative stress gained the attention recently in behavioral medicine and been contributes to various psychological disturbances and mental stress in Medical and Dental students. This study is to evaluate the level of oxidative stress markers in undergraduate students caused by examination stress.

*This cross sectional study was conducted at Priyadharshini Dental College. Fifty healthy final year dental students were participated and the level of oxidative stress (Lipid peroxidation and Antioxidant status) were assessed on the day of examination “stress condition” and compared with the same parameters obtained from the same students after the exam “non-stress condition”.*

*The results shows that oxidative damage and sensitivity to lipid oxidation were significantly increased in “stress condition” ( $P<0.05$ ) when compared with the same parameters in “non-stress” conditions. A significant decrease in plasma antioxidant level was observed ( $P<0.05$ ) under stress.*

*There is significant higher in oxidative stress its adversely affects the student performance and mental health during the examination.*

**Keywords:** Examination Stress, Antioxidant status, Lipid peroxidation, Oxidative stress and Reactive oxygen species (ROS).

## Introduction:

Biological stress is a complex phenomenon involving many biochemical changes. There is some information on the contribution of oxidative stress in the etiology or manifestation of psychiatric disorder [1, 2]. A connection has been reported between psychological stress and the toxic

effects of reactive oxygen species, resulting in oxidative stress [3].

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to detoxify the reactive normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and nucleic acids. In humans, oxidative stress is involved in many diseases, such as atherosclerosis, Parkinsonism, myocardial infarction (MI), Alzheimer's disease, fragile X-Syndrome, and chronic fatigue syndrome; but short-term oxidative stress may also be important in prevention of aging. Oxidative stress contains large numbers of free radicals that are capable of initiating or promoting oxidative injury [4]. It is

Suggested that oxidative injury may play a major role in mediating the health risks associated with oxidative stress.

Reactive oxygen and nitrogen species ( $O_2^-$ , OH,  $H_2O_2$ , NO), including free radicals, are produced in the body in both physiological and pathological conditions. In physiological conditions, they may arise as regulatory mechanisms, intercellular signaling species and bactericidal agents. Their production is normally controlled by the antioxidant defense systems that include intracellular enzymes, such as SOD, CAT, GPx and low molecular weight antioxidants (e.g. ascorbic acid, glutathione, vitamin E). Although repair mechanisms exist, some steady-state basal oxidative damage occurs in all individuals.

Moller *et al.*, [5] found that psychological stress increases the oxidative stress likewise, Cernak *et al.*, [6] found that increased formation of superoxide  $O_2^-$  and malondialdehyde (a marker of oxidative damage mainly to lipids) modified the antioxidant defense system in stress condition.

The aim of our study was to investigate oxidative stress through the determination of its markers (oxidative damage to lipid oxidation and antioxidant status) in dental students exposed to the psychological stress induced by examinations, compared to non-stress conditions (After the exam).

### **Materials and Methods:**

The present study was undertaken in Priyadarshini Dental College and it was approved by institutional ethics committee (11/Jan/2019). Study participants signed an informed consent. 50 healthy volunteers are participated at the average age of 19-22 years. During the experiments students were instructed not to take any antioxidant vitamins, minerals or other such natural products, and to avoid excessive intake of wine, alcoholic drinks, juices, fruits and vegetables. Blood samples (5ml) were obtained using aseptic technique, after the centrifugation serum sample was collected, which then used for the determination of oxidisability of lipids and antioxidant status for both the "stress" and "non-stress" conditions.

### **Biochemical Investigations:**

#### **Estimation of lipid peroxidation**

The levels of lipid peroxidation, MDA were estimated by the modified method of Satoh [7]. This assay is based on the action of thiobarbituric acid (TBA) with MDA, one of the aldehyde products of lipid peroxidation. 0.5ml of serum was mixed with a reagent containing 20% TCA and centrifuged at 3,500 rpm for 10 min. The precipitate was washed with 2 ml of 0.05 M

H<sub>2</sub>SO<sub>4</sub> and centrifuged at 3,500 rpm for 10 min. 3ml of TBA was added and the tubes were kept in boiling water bath for 30 min and cooled under tap water. 4ml n- butanol was added then mixed well or vortex for 5–10 min and centrifuged at 3,000 rpm for 10 min. The supernatant fluid was taken and measured the optical density at 532 nm in the spectrophotometer. The serum values were calculated from standard graph.

### **Activity of Antioxidant enzymes**

Antioxidant status was evaluated by the enzyme activities of SOD, CAT, GSH and GPx with standard methods. Total SOD activity was determined by the method of Kakkar [8] in which reduction of the substrate, nitrobluetetrazolium (NBT), is used to indicate O<sup>2-</sup> production. NBT is reduced to 50% by one unit of SOD at 240nm [9]. Catalase activity was measured by breakdown of H<sub>2</sub>O<sub>2</sub>. In a minute 1 unit of catalase breaks up 1μ/mole of H<sub>2</sub>O<sub>2</sub> and CAT activity is written as U/ml. The GPx level was estimated by oxidation of glutathione by H<sub>2</sub>O<sub>2</sub>. This chemical change is coupled to the reduction of oxidized glutathione by glutathione reductase, which oxidizes NADPH to NADP<sup>+</sup> at the absorbance of 340 nm [10].

### **Statistical analysis**

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

### **Result:**

In this study 50 students at the average age of 19 to 22 years were participated. After the signed informed consent form 5ml of blood sample was collected at aseptic condition. The Levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) & glutathione peroxidase (GPx) were measured with standard procedure.

The Fig.1 shows the levels of malondialdehyde (MDA) were increased during stress than the relax state. The value of MDA at (stress state) 2.95±0.08 and (Non stress) was 1.74±0.18. Therefore our findings suggest that lipids in “stress” are more sensitive to the oxidation compared to the “non-stress” condition.

The levels of antioxidants (SOD, Catalase, GPx and GSH) were significantly increased in stress condition compared to non-stress. Fig.2 shows the mean±SD value of SOD on the day of exam is 0.66±0.09 and significantly lowered after the exam 0.40±0.02. Same in the Catalase concentration determined during the stress 4.31±0.03 raised and lowered in non-stress 3.82.0.04 indicates a significant difference.

Glutathione showed a significant increase in stress level as compare to non-stress, which is 9.47±0.21 and 9.13±0.13 respectively. The values of glutathione peroxidase during the stress state and relax state were 0.48±0.03 and 0.63±0.04 respectively showing a significance difference depicting higher lipid peroxidation.

Fig.3 shows the comparison of oxidative stress and lipid peroxidation between male and female students, show a significant difference in the level of MDA, SOD, CAT, GPx and GSH, which indicate that males have moderate level of oxidative stress, and depression; while female of same batch are emotionally unstable, tended to be neurotic, anxious, and stressed. But do not show

any significant difference.

### **Discussion:**

Increased sensitivity of lipids to oxidative stress and reduced plasma antioxidant capability of students on their examinations, supports our findings which could be psychological stress and oxidative stress. This could be influenced also by various exogenous factors (e.g. seasonal nutrition habit, smoking, no physical activity).

This study showed significant increase of oxidative damage during the stress just before university examinations support the findings of Irie *et al.*, [11] who showed elevated production of 8-oxo-dG (a marker of oxidative damage to DNA) during psychological stress in rats and humans.

Similarly, increased sensitivity of lipoproteins to oxidation in students before examinations are in accord with results published by Cernak *et al.* [12]. They found elevated production of malondialdehyde (marker of oxidative damage to lipids) in young volunteers exposed to chronic or sub-chronic stress for more than 3 months. In addition, they found increased production of superoxide in plasma and modified antioxidant defense in these individuals. Together, the changes in these markers increased the oxidative stress during examination.

The present study was to prove the effect of long-term oxidative stress on dental students by estimating their lipid peroxidation (MDA levels). We can conclusively prove that there is significant raise in serum MDA levels were observed which may be due to high oxidative stress. This finding is supported by the study performed by Nakhee *et al.*, [13] who also reported significantly high levels of MDA during exams than those in post exam period.

The levels of Antioxidants (SOD, CAT, GSH and GPx) were significantly increased in students who are appeared for the exam compare to post exam period. Examination stress can significantly increase the pro-oxidant MDA while decreasing the anti-oxidant levels [14-16]. As SOD is the first enzyme involved in the metabolism or destruction of superoxide anion radicals, the decreased activity of SOD initiate the formation of high levels of ROS, and subsequently increase in membrane lipid peroxidation.

Other antioxidant markers, CAT, GPx and GSH, also showed higher levels during the examination period, and these might contribute to the accumulation of H<sub>2</sub>O<sub>2</sub> in the body. Thus, examination stress apparently shifts the delicate pro- and anti- oxidation balance to a more pro-oxidative state. Continuous exposure to oxidative stress may causes risk of ROS related disease. [17-19]. In a study performed by Rostami *et al.*, [16] also reported a higher level of SOD & Glutathione peroxidase during stressful conditions and a decline in these levels after the stress was over. This may also suggested to consume antioxidant-rich supplementation during an examination period.

### **Conclusion:**

The present study is revealed that examination stress has a direct effect on lipid peroxidation and antioxidant status, as its levels were found to be significantly high in students. It is suggested that this study indicates that those students who are high on stress due to high study

burden, if detected at the earliest can be saved them from advance health disorders due to the oxidative stress.

### **Acknowledgement:**

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### **Conflict of Interest:**

All the Authors declare that they have no conflict of interest.

### **Reference:**

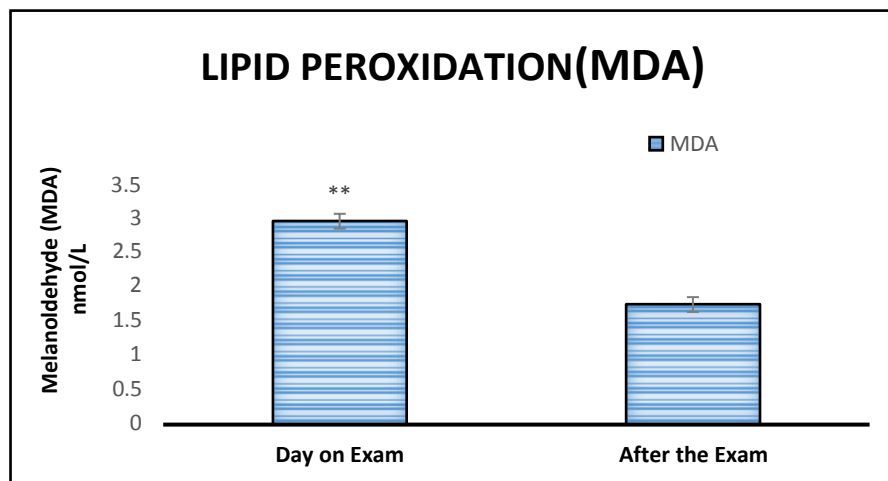
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**Results:**

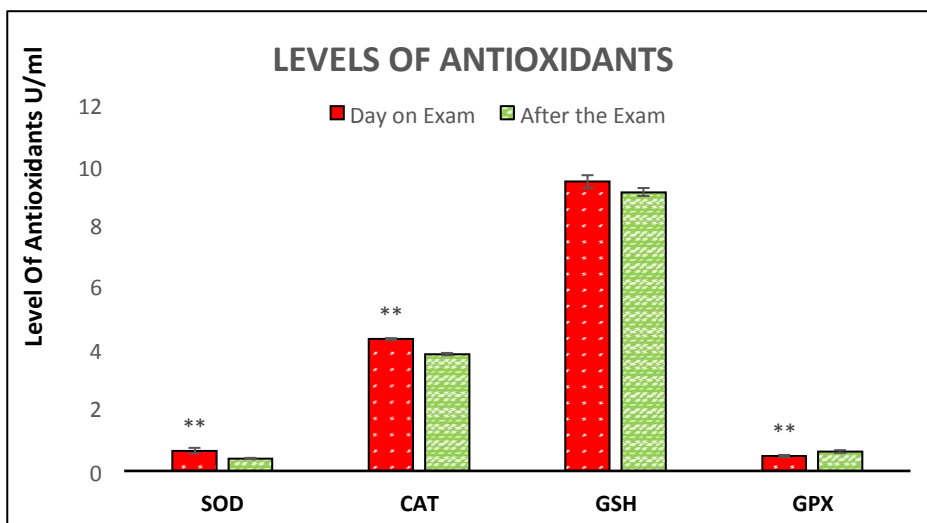
Fig.1 level of Lipid Peroxidation Dental students during the examinations and after the examinations.



Data presented are means±SD. Analysis of data was done by one-way ANOVA and student‘t’ test.

The significant difference (P<0.05) are flagged with \* and (P<0.001) is \*\*.

Fig.2 level of Antioxidant status in Dental students during the examinations and after the examinations.

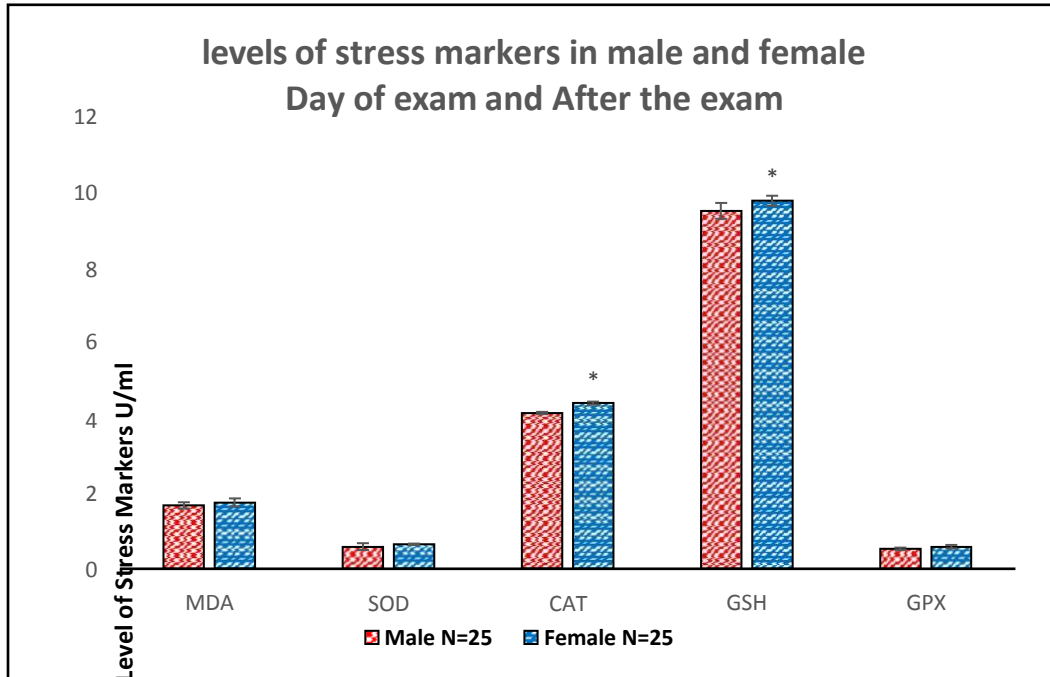


Data p

resented are means±SD. Analysis of data was done by one-way ANOVA and student‘t’ test.

The significant difference (P<0.05) are flagged with \* and (P<0.001) is \*\*.

Fig. 3 Levels of Stress Markers in male and female students during examination and after the examinations.



Data presented are means $\pm$ SD. Analysis of data was done by one-way ANOVA and student 't' test.

The significant difference ( $P < 0.05$ ) are flagged with \* and ( $P < 0.001$ ) is \*\*.