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# A study of oxidative stress and influence of antioxidant vitamins supplementation in patients with major depression.

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**Abstract**

**Many lines of evidence suggest that oxidative stress is important in the pathogenesis of major depression (MD). In the particular, catecholamine's and increased metabolism seems to be associated with tissue damage involving free radicals in the brain. The aim of this study was to investigate the lipid peroxidation and total antioxidant capacity in the patients suffering from the MD as compared to the healthy controls. Specifically we wanted to estimate the effective influence of antioxidant supplementation on the oxidative stress parameters including antioxidant defense system in MD patients. Thirty MD patients and thirty healthy controls were participated in the study. Lipid peroxidation was assessed by measuring concentration Malondialdehyde (MDA) and NO metabolites in plasma. Antioxidant activity was measured by determination of erythrocyte-superoxide dismutase (SOD) as a powerful enzymatic antioxidant and the non – enzymatic antioxidants like vitamin E, C and uric acid along with total antioxidant capacity in plasma. All measurements were taken in newly diagnosed patients and then during depression remission after 12 weeks treatment along with antioxidant supplementation. The concentration of MDA and NO metabolites were significantly higher in patients before treatment (newly diagnosed). Levels of antioxidants such as SOD, vitamin E, C and uric acid were significantly decreased in patients along with decrease in plasma TAC as compared to controls. After 12 weeks antidepressant treatment adjunctive to antioxidant vitamin E and C supplementation showed reverse changes in above parameters significantly. MD is accompanied by imbalance in pro- and anti-oxidative processes and finally, combined therapy with antioxidants and antidepressant has an improved potential in preventing oxidative damage and repairing already existing damage, but this has to be confirmed in future clinical studies.**

**Keywords:** Major Depression (MD), Lipid peroxidation, Oxidative stress, Malondialdehyde (MDA), Total Antioxidant Capacity (TAC).

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## Introduction

Reactive oxygen species (ROS) are produced in metabolic and physiological processes and harmful oxidative reactions may occur in organisms, which remove them via enzymatic and non- enzymatic antioxidative mechanism. Under some conditions, the increase in oxidants and decrease in antioxidants cannot be prevented, and the oxidant/ antioxidant balance shifts towards oxidative stress status. Consequently, oxidative stress, which has been implicated in over many neuropsychiatric disorders including MD. Neurons are especially susceptible to free radical attacks, and insufficient defenses of exposure to excess ROS can lead to neuronal dysfunction and death [1], Oxidative stress, which is one of the important mechanisms, causes the destruction of cells by decreasing the volume of hippocampus in patients with MD [2]. There is evidence for oxidative disturbances in major depression, as demonstrated by oxidative marker studies and those examining the antioxidant effects of antidepressant. Human studies have reported a number of oxidative disturbances in patients with MD, including oxidative damage in erythrocytic membranes suggested by the depletion of omega 3- fatty acids (Peet,1998), elevated lipid peroxidation product [3,4,5,6] reduced vitamins E and C (4, 7, 8). Increase concentration of the endogenous inhibitor of

endothelial NO synthase asymmetric dimethylarginine (ADMA and decreased NO [6, 9]. The findings of altered antioxidant enzyme levels have been mixed with reports of elevated SOD [3, 4, 5], diminished SOD activity [10], and no change [9].

In one study of MD patients, who had been medication free for at least 2 months, the plasma total antioxidant potential and uric acid were reduced in patients compared with controls; where as their total plasma peroxide levels and oxidative stress index were both higher [11]. There are no enough clinical trials of antioxidants therapies have been published for MD disorder. A small, open-label study adjunctive ginkgo biloba extract in treatment of patients with MD has been published, reporting the positive outcomes in terms of improved sleep efficiency and awakenings, but depressive outcomes were not reported [12].

The illness has a poor outcome in spite of best available treatment. However, the majority of patients respond to pharmacological treatment if diagnosed early. Antidepressant like fluoxetine protects from oxidative damage at only certain limits, but the mechanism of this protection is not known. Hence development of novel strategies to improve outcome which include early diagnosis and treatment will be of great benefit to patients of MD.

This study was undertaken to find out the association of oxidative stress with the antioxidant balance in the newly diagnosed MD patients and in healthy controls and the effect of antioxidant vitamins E and C supplementation on it. To support this working hypothesis we measured MDA as the index of lipid peroxidation, NO as nitrates and nitrites, and individual antioxidant such as erythrocytes SOD activity, plasma vitamin E, C, uric acid and TAC to evaluate the antioxidant status in both pre- and post-treatment periods, and in a control group.

## Materials and Methods

The study was conducted at the department of Biochemistry and psychiatry, PDVVPF's Madical College and Hospital, Ahmednagar. The total of 30 patients with MD and 30 age and sex matched controls with an average age of 32.20 ± 6.4 years were included in the present study. A diagnosis of MD was made by DSM-IV criteria [13]. All the patients and controls were free of any medication at least one month. Patients with a history of drug abuse or dependence, serious medical illness, severe head injury or seizure disorders were excluded from the study. None of the control subjects had a history of psychiatric disorders, severe head injury, seizure, diabetes mellitus, chronic renal failure, hypertension etc both groups consisted of no smokers. Oxidative stress markers such as MDA, NO and antioxidant activity were assessed before and after antioxidant supplementation along with routine antidepressant therapy. The supplementation was done with vitamin E (400IU/ day) and C (250mg/day) for 12 weeks. The overall study was carried out in accordance with Helsinki declaration made in 1975 (revised in 2000) and was approved by our institutional ethical committee. Written informed consent was obtained from all the subjects. The fasting blood samples were collected in 5 ml heparinized tubes on the initial test day and after 3 months of supplementation treatment. Plasma was separated by centrifugation at 3000 g for 15 minutes. Separated plasma was used for the estimation of MDA, NO, vitamin E, vitamin C, TAC. The buffy coat was removed and the packed cells were washed three times with physiological saline. The erythrocytes suspension was prepared by the method of Dodge et. al. [14], modified by Quist [15]. SOD activity was measured in hemolysate, according to the method of Kajari Das [16]. Plasma vitamin E was determined by the method of Baker and Frank [17]. Vitamin C (Ascorbic acid) levels were estimated in plasma by the method of Tietz [18]. Plasma MDA and NO concentrations were determined as the measure of TBARS [19] and Najwa and Cortas [20] respectively. TAC was measured by the assay of FRAP [21]. All the reagents used were of analytical reagent grade.

### *Statistical analysis*

Statistical analysis between controls and patients was performed by students't' test using Graph Pad Prism, Version 3.02 software. The data were expressed as mean± SD, $p< 0.05$ was considered as significant.

## Results

Analysis of mean values of the parameters for oxidative stress and antioxidant status between pre and post supplementation in MD group are shown in the Table 1. All the individual parameters of antixidative status tend to decrease, the decrease of vitamin E ($p<0.0001$) and vitamin C ($p<0.0001$) was statistically significant as compared to control subjects. Oxidative stress indicator MDA was significantly higher ($p< 0.001$) in newly diagnosed MD patients than the control population. However, the mean plasma level was found to be significantly decreased ($p< 0.04$) after supplementation.

he NO levels showed statistically significant ($p<0.01$) increase in patient before supplementation. But there is no significant improvement in their levels after supplementation. Erythrocyte –SOD activity was significantly lower ($p<0.02$) in depressed patients before supplementation as compared to healthy controls. Depressed patients showed no significant difference before and after antidepressant treatment along with antioxidant supplementation. The plasma vitamin E, C and TAC levels were also found to be increased significantly after supplementation. Plasma TAC in patients before supplementation was significantly

lower ($p<0.001$) that the controls group. It was statistically different before and after supplementation ($p<0.03$).

***Table 1.*** *The mean ± SD values of Plasma MDA, Erythrocyte-SOD, GSH, Plasma VITAMIN E, VITAMIN C, and TAC in controls and patients with Major Depression before and after intervention.*

| **Parameters** | **Healthy controls** (n=30) | **Before supplementation** (BS) (n=30) | **After supplementation** (AS) (n=30) | **p value** Controls vs BS | |
|---|---|---|---|---|---|
| | | | | Controls vs BS | BS vs AS |
| MDA (nmol/L) | 267.24 ± 34.12 | 296.27 ± 32.50 | 278.25 ± 36.30 | 0.001 | 0.04 |
| NO (mmol/L) | 24.45± 10.30 | 31.67 ± 10.78 | 26.02 ± 9.28 | 0.01 | 0.05 |
| SOD (U/gm Hb) | 34.14 ± 9.45 | 29.19 ± 7.67 | 31.46 ±10.23 | 0.02 | NS |
| Vitamin E (mg/L) | 12.58 ± 3.15 | 9.12± 2.56 | 10.87 ± 2.95 | 0.0001 | 0.01 |
| Vitamin C (mg/dL) | 0.97 ± 0.11 | 0.65± 0.18 | 0.75 ± 0.14 | 0.0001 | 0.01 |
| TAC (nmol/L) | 845.25 ± 175.50 | 677.10± 210.34 | 785.55 ± 168.50 | 0.001 | 0.03 |

*P < 0.05 considered statistically significant, NS=Not Significant*

## Discussion

This is the first study to show that combined supplementation of antioxidant vitamins E and C with regular antidepressant treatment is effective in improvement of oxidative stress parameters in the patients with MD. This study is also unique since it involved the patients and normal controls with same racial background with similar lifestyle and dietary patterns, which may seems to alter the oxidative indicators [22].

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The important measure of oxidative stress in our study was the concentration of MDA, which is a product of the free radical process. Our results showed a highly significant increase in the concentration of MDA in plasma of the patients with depression in comparison to that in healthy controls. Similar results were obtained by Bilici et. al., Sarandol et al. and Khanzode et. al. [3, 4, 5]. An increase in lipid peroxidation in the plasma of the depressed patients was also demonstrated by Selley et al.[6] and Forlenza and Millar [23] who measured the concentration of other end products of lipid peroxidation such as 4-hydroxy 2-nonenal (HNE) and 8- hydroxyl 2-deoxyguanosine. The raised levels of MDA could be due to increased generation of ROS due to the excessive oxidative damage generated in these patients. This also reflects the oxidative injury due to schizophrenia, which is attributed to free radicals formation that abstract hydrogen atoms from lipoproteins, causing lipid peroxidation [24].

The level of NO metabolites was found to increase in patients with MD in comparison with controls. These results are similar to that of number of studies [25]. However some studies showed no changes in the levels of NO metabolites [10]. There was no difference was seen in the levels of NO metabolites in the patients before and after supplementation.

An important finding of our study was that the patients with depression disorder have lower activity of antioxidant enzyme i.e. SOD in erythrocyte, before supplementation as compared to the healthy controls. Disturbances in the overall mechanism of generation of free radicals and their consequent neutralization due to oxidative stress in schizophrenia, where SOD is utilized for neutralizing free radical superoxide ion to $H_2O_2$ and oxygen [24].

In present study, our results indicated that the levels of vitamin E, vitamin C and uric acid were found to be decreased significantly in depressed patients as compared to controls which are supported by many studies [4, 7, and 11]. This indicates that increased utilization of all these free radical scavengers in the condition of excessive lipid peroxidation [4, 7]. The link between vitamin C deficiency and depression may be caused by lower neurotransmitter levels. Vitamin C works together with the enzyme dopamine-beta-hydroxylase to convert dopamine into nor-epinephrine, which plays an important role in the regulation of mood [28].

After supplementation these levels were significantly improved in patients with depression. Our finding regarding vitamin E and vitamin C suggested that, there was increased oxidative stress induced lipid peroxidation in the patients that resulted in increased consumption of the antioxidants vitamins leading to significant reduction. Moreover due to a prevailing decreased ascorbate level in them, oxidatively modified, inactive α-tocopherol could not be regulated into its active form. This further exaggerated the oxidative stress induced lipid peroxidation in the brain cell membrane. The free radical scavenging function of ascorbate most probably protects the SOD enzyme integrity and activity against the free radical induced damage. Thus data analyses suggested that SOD activity became dependent primarily on the plasma ascorbate level in the schizophrenic patients in the present study. Also ascorbate was found as an important antioxidant that pre-

vented dopamine against the oxidation by RNS derived from NO [29]. In present study we obtained significantly reduced level of TAC in plasma in MD patients compared to that of control subjects. Decline in TAC is also associated with increased production of free radicals and decreased levels of antioxidant defenses. Our data regarding the TAC is consistent with the previous reports showing depletion of main antioxidants: vitamin E, C, GSH and decreased activities of antioxidant enzyme in depression [30, 31]. This provides the indication that the mechanisms of free radical induced damage to lipids, proteins and DNA may be involved in the pathogenesis of depression. These components of TAC prevent the reaction that causes lipid peroxidation. An overall result suggests the disturbance in pro and antioxidant balance in depression. Lipid peroxidation can be prevented by antioxidant supplementation. Vitamin E and vitamin C are well known antioxidants that are postulated to protect against damage to biological membranes by their ability to scavenge free radicals. Accordingly, several studies have examined the efficacy of vitamin E or vitamin C in the treatment. Since antioxidants alone may stop ongoing oxidative damage and EFA have the potential to restore the cellular structure their combined use may be necessary for optimal treatment of oxidative cell damage [32]. Vitamin E is a lipid soluble antioxidant with the potential to prevent oxidative damage. However, vitamin E cannot prevent oxidative damage to cytosolic proteins, mitochondria, and nuclei, where most of the ROS are generated. Therefore, it may be important to use vitamin E in combination with vitamin C, a water soluble antioxidant. Even moderately low levels of vitamin C have been linked to depression. [33].

However, since our endogenous antioxidant defenses are not always completely effective, and since exposure to damaging environmental factors is increasing, it seems reason- able to propose that exogenous antioxidants could be very effective in diminishing the cumulative effects of oxidative damage.

Some of the studies demonstrated the antioxidant effects of antidepressant to improve the clinical conditions related to depressive disorders along with increase in the levels of antioxidants such as vitamin C and SOD (4). But not a single study that shown an exogenous antioxidant supplementation have reported. So the present study also suggests the combined therapy of antioxidant vitamin E and C with antidepressant may be more effective than monotherapy with antidepressant.

## Conclusion

In conclusion, oxidant/antioxidant imbalance may be involved in the pathogenesis of MD. The present study has clearly shown higher MDA level and reduced total antioxidant capacity which supports the hypothesis of oxidative damage in depression. The 12 weeks supplementation of antioxidants significantly influences the above parameters. Although the results of these studies are encouraging, further studies with placebo- controlled and at least two doses need to be done with larger number of patients with longer duration of supplementation. Apart from this, it is essential to mention that some confounding factors related to the patients habits, i.e. life style, dietary changes and exercise which may affect the levels of antioxidant system. Of course, we got all the results from peripheral blood in this study; further advanced technique should be adopted to make sure whether it mirrored the exact status in the brain.

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