

## ANTIOXIDANT ENZYME ACTIVITIES IN RECURRENT MAJOR DEPRESSION

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### Abstract:

The aim of this study was to investigate the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde (MDA) in patients suffering from recurrent major depressive disorder (MDD) as compared to healthy controls. There was an increased oxidative stress in patients with depression demonstrated by a decrease of the main antioxidant enzymes (SOD and GPX) and an increase of MDA, as a lipid peroxidation marker. The oxidative stress is more pronounced, mainly in severe depressed patients subgroup.

**Key words:** antioxidant enzymes, recurrent major depressive disorder

### INTRODUCTION

There are evidences that oxidative stress, due to dysfunction of oxidative and anti-oxidative systems is implicated in the pathogenesis of several neuropsychiatric disorders such as dementia, schizophrenia or Parkinson's disease.<sup>1,2</sup> It has been suggested that depression has been likened to “accelerated aging” affecting the hippocampus and the cerebrovascular, neuroendocrine, metabolic, and immune systems.<sup>3,4</sup> There are a few studies which investigated the role of oxidative stress in major depressive disorder and the results have been variable and contradictory. It was reported that

during depressive episodes, the concentration of malondialdehyde (MDA), a lipid peroxidation marker has increased<sup>5</sup>. The specific activities of some enzymatic antioxidant defenses like glutathione peroxidase (GPX) has decreased.<sup>6</sup> However, some studies reported an increased activity of catalase<sup>5</sup> and superoxide dismutase (SOD)<sup>6</sup> in depressive episodes. The aim of this study was to investigate the activities of SOD, GPX and MDA in patients suffering from recurrent major depressive disorder (MDD) as compared to healthy controls. These antioxidant enzymes act at different sites in the metabolic pathway of free radicals and any functional imbalance may result in oxidative stress.

## MATERIALS AND METHODS

### *Subjects*

Twenty six patients, who had applied to inpatient clinic of “Socola” University Hospital, and diagnosed with recurrent major depression disorder according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text Revision (DSM-IV TR), were investigated in this study. The scale used to assess depression severity was 17-item Hamilton Depression Rating Scale (HAM-D).<sup>7</sup> Scores between 18 and 24 indicate moderate depression, and scores over 24 indicate severe depression. Patients with an Axis I disorder, other than MDD or with an Axis II disorder were excluded (n = 3). Patients with a history of known major health problems, including endocrine, metabolic and cardiovascular system disorders (n = 1) or any neoplastic disease were excluded. Laboratory examinations, including complete blood count, serum electrolyte assay, liver function tests and thyroid function tests, were performed on all subjects. One patient was excluded because of the abnormalities in the thyroid function tests. The total number of recruited patients was 21 (12 females, 9 males; age range, 21 – 62 years). Eleven control subjects who were matched according to age, gender and smoking status were recruited among the hospital staff. The same laboratory test protocol was applied to the control subjects as it was applied to the patient group. The control subjects were free of any medication for at least 2 weeks prior to blood sampling. Exclusion criteria for the control group were: having a physical or psychiatric disorder as judged from their clinical and laboratory examinations. All assessments of a patient were performed by the same psychiatrist.

The study was conducted according to provisions of the Helsinki Declaration. All the patients signed the consent for the participation in this study.

### *Biochemical estimations*

Blood samples were collected in the morning, prior to first antidepressant dose, before breakfast, allowed to clot and centrifuged immediately. Sera were aliquoted into Eppendorf tubes and stored at -35°C until measurement.

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 substrate (a water soluble tetrazolium dye) and xanthine oxidase using a SOD Assay Kit (Fluka, 19160) according to the manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide) after 20 min of reaction time at 37°C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (Sigma Chemicals). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP<sup>+</sup> is indicative of GPX activity.

MDA levels were determined by thiobarbituric acid reactive substances (TBARs) assay. 200 µL serum was added and briefly mixed with 1 mL of trichloroacetic acid at 50%, 0.9 mL of TRIS-HCl (pH 7.4) and 1 mL of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100°C for 20 minutes. Afterwards, samples were centrifuged at 3000 rpm for 10 min and super-

natant read at 532 nm. The signal was read against an MDA standard curve and the results were expressed as nmol.<sup>8,9</sup>

### Statistical analysis

Results were expressed as mean  $\pm$  SD. MDD group was compared with the control group by using t-test for independent groups. Moderate and severe MDD patients and the control group were compared by using Mann – Whitney test. A p value  $< 0.05$  was taken as the criterion for significance.

## RESULTS AND DISCUSSION

We find out a significant decrease in the specific activity of SOD and GPX in depressed patients compared to control group (fig. 1 – fig. 4).

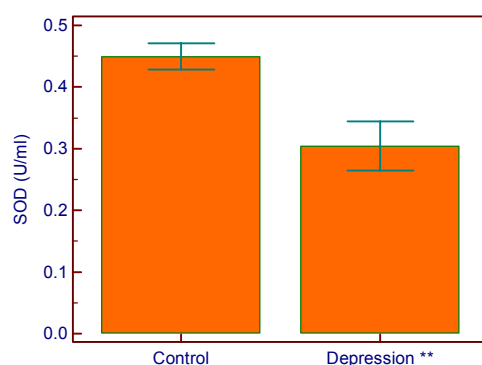


**Fig. 1.** SOD specific activity in control ( $n = 11$ ) and depressed patients serum ( $n = 21$ ,  $p = 0.02$ )

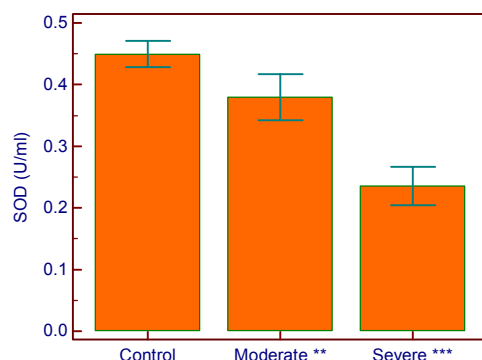


**Fig. 2.** SOD specific activity in control ( $n = 11$ ), moderate ( $n = 10$ ,  $p = 0.014$ ) and severe depressed patient groups ( $n = 11$ ,  $p < 0.001$ )

SOD activity was significantly increased in patients with MDD and furthermore there was a significant positive correlation between the severity of the disease and SOD activity (fig. 2).

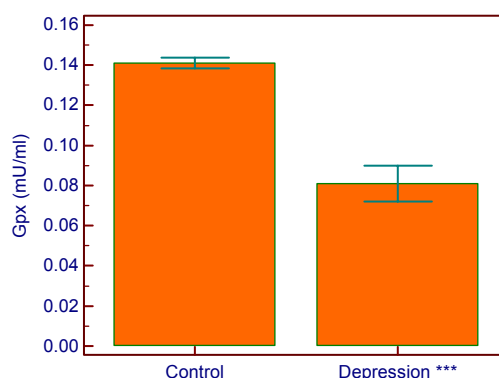


**Fig. 3.** Glutathione peroxidase specific activity in control ( $n = 11$ ) and depressed patients serum ( $n = 21$ ,  $p < 0.001$ )

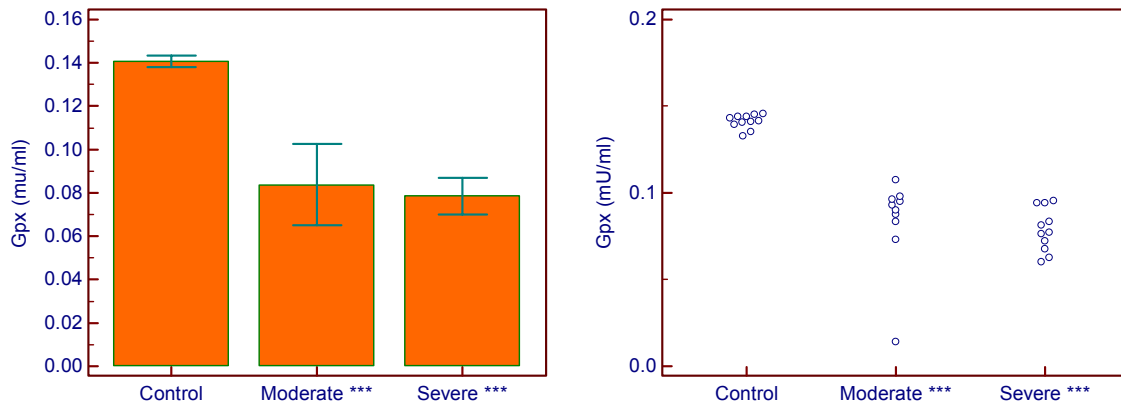


**Fig. 4.** Glutathione peroxidase specific activity in control ( $n = 11$ ), moderate ( $n = 10$ ,  $p < 0.001$ ) and severe depressed patient groups ( $n = 11$ ,  $p < 0.001$ )

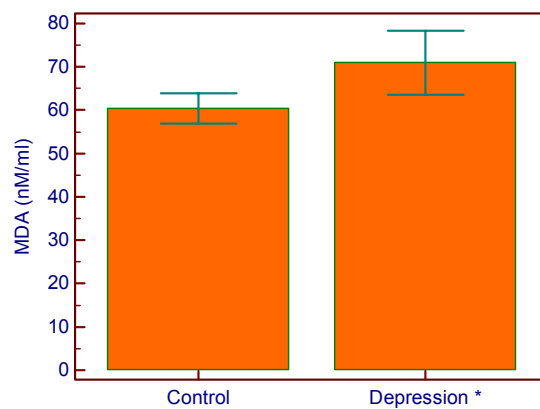


**Fig. 5.** Levels of malondialdehyde in control ( $n = 11$ ) and depressed patients serum ( $n = 21$ ,  $p = 0.0373$ )

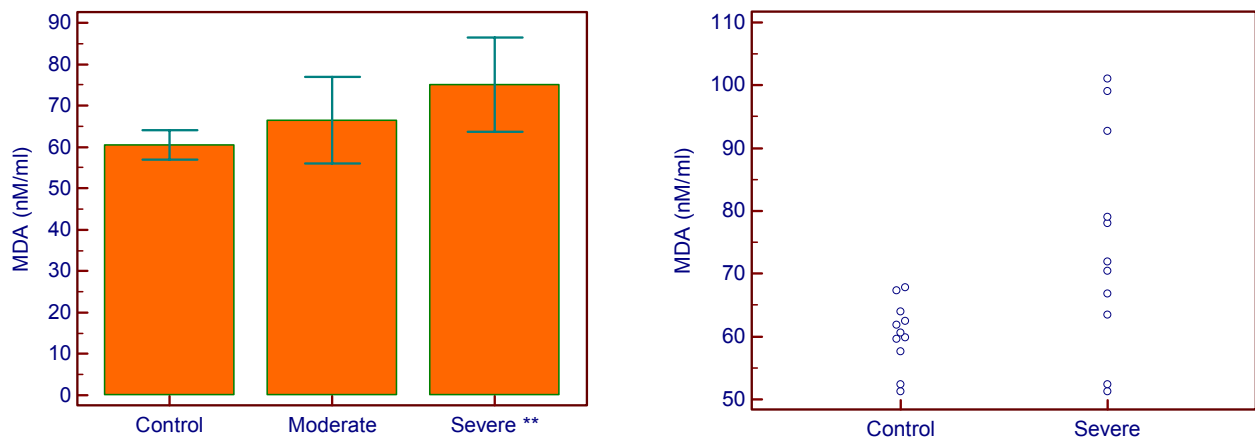


**Fig. 6.** Levels of malondialdehyde in control ( $n = 11$ ), moderate ( $n = 10$ ,  $p = 0.2599$ ) and severe depressed patient groups ( $n = 11$ ,  $p = 0.0165$ )

An increase in serum concentration of MDA was found in depressed patients serum, with higher levels in severe depressed subgroup (fig. 5, fig. 6). Increased plasma MDA levels observed in the present study are similar with data published by Selley et al.<sup>10</sup> and Ozcan et al.<sup>11</sup> Oxidative stress occurs when the production of oxygen-free radicals exceeds the capacity of the body's antioxidants to neutralize them. Oxidative stress damages the DNA, protein, lipids, and other macromolecules in many tissues and the brain being particularly sensitive.<sup>12</sup> Elevated plasma oxidative stress markers and decreased antioxidant compounds have been linked with depression and chronic psychological stress.<sup>2,13</sup> The severity and recurrence of depression are correlated with the oxidative

stress markers.<sup>14, 15</sup> Because cellular oxidative damage is an important component of the aging process, prolonged or repeated exposure to oxidative stress could accelerate aspects of biological aging and promote aging-related comorbid diseases in depression.<sup>16</sup>

## CONCLUSIONS

Our study findings showed that recurrent major depression is correlated with oxidative stress. This was demonstrated by a decrease of the main antioxidant enzymes (SOD and GPX) and an increase of MDA, as a lipid peroxidation marker. The oxidative stress is more pronounced, mainly in severe depressed patients subgroup. Previous studies have demonstrated a significant correlation between oxidative stress and depression.<sup>2,15</sup> This study attempts to raise some questions regarding the role of oxidative stress in pathophysiology of depression. Furthermore, antioxidant compounds could be used as add-on strategy in difficult-to-treat and recurrent depressive states.

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