The Effects of Escitalopram Treatment on Oxidative/Antioxidative Parameters in Patients with Depression

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ABSTRACT:
The effects of escitalopram treatment on oxidative/antioxidative parameters in patients with depression

Objective: In this study, we aimed to investigate the effects of escitalopram, an antidepressant drug of the selective serotonin reuptake inhibitor group, on lipid peroxidation, nitric oxide (NO) level and superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities in patients with major depressive disorder.

Method: Eighteen patients (11 women, 7 men) diagnosed with major depressive disorder (MDD) according to DSM-IV criteria and eighteen healthy controls (10 women, 8 men) were included in the study. The relevant parameters were measured before and after treatment with 20 mg/day escitalopram for 6 weeks in patients and only once in the controls.

Results: Plasma SOD, CAT, malondialdehyde (MDA) and NO levels were significantly higher before treatment in patients with major depression compared to healthy controls; there was no significant differences in GPx levels. Treatment with 20 mg/day escitalopram for 6 weeks reduced plasma SOD, CAT, MDA and NO levels statistically significantly; it had no effect on GPx levels.

Conclusion: The results provide evidence for the role of oxidative stress in the pathogenesis of MDD and revealed that subchronic treatment with escitalopram significantly decreased the activity of antioxidant enzymes and MDA values. It may be argued that antioxidant enzymes such as SOD, CAT and oxidative stress markers such as MDA and NO are state markers of MDD, because values came close to the results of healthy subjects after treatment.

Keywords: escitalopram, major depression, lipid peroxidation, antioxidant

INTRODUCTION

Major depressive disorder (MDD) is a serious psychiatric mood disorder resulting in detrimental effects, including increased health care expenditure and elevated suicide rates; about one-third of MDD patients have failed two or more conventional antidepressant drug trials within the first year of treatment¹⁻². Selective serotonin reuptake inhibitor group (SSRI) drugs have been widely used in the treatment of MDD because of their efficacy, safety, and tolerability, as they modify both enzymatic antioxidants and lipid peroxidation³⁻⁴. Escitalopram is an SSRI whose absolute bioavailability of about 80% absorption of escitalopram is independent of food; it is almost entirely absorbed from the intestine. It takes a week to raise constant plasma levels². Current evidence suggests that the pathophysiology of MDD is multifactorial and that inter-related mechanisms effect genetic, neurotransmitter, immune, oxidative, and inflammatory systems.
with increased production of procytokines. The oxidation of catecholamines such as dopamine and serotonin by monoamino oxidase (MAO) may result in increased radical burden. There are numerous studies indicating that reactive oxygen species (ROS) inducing neuronal damage have an important role in the pathophysiology of depression, probably via membrane omega 3 polyunsaturated fatty acids (PUFAs) pathology, by decreasing the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Abnormal levels of antioxidant enzymes and lipid peroxidation in MDD further substantiate the role of free radicals in major depression. Insufficient defenses against exposure to excess ROS can lead to neuronal dysfunction and the death of neurons. Because ROS have short half-lives, most studies measure the products of oxidative damage. Malondialdehyde (MDA) is the most studied product of lipid peroxidation. Numerous studies reported increasing levels of MDA and other products of lipid peroxidation in depressed patients. Nitric oxide (NO) is responsible for the formation of many end products involved in oxidative stress. Increased plasma NO levels have also been reported in depressive patients. The major antioxidative defenses include both enzymatic and non-enzymatic antioxidants. The levels of enzymatic antioxidants like SOD, CAT, GPx etc. are altered in major depressive disorder patients.

Several preclinical and clinical studies have investigated the effects of antidepressants on the oxidative/antioxidant system. Most of them have suggested a potential antioxidant effect of antidepressants and revealed that treatment with antidepressants can reverse the increased oxidative stress observed in depressive patients. However, there are contradictory findings on this issue. For example, some studies reported a decrease with antidepressant treatment of increased MDA and SOD levels in depressive patients. Others found no effects or any increase in SOD after antidepressant treatment. It is also not clear whether there is a difference in antioxidant potential between different antidepressants. Considering these aspects, we hypothesized that the oxidative/antioxidant activities may be altered by depression; and antidepressant therapy with escitalopram may attenuate these activities. Therefore, in this study, we aimed to measure MDA, NO, SOD, CAT, and GPx levels in patients with MDD before and after treatment with escitalopram.

**MATERIAL AND METHODS**

**Subjects**

The study group consisted of 18 patients aged 18-57 years (11 women, 7 men) who were admitted to the Psychiatry Outpatient Clinic of Erciyes University School of Medicine. The patients were diagnosed with major depressive disorder according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, (DSM-IV) criteria and met the inclusion criteria for the present study. Eighteen healthy controls (10 women, 8 men) were included in the study.

**Study Design**

Written informed consents to participate in the study was received from the subjects after they head been thoroughly informed about the research details. This study has been supported by Erciyes University Scientific Research Projects Unit. The research protocol was approved by the Ethics Committee of Medical Ethics at Erciyes University. The study was carried out according to the principles of the Helsinki Convention on Human Rights and good clinical practice. All subjects were included in the study after evaluation of their baseline laboratory test results (routine biochemical and hematological tests) in addition to physical and psychiatric examinations. Control group samples were measured only once because no drugs were administered to the control group for 6 weeks; thus, we did not expect any changes in the measured parameters.
Exclusion criteria were as follows: other comorbid psychiatric disorders, bipolar disorder, alcohol and substance use disorder, having received electroconvulsive therapy within the last 6 months, presence of a severe physical disorder of neurologic, endocrine, or hematologic nature. Patients and controls were undergone to standard clinical evaluation including psychiatric and physical evaluation on the first day of the study. Psychiatric diagnosis was determined via clinical interviews. Patients with first-episode unipolar depression were included. Patients were included in the study after a one-week drug washout period in case they were on any medications. The patients were assessed by the Montgomery-Asberg Depression Rating Scale (MADRS) to determine the severity of the disorder and response to treatment. The patients received 20 mg/day escitalopram, an SSRI, for 6 weeks; the controls did not. The patients did not receive any other medication or non-medication therapies.

**Laboratory Measurement**

Venous blood samples were collected from the left forearm vein into 5-ml vacutainer tubes containing potassium EDTA after overnight fasting between 7 and 8 AM. Blood specimens were allowed to clot for 30 min. SOD activity levels were assayed by the method of Sun et al. This method is based on the reduction of superoxide, which is produced by the xanthine oxidase enzyme system, by nitroblue tetrazolium. A unit of SOD was determined as the amount that decreased nitroblue tetrazolium reduction by 50%. GSH-Px activity levels in the hemolysates of erythrocytes were measured using the method of Paglia and Valentine, in which GSH-Px activity was coupled to the oxidation of NADPH by glutathione reductase. The oxidation of NADPH was followed spectrophotometrically at 340 nm at 37°C. The absorbance at 340 nm was recorded for 5 min. Activity was observed as the slope of the lines as μmol of NADPH oxidized per min. CAT activity was determined by the method described by Yasmineh et al. The principle of the assay is based on the determination of hydrogen peroxide decomposition. By measuring the absorbance changes per minute, the rate constant of the enzyme was determined. Levels of plasma MDA were measured by the thiobarbituric acid method, which was modified after the methods of Yoshiko et al. Peroxidation was measured as the production of MDA, which forms a pink chromogen compound in combination with TBA, whose absorbance at 532 nm was measured.

Serum nitrate plus nitrite levels were measured as an index of NO generation. To measure total nitrate level, nitrate is converted to nitrite by using nitrate reductase enzyme. Total nitrite was measured enzymatically with a modified Griess method defined by Smarason et al. The color that resulted from the reaction of the nitrite with Griess reagent was measured. Nitrite, as an end product, had a pink color and concentration determined by spectrophotometric absorbance at 548 nm.

**Statistical Analyses**

Statistical analyses were performed using statistics packages with SPSS software version 15.0 and SigmaStat 3.5. Normality of data distribution was assessed by Kolmogorov-Smirnov test. The mean values for the groups were compared using independent-samples t-test. Non-parametric analysis of the continuous data that did not fit the normal distribution was done with Mann-Whitney U test (for between-groups comparisons) and Wilcoxon test (for within-group comparisons before and after treatment). Group mean differences were examined by means of unpaired (for between-groups comparisons) or paired (for within-group comparisons before and after treatment) t-test. The difference in the distribution of categorical variables was tested using the Chi-square test. The Pearson correlation test was performed to investigate the relationship between oxidative stress parameters and MADRS scores before and after treatment, respectively. Categorical variables were expressed as number, and continuous variables were expressed as mean±SD.
or median (25th-75th percentile), as appropriate. Statistical significance was set at P value of 0.05.

RESULTS

The demographic and clinical data are summarized in Table 1. The mean age was 42.17±10.16 years for patients and 37.89±10.62 years for the controls. There were no significant differences between the patients and controls (p>0.05) in terms of age, sex, body mass index (BMI), smoking, and the amount of time and smoking status (rate and duration). The duration of education in the control group was significantly higher than in patients (Table 1).

Data are expressed as numbers for categorical variables and mean±SD or median (25th-75th percentile) for continuous variables.

When the distribution of values of the control group was analyzed, different variations were observed. For example, although SOD and MDA levels of the control group showed more variability, NO levels showed less variability than values of patients before and after treatment. The SOD, CAT, MDA and NO levels of the patients before treatment were found to be significantly higher than those of the controls. The SOD, CAT, MDA and NO levels in post-treatment were significantly lower than those in pre-treatment. After the treatment, SOD and CAT levels were higher than those of the controls, and the MDA level was significantly lower than that of the controls. The NO level of the patients in pre-treatment did not significantly differ from that of the controls (Table 2).

When the SOD, CAT, MDA and NO levels of the patients were compared with regard to gender differences, there was no statistically significant differences between women and men either before or after treatment. The GPx levels of women after treatment were found to be significantly higher than those of men after treatment (Table 2). There were no correlations between severity of depressive symptoms and oxidative stress parameters before or after treatment.

### Table 1: Socio-demographic and clinical characteristics of the patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Patient (n=18)</th>
<th>Controls (n=18)</th>
<th>p</th>
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<tbody>
<tr>
<td>Age</td>
<td>42.17±10.16</td>
<td>37.89±10.62</td>
<td>0.182a</td>
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<tr>
<td>Gender (F/M)</td>
<td>11/7</td>
<td>10/8</td>
<td>0.379b</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.40±5.31</td>
<td>25.26±3.06</td>
<td>0.120a</td>
</tr>
<tr>
<td>Duration of disease (months)</td>
<td>3.93±2.42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Duration of education (years)</td>
<td>8.0 (5.0-11.75)</td>
<td>12.0 (10.5-15.5)</td>
<td>0.005c</td>
</tr>
<tr>
<td>Duration of smoking (years)</td>
<td>0.0 (0.0-11.75)</td>
<td>1.0 (0.0-14.5)</td>
<td>0.483c</td>
</tr>
<tr>
<td>Number of cigarettes (pcs/day)</td>
<td>0.0 (0.0-10.75)</td>
<td>0.0 (0.0-27.5)</td>
<td>0.267c</td>
</tr>
</tbody>
</table>

Data are expressed as numbers for categorical variables and mean±SD or median (25th-75th percentile) for continuous variables. aVariables were tested using the independent samples t test, bvariables were tested using the Chi-square test and cvariables were tested using the Mann-Whitney U test. F: Female, M: Male, pcs: Pieces.

### Table 2: SOD, CAT, GPx, MDA, and NO levels of the patients and the controls

<table>
<thead>
<tr>
<th></th>
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<th>Controls</th>
<th>Comparisons</th>
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<tr>
<td></td>
<td>Pre treatment</td>
<td>Post treatment</td>
<td>Controls</td>
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<tr>
<td>MADRS score</td>
<td>36.70±7.93</td>
<td>15.00±5.95</td>
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<td>MDA (mmol/ml)</td>
<td>4.90±0.07a</td>
<td>3.70±0.04abc</td>
<td>3.80±0.08</td>
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<td>NO (µmol/L)</td>
<td>52.27±8.22a</td>
<td>43.66±7.89abc</td>
<td>42.93±7.15</td>
</tr>
<tr>
<td>CAT (U/ml)</td>
<td>3.82±0.10a</td>
<td>3.23±0.11abc</td>
<td>3.10±0.10</td>
</tr>
<tr>
<td>GPx (U/ml)</td>
<td>136.89±6.96</td>
<td>139.45±4.17</td>
<td>140.43±6.07</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>10.43±(10.12-10.51)</td>
<td>8.27±(8.2-8.39)</td>
<td>8.19±(8.15-8.23)</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD or median (25th-75th percentile) for continuous variables. The significance of differences in continuous variables between groups was tested using independent samples t test or the Mann-Whitney U test for SOD. Comparison of the values in pre-treatment and post-treatment was performed with paired samples t test and Wilcoxon test for SOD.

aHigher than those of the controls, bLower than those of the controls, cLower than those in pre-treatment.
DISCUSSION

Our results provide evidence of an increased oxidative stress status in MDD as shown by increased concentrations of MDA. The MDA levels of the patients before treatment were significantly higher than those of the controls and significantly decreased with treatment. After treatment, the MDA levels of the patients were significantly lower than those of the controls. Some other studies found significantly increased MDA levels in depressive patients, consistent with our results. Sarandol et al. reported significantly increased levels of MDA in MDD, but they did not find a significant difference after treatment.

It may be suggested that increased production of lipid peroxidation or decreased detoxification ability might cause increased oxidative stress in neuronal and glial cells. Moreover, MDA has an inhibitory effect on the receptor binding sites of serotonin. As a result, it may be tempting to speculate that these relationships support a possible etiopathogenetic association between oxidative stress and monoamine oxidase (MAO) activity in MDD. Catecholamines including dopamine and norepinephrine are associated with oxidative stress, thus conditions causing increased catecholamine metabolism may increase the radical burden as observed in MDA. Moreover, it has been reported that increased free radical production may cause the destruction of phospholipids and alter viscosity of neuron membranes, and consequently changes in membrane viscosity may affect receptor functions.

In addition to these results, this study secondly shows significantly increased stress in MDD with nitrogen-reactive species. The NO levels of patients before treatment were found to be significantly higher than those of controls. NO levels were significantly decreased by treatment with escitalopram. Most importantly, the NO levels of patients after treatment were similar to those of the control group, which shows the success of antidepressant therapy in reducing oxidative stress. Some other studies found significantly decreased levels of NO in depressed patients with treatment, consistent with our results.

Lu et al. reported significantly increased levels of NO in depressed patients and showed significantly decreased levels of NO after 2 months of fluoxetine treatment. Chrapko et al. reported significantly increased levels of NO in depressed patients and showed significantly decreased levels of NO after 2 months of treatment with paroxetine. Moreno et al. reported that MDD patients significantly differed from controls in NO levels. Individuals may have an inadequate antioxidant enzymatic activity that is incapable of responding to increased free radical production, which could lead to some of the various pathological alterations originating from having MDD.

Our findings provided evidence of an increase not only of oxidative stress markers but also of antioxidant activity. However, the other important finding is the significantly lowering of oxidative stress markers and antioxidants in MDD after treatment with escitalopram. The SOD and CAT levels of patients before treatment were significantly higher than the SOD and CAT levels of controls. At the same time, SOD and CAT levels were significantly decreased with treatment with escitalopram, but still higher than those of the controls. Nevertheless, it was not possible to say the same thing for GPx, which was another antioxidant examined in our study: There were no statistically significant differences between the GPx levels of controls and those of patients before treatment. When the GPx levels in patients were compared before and after treatment, there were no statistically significant differences in terms of GPx levels. Among the antioxidant enzymes; SOD dismutates superoxide radicals to form hydrogen peroxide, which in turn is decomposed to water and oxygen by GPx and CAT, thereby preventing the formation of hydroxyl radicals. Therefore, these enzymes act cooperatively at different sites in the metabolic pathway of free radicals. Failure of this antioxidant defense may lead to oxidative damage and the initiation of lipid peroxidation. Therefore, our findings of significantly decreased activity of two antioxidant defense enzymes (SOD and CAT) in
treatment with escitalopram in the present study indicate increased oxidative stress in MDD.

Sarandol et al. similarly reported significantly increased antioxidant activity of SOD in MDD, but they did not find a significant difference after treatment14. Galecki et al. indicated a statistically significant decrease in the activity of SOD and CAT as well as in MDA concentration after combined therapy24.

Some speculations can be made regarding this issue. It has been reported that antidepressant treatment may suppress immune cells, including natural killer cells32. Suppression of immune cells by means of treatment with escitalopram may cause a decrease in oxidative stress. Moreover, SOD and CAT are two key antioxidant enzymes that protect against oxidative tissue damage. It is therefore conceivable that down-regulation of these two enzymes (SOD and CAT) could lead to further free radical-induced neurotoxicity/neurodegeneration in MDD. Based on our results, we can say that an increased generation of oxygen and nitrogen reactive species in MDD may lead to an increase in antioxidants. These findings are consistent with some recent studies that found significantly increased levels of MDA, SOD and GPx activity in patients with MDD and schizophrenia15,33-36. Atmaca et al. found increased levels of MDA, SOD, CAT and GPx activity in patients with social phobia37. Moreover, some studies found reduced levels of plasma MDA and SOD in patients with MDD and schizophrenia11,38-39, while others failed to find any difference in SOD, CAT and GPx activity31,34,40,41 between patients and normal controls.

As can be seen from the literature, the results seem to conflict with one other. Several factors, such as differences in techniques of measuring SOD levels, sampling of patients in different stages of disease progression (acute or chronic), differences in testing material (red blood cells or plasma), exposure to neuroleptic treatment (currently treated or ceased medication treatment), different illness courses of patients or different ethnic origin and lifestyle may be responsible for the discrepancy. The findings of the present study may contribute to the literature providing information on oxidative stress and antioxidant activity in MDD patients with treatment. The treatment for 6 weeks with 20 mg/day escitalopram significantly decreased plasma levels of SOD, CAT, NO and MDA; on the other hand, GPx levels were unaffected by treatment. Post-treatment plasma levels of SOD and CAT were significantly higher, while MDA levels were significantly lower than those of the controls. Levels of NO and GPx after treatment were not significantly different from the controls. After treatment; GPx, CAT, NO, SOD and MDA showed 1.8%, 15%, 16.5%, 19.5%, 24% variations, respectively. According to these findings, it may be speculated that MDA is superior to other parameters for predicting treatment response. Increased levels of MDA have been found in depression and antidepressant treatment has been able to lower the concentrations of lipid peroxidation4,11,42. Abdel-Wahab et al. showed that long-term venlafaxine treatment at effective antidepressant dosages can protect against stress-induced oxidative cellular and DNA damage in male mice43. They also showed that at all doses tested, venlafaxine decreased MDA. Increased levels of MDA have also been found in our patient group, and escitalopram treatment has been able to lower the concentrations of MDA. Of the many biological targets of oxidative stress, lipids are the most susceptible class of biomolecules and MDA serves as a diagnostic indicator of lipid peroxidation in many diseases. Therefore, when we compare them with other parameters, we may say that MDA is superior to other parameters for predicting treatment response due to MDA being the most susceptible classes of biomolecules.

There are several limitations in the present study. Therefore, it should be considered as a preliminary study from our group. The sample size was small and control group samples were not analyzed after treatment, since no differences were expected in terms of the control values. Furthermore, no placebo control group was used. Our results need to be confirmed by placebo-
controlled studies with a larger number of samples. Additionally, we were not able to perform Structured Clinical Interview for DSM Disorders (SCID) for diagnosis and determination of specifiers such as melancholic depression. An association between melancholic depression and antioxidative enzyme activities and lipid has been reported. It is a limitation of the present study that we were not able to assess anxiety symptom severity in depressive patients and define depression subtypes such as melancholic, atypical etc. Recent studies suggested a relationship between anxiety and oxidative stress. Another limitation of the study was the short duration of medication washout. Finally, some confounding factors that we were not able to notice might have influenced oxidative stress parameters.

In conclusion, our results may provide encouraging evidence for the role of oxidative stress in the pathogenesis of MDD and suggest that subchronic treatment with escitalopram may decrease activity of SOD, CAT enzymes and levels of MDA and NO. Most importantly, the NO levels of patients after treatment were closer to the NO levels of healthy individuals, which shows the success of antidepressant therapy in reducing oxidative stress.

Acknowledgements

This research was supported by the scientific research project unit of Erciyes University (TSY-09-887). We are grateful to all participants and everyone who contributed to this research.

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