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Lipid peroxidation and antioxidant protection in patients during acute depressive episodes and in remission after fluoxetine treatment

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Abstract:
Increasing numbers of studies indicate that free radicals and their derivatives play a role in some neuropsychiatric disorders, such as depression. The aim of this study was to investigate the activities of antioxidant enzymes, lipid peroxidation and total antioxidant status (TAS) in patients suffering from major depressive disorder (MDD) as compared to healthy controls. Specifically, we wanted to estimate how fluoxetine influences antioxidant defense and lipid peroxidation.
Fifty MDD patients and thirty healthy controls participated in the study. Antioxidant enzyme activities and lipid peroxidation levels were measured in erythrocytes, while TAS was measured in plasma. All measurements were taken during an acute depressive episode and then again during depression remission after a three-month fluoxetine treatment.
During acute depressive episodes, patients had significantly higher activity levels of antioxidant enzymes, such as copper-zinc superoxide dismutase (SOD1) and catalase (CAT), as compared to healthy controls. Concentrations of malondialdehyde (MDA) were also significantly higher during depressive episodes. Activity levels of glutathione peroxidase (GPx) did not differ significantly between depressed patients and healthy control subjects. Moreover, the plasma total antioxidant status of the depressed patients was decreased in comparison to control subjects. After three months of fluoxetine treatment, the above parameters did not change significantly.
Major depressive disorder is accompanied by disturbances in the balance between pro- and anti-oxidative processes; however, these disturbances do not improve in patients in remission after three months of fluoxetine therapy.

Key words:
antioxidant defense, lipid peroxidation, depressive disorder, fluoxetine

Abbreviations: γGT – glutamyl transferase gamma, AA – arachidonic acid, ACTH – adrenocorticotrophic hormone, ALT – alanine transaminase, AST – aspartate transaminase, CAT – catalase, CRH – corticoliberin, CuZnSOD – cooper-zinc superoxide dismutase, $cPLA_2$ – cytosolic phospholipase A_2, GPx – glutathione peroxidase, GSH – reduced glutathione, HDRS – Hamilton Depression Rating Scale, HNE – 4-hydroxy-2-nonenal, H_2O_2 – hydrogen peroxide, HOCl – hypochlorous acid, HPA – hypothalamo-pituitary-adrenal axis, IL-1 – interleukin-1, IL-2 interleukin-2, IL-6 – interleukin-6, INF-γ – interferon gamma, iNOS – inducible nitric oxide synthase, LPS – lipopolysaccharide, MDA – malondialdehyde, MDD – major depressive disorder, $NO^{\bullet}$ – nitric oxide, $O_2^{\bullet-}$ – superoxide radical, $OH^{\bullet}$ – hydroxyl radical, PGE_2 – prostaglandin E_2, PLA_2 – phospholipase A_2, PUFAs – polyunsaturated fatty acids, $ROO^{\bullet}$ – peroxyl radical, ROS – reactive oxygen species, SOD1 – cooper-zinc superoxide dismutase, SSRI – selective serotonin reuptake inhibitor, $sPLA_2$ – secreted phospholipase A_2, TAS – total antioxidant status, TBARS – thiobarbituric acid reactive species, TNF-α – tumor necrosis factor alpha

Introduction

Free radicals are very reactive species with one unpaired electron. This large group of molecules is represented mainly by the superoxide radical ($O_2^{\bullet-}$), peroxyl radical ($ROO^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), and nitric oxide ($NO^{\bullet}$). All of these molecules and their derivatives, such as hydrogen peroxide (H_2O_2) or hypochlorous acid (HOCl), are referred to as reactive oxygen species (ROS) [17, 73].

Overproduction of ROS results in an imbalance of pro- and anti-oxidative processes, which creates a phenomenon known as oxidative stress. This kind of burden is involved in many neuropsychiatric disorders, such as Alzheimer's disease, Parkinson's disease, schizophrenia, bipolar disorder and major depressive disorder (MDD) [5, 6, 26, 38, 46]. Oxidative stress is harmful to lipids, proteins, nucleic acids, and other cell structures, and consequently disturbs normal metabolism and may even cause cell death. The lipid peroxidation process, which also results in free radical production, is the best known of the above types of damage [24, 30, 53]. Malondialdehyde (MDA) is the most well-studied product of lipid peroxidation. This aldehyde is a highly toxic molecule that interacts with DNA and proteins and is often referred to as mutagenic [16]. Numerous studies assessing lipid peroxidation in depressed patients describe increased levels of MDA and other products of lipid peroxidation [13, 21, 54, 60, 61, 72].

Studies on depression suggest that the disorder is characterized by overproduction of free radicals by different biochemical processes. One of these processes enhances glutaminergic transduction and increases glutamate concentration to excitotoxic levels [39]. Prolonged activation of neurons by glutamate may be damaging due to the resultant production of ROS [28]. Glutamate can stimulate intracellular production of superoxide radicals [14, 63]. Studies investigating depression show that patients suffering from this disorder have higher levels of proinflammatory cytokines, such as interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), interferon gamma (INF-γ), and tumor necrosis factor alpha (TNF-α), than healthy subjects [18, 36, 59, 62]. Proinflammatory cytokines increase hypothalamo-pituitary-adrenal axis (HPA) activity, which results in the generation of free radicals. IL-1 plays a key role in HPA stimulation [39, 40, 51]. Major depressive disorder is accompanied by increased activities of immune cells, such as neutrophils, macrophages, and monocytes [36, 47, 59, 67]. Interaction of proinflammatory cytokines with inflammatory cells (i.e., neutrophils, macrophages, monocytes, astrocytes) results in the production of free radicals [22, 23]. Psychological and physical stress, both common in depression, activate these cells [45]. Inflammatory cells use multi-complex enzymes, such as NADPH oxidase, to produce free radicals and their derivatives [20]. Another mechanism underlying the production of free radicals involves myeloperoxidase converting chloric ions to HOCl, a ROS very toxic to cell structure. Furthermore, HOCl decreases levels of reduced glutathione (GSH), the most important non-enzymatic antioxidant [74]. Activation of inducible nitric oxide synthase (iNOS) in neutrophils, monocytes and macrophages results in the conversion of L-arginine to free radicals such as nitric oxide [23]. Activated macrophages in large quantities secrete glutamate, which is an activator of phospholipase A_2 (PLA_2) [28]. These enzymes are normally present in the cell membrane, but they also influence the release of arachidonic acid (AA), a precursor of prostaglandin synthesis. Prostaglandin synthesis in turn produces free radicals [1, 7]. Metabolism of AA in the central nervous system increases with increased glutamate transduction. Glutamate stimulates activation of cytosolic phopholipase A_2 (PLA_2) and production of prostaglandin E_2 (PGE_2)

and free radicals [31, 70, 71]. The secreted phospholipase A_2 ($sPLA_2$) plays an important role in AA disturbances in immune cells. When this enzyme is expressed under inflammatory, autoimmune or allergic conditions, $sPLA_2$ begins producing superoxide radicals with the help of cyclooxygenase [1]. One indication confirming this process is the increase in levels of PGE_2 seen in patients suffering from depression [41].

Enzymatic and non-enzymatic systems that are synchronized with free radical processes protect cells from damage caused by the free radicals. This antioxidant system is expressed in the periphery and in the brain. The major antioxidant enzymes include copper-zinc superoxide dismutase (CuZnSOD), catalase (CAT), and glutathione peroxidase (GPx) [73]. According to some research, the activities of the above enzymes in patients with MDD are different from those observed in healthy subjects [13, 29, 33, 60]. Non-enzymatic antioxidants include bilirubin, uric acid, glutathione, and vitamins A, C, E [32, 73]. The aforementioned non-enzymatic antioxidants work separately, but their cooperation with the antioxidant enzymes is referred to as the total antioxidant status [57].

Based on the inflammatory components involved in the etiology of depression, researchers have suggested that antidepressant drugs work at the level of proinflammatory cytokines [15, 37]. However, there is scarce information about the influence of antidepressants on antioxidant enzyme activities, lipid peroxidation levels, and total antioxidant status in depressed individuals.

The aim of this study was to explore the effects of fluoxetine, a selective serotonin re-uptake inhibitor (SSRI), on TAS, MDA concentration, and antioxidant enzyme activity levels in MDD patients in remission.

Materials and Methods

Patients

The study was carried out in a group of 50 patients who had achieved remission from their first episode of MDD after three months of treatment with 20 mg of fluoxetine. They were selected from a group of 82 patients who were diagnosed with MDD according to the DSM IV [3]. Their symptoms were assessed using the Hamilton Depression Rating Scale (HDRS), a 21-item scoring system [27]. The criterion for remission was an HDRS score of less than seven. The healthy control group consisted of 30 subjects. To provide a comparable sample, control subjects were matched according to age and gender. The mean age of patients treated with fluoxetine was 36.7 ± 5.2 years. In the healthy control group, the mean age was 32.1 ± 4.3 years. Written consent to participate in the study was obtained from the subjects after they were thoroughly informed about the details of the research. The study protocol was approved by the Local Ethics Committee of the Medical University of Łódź (No. RNN/207/06/KB). MDD patients with another Axis I or II diagnosis or significant suicide risk were excluded. Subjects were free of all medications at least 3 weeks prior to blood sampling. All participants underwent routine blood tests looking at serum electrolytes, urea, creatinine, γ-glutamyl transferase (γGT), alanine transaminase (ALT), aspartate transaminase (AST), plasma lipids, and thyroid levels. All subjects were free of medical illness, including infections and inflammatory or allergic reactions. None of the control subjects or depressed patients were treated with drugs known to influence lipid metabolism, immune response, or endocrine function. The control subjects were free of all medication for at least 2 months prior to blood sampling. None of the control subjects were drinkers, heavy smokers or had ever taken psychotropic drugs. Control volunteers with a family history of psychiatric disorders were excluded from the study.

The patients received 20 mg of oral fluoxetine a day for three months; controls did not. Blood samples from the patients, as well as from the healthy controls, were collected in 5 ml EDTA-containing tubes after overnight fasting on the initial test day and after 3 months of treatment. The blood samples were centrifuged at 4000 rpm for 10 min at 4°C to remove plasma. Plasma samples for TAS determinations were kept at –70°C until they were analyzed. Red blood cells for SOD, CAT, GPx and MDA determinations were washed three times in saline and frozen after hemolysis.

Drop-outs

All 82 (100%) patients started treatment with 20 mg of fluoxetine at the initial day of the study, but only 50 (61%) of them were in remission from depression after three months of therapy and completed the study

according to our project guidelines. Of the 32 dropouts, 12 were due to fluoxetine side effects (headache, sleep disturbances, nausea), 16 were due to lack of improvement in depression symptoms after 6 weeks of treatment, and four were due to incomplete remission of MDD (HDRS > 7) after 12 weeks of treatment. No serious adverse events were reported and no abnormalities in laboratory tests were found during the trial period.

Methods

The obtained hemolysate was used to determine the parameters of oxidative stress and antioxidative defense. Erythrocytic CuZnSOD, CAT, GPx, and MDA were assayed using the methods of Misra and Fridovich [52], Beers and Sizer [9], Little and O'Brien [44] and Placer et al. [56], respectively. TAS was measured according to Benzie and Strain [11, 12] methods with some modification of Bartosz [8].

CuZnSOD activity was determined at 37°C by recording the increase in absorbance at 480 nm following the auto-oxidation of adrenaline inhibited by CuZnSOD. One unit (U) of this activity is defined as the amount of enzyme capable of inhibiting 50% of adrenaline auto-oxidation. CAT activity was measured at 25°C by recording H_2O_2 decomposition at 240 nm. One Bergmeyer unit (BU) of this activity is defined as the amount of enzyme required to decompose 1 μmol H_2O_2 per min. GPx was determined at 25°C by recording the absorbance rate at 412 nm. One unit of GPx activity (U/gHb) is the amount of enzyme required to decrease the amount of reduced glutathione by 10% of the initial level in 1 min at 25°C. MDA level was expressed as the concentration of thiobarbituric acid reactive species (TBARS) measured at 532 nm. TAS was measured by using the determination of ferric reducing ability power (FRAP). FRAP was estimated following the process in which Fe^{3+} reduces to Fe^{2+} at low pH, which causes the formation of a colored ferrous –TPTZ (2,4,6- tripyridyl-s-triazine) complex resulting in the increase in absorbance at 593 nm. The results were expressed in Trolox equivalents (mmol/L).

Methods of statistical analysis

The data results were analyzed using simple descriptive statistics (mean, median, standard deviation) and non-parametric tests of significance.

For independent groups, the Mann-Whitney *U* test or Kruskal-Wallis ANOVA (for more than two groups) were used. For paired data (before and after treatment), the Wilcoxon test for matched observations was used. Associations between the variables of interest were qualified using the Spearman rank correlation method.

Non-parametric methods and rank correlations were used because of lack of normality in distributions of some of the analyzed variables.

In all analyses, differences were considered to be statistically significant with $p \leq 0.05$.

All calculations were derived using Statistica v. 6.0 software.

Results

The control group (n = 30) (C) and the fluoxetine treatment group (n = 50) (FLU) were homogenous in age and sex distribution (C: 16F, 14M; FLU: 28F, 22M). There was no correlation between variables of interest and age or gender of the study participants ($p > 0.05$). Fluoxetine treatment (FLU) seemed to cause a significant decrease in HDRS scores from an initial mean score of 32.3 points to a post-treatment

Tab. 1. Descriptive statistics for the Hamilton Depression Rating Scale in the FLU group during an acute depressive episode (before treatment) compared to in remission (after treatment). The Wilcoxon matched paired test was used to compare patients before and after FLU treatment

Variable	Group	n	Mean	95%CI	MED	SD	Comparison ADD* *vs.* R** p value
HAMILTON	FLU_ ADD*	50	32.3	(31.5; 33.1)	32.0	2.9	< 0.0001
	FLU_R**	50	6.2	(5.9; 6,6)	6.0	1.3	

* group on FLU therapy – during acute depressive episode, ** group on FLU therapy – in remission after fluoxetine treatment

mean score of 6.2 points ($p < 0.0001$). Detailed results are presented in Table 1.

Biochemical parameters

In the control group and treatment group before any medication, the mean levels of biochemical parameters such as SOD1, CAT, TAS, MDA were significantly different (Kruskal-Wallis ANOVA: $p = 0.04$, $p = 0.0001$, $p = 0.00001$, $p = 0.0002$, respectively).

After 3 months of treatment, differences in the mean values of those parameters were still significant for CAT ($p = 0.0001$) and TAS ($p < 0.0001$), were not significant for SOD1 ($p = 0.08$) and for MDA ($p = 0.17$).

The only exception was GPx, the levels of which did not differ between the treatment and control groups before and after medication (ANOVA: $p = 0.30$ and $p = 0.19$, respectively).

Table 2 shows the mean erythrocyte activity of SOD 1, CAT, GPx, erythrocyte levels of MDA and plasma levels of TAS.

Erythrocyte SOD1 activity was significantly higher in depressed patients before treatment (2078 U/gHb) as compared to healthy controls (1978 U/gHb) ($p = 0.04$). Depressed patients showed no significant difference before (2078 U/gHb) and after treatment (2028 U/gHb) ($p = 0.08$). Red blood cell CAT activity was significantly higher in depressed patients before treatment (17.4 U/gHb) in comparison to healthy controls (14.2 U/gHb) ($p = 0.0001$). In depressed patients, these levels were not significantly different after treatment (17.6 U/gHb; $p = 0.8$). The activity of GPx in depressed patients before treatment (74.2 U/gHb) was not statistically different than that in healthy controls (68.7 U/gHb; $p = 0.13$). The MDA level was significantly higher in depressed patients before treatment (0.739 nmol/gHb) compared to controls (0.549 nmol /gHb) ($p = 0.0002$), while MDA values in the depressed group after treatment were statistically different ($p = 0.0003$). Total antioxidant status in depressed patients before therapy (0.433 mmol/L) was statistically lower that the control group (0.737 mmol/L) ($p < 0.0001$). It was not statistically different before and after treatment (0.485 mmol/L) in depressed patients ($p = 0.1$).

Tab. 2. Descriptive statistics for the levels of biochemical parameters in the control group and FLU group before and after treatment. The Mann-Whitney U test was used to compare patients to controls and the Wilcoxon matched paired test was used to compare depressed patients before and after the treatment

Variable	Group	n	Mean	95%CI	MED	SD	Before treatment: FLU group *vs.* Control group p-value	FLU group before *vs.* after treatment p-value
SOD1	CONTROL	30	1978	(1905; 2051)	1903	196	–	–
	FLU_before	50	2078	(2022; 2135)	2002	199	0.04	
								0.17
	FLU_after	50	2028	(1993; 2064)	2007	124	0.08	
CAT	CONTROL	30	14.2	(12.9; 15.5)	13.0	3.5	–	–
	FLU_before	50	17.4	(16.5; 18.3)	17.0	3.1	0.0001	0.80
	FLU_after	50	17.6	(16.7; 18.5)	17.5	3.2	0.0001	
GPx	CONTROL	30	68.7	(62.4; 74.9)	71.0	16.7	–	–
	FLU_before	50	74.2	(70.3; 78.0)	76.0	13.5	0.13	0.18
	FLU_after	50	70.2	(66.0; 75.0)	67.0	15.5	0.70	
TAS	CONTROL	30	0.737	(0.666; 0.808)	0.740	0.191	–	–
	FLU_before	50	0.433	(0.388; 0.478)	0.437	0.158	<0.0001	0.1
	FLU_after	50	0.485	(0.441; 0.529)	0.476	0.153	<0.0001	
MDA	CONTROL	30	0.549	(0.465; 0.632)	0.550	0.224	–	–
	FLU_before	50	0.739	(0.692; 0.786)	0.755	0.164	0.0002	0.0003
	FLU_after	50	0.607	(0.568; 0.646)	0.640	0.136	0.17	

The detailed results of comparisons between groups are presented in Table 2 and Figures 1–5.

Discussion

An interesting finding of our study was the low correlation between biochemical parameters and HDRS scores in patients that were in remission from MDD after three months of treatment with 20 mg of fluoxetine. No correlation was found in by Herken et al. either [29]. Khanzode et al. [33] found improvement of HDRS with fluoxetine and citalopram treatment, but they also found a significant decrease in SOD1 and MDA; the same findings were reported by Bilici et al. [13] after SSRI treatments. In our study, 61% of patients went into remission. Our results confirm information in literature about the effectiveness of treatment with 20 mg of fluoxetine per day in alleviating depression [58].

An important finding of our study is that patients with depressive disorder have statistically higher ac-

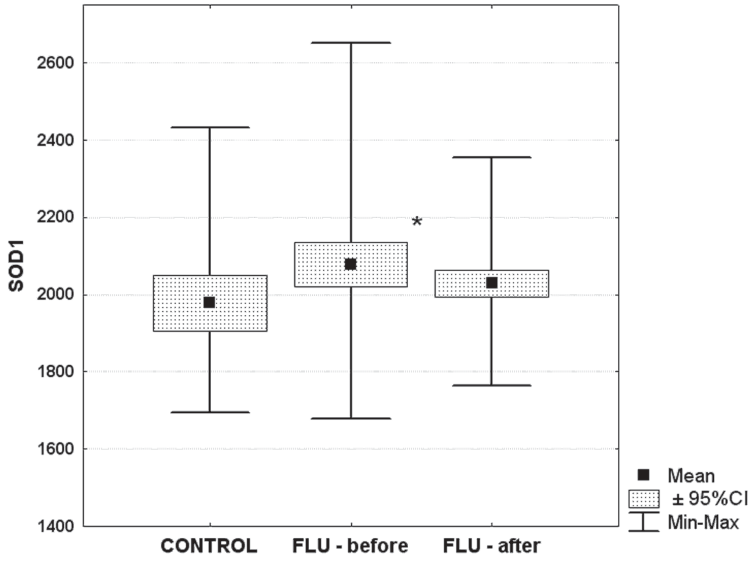

Fig. 1. Distribution of SOD1 levels in the control and the treatment group before and after therapy. Statistically significant differences between controls and patients are indicated by an asterisk: * ($p \leq 0.05$). SOD1 values in depressed patients before treatment were statistically higher than SOD1 values in control subjects. These values did not differ significantly in depressed patients before and after treatment ($p = 0.17$)

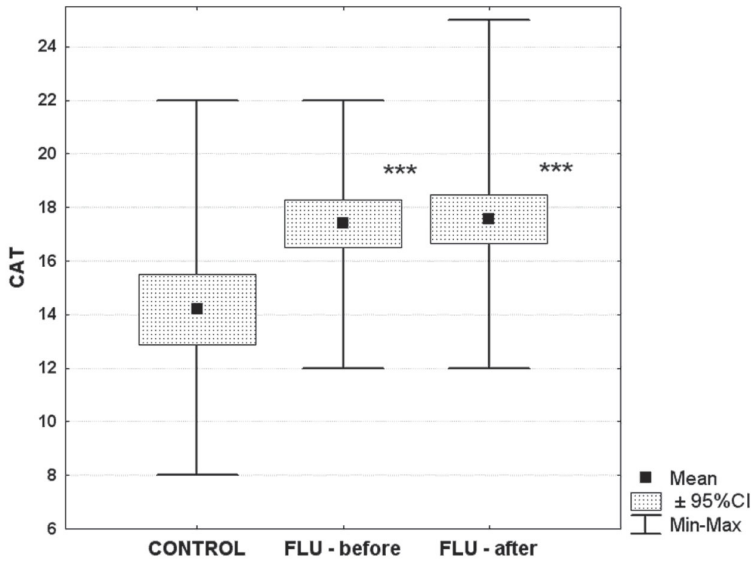

Fig. 2. Distribution of CAT levels in the control and the treatment group – before and after the therapy. Statistically significant differences between controls and patients are indicated by asterisks: ***($p \leq 0.001$). CAT values in depressed patients before treatment were statistically higher than in control subjects. CAT values in depressed patients before and after treatment were not significantly different ($p = 0.8$)

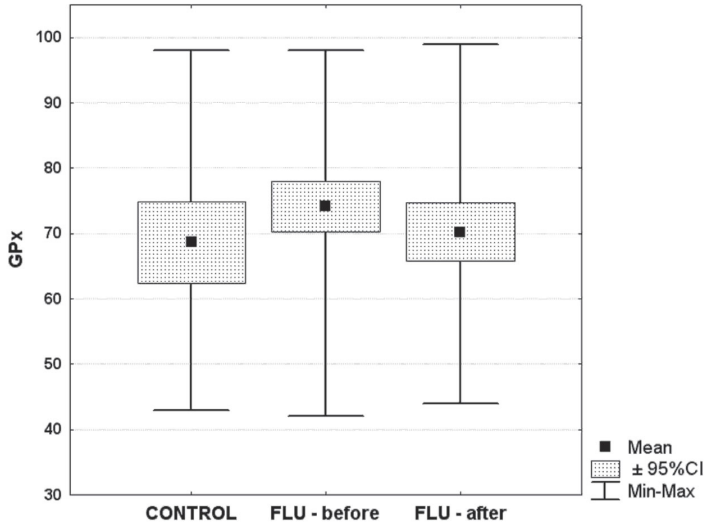

Fig. 3. Distribution of GPx levels in control subjects *versus* depressed patients before and after treatment. No significant differences were observed

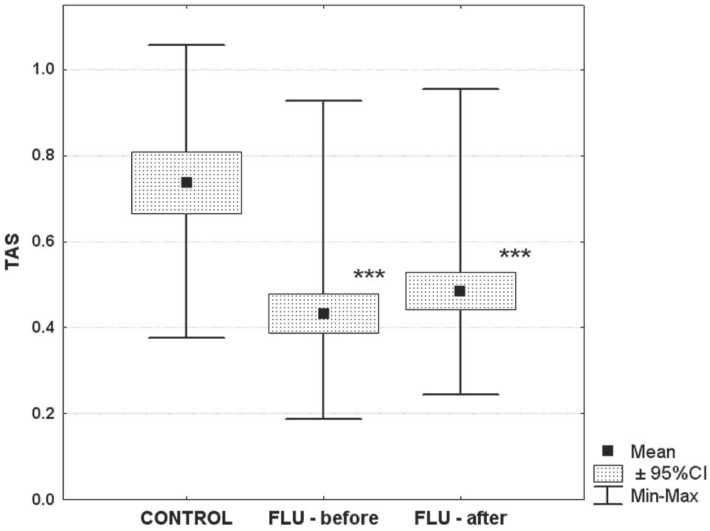

Fig. 4. Distribution of TAS in the control *vs.* treatment group before and after therapy. Statistically significant differences between controls and patients are indicated by asterisks: ***($p \leq 0.001$). TAS values in depressed patients before and after treatment were significantly lower than controls ($p = 0.1$)

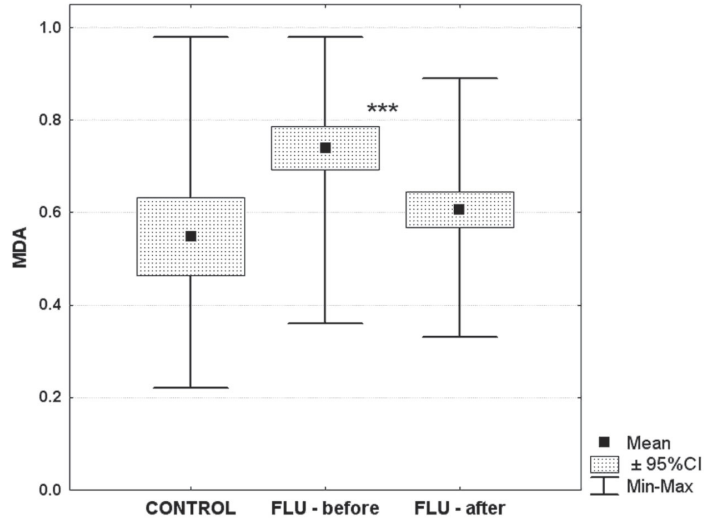

Fig. 5. Distribution of MDA levels in the control group and the treatment group before and after therapy. Statistically significant differences between controls and patients are indicated by asterisks: ***($p \leq 0.001$). MDA levels in depressed patients before treatment were statistically higher than in controls ($p = 0.0002$). MDA values in depressed patients before and after treatment were significantly different ($p = 0.0003$)

tivities of antioxidants enzymes, such as CuZnSOD and CAT in erythrocytes, before treatment as compared to healthy controls. This suggests a disturbance in pro- and anti-oxidant balance in depression. The higher activities of SOD1 and CAT might be a compensatory mechanism to excessive production of ROS in depressed patients. Results that also confirm the increase in erythrocyte antioxidant enzyme activities, especially in CuZnSOD and GPx, were presented by Bilici et al. [13]. Moreover, Khanzode et al. [33] and Sarandol et al. [60] obtained the same results by measuring the serum activity of SOD1 in patients with depressive disorder. In a small group of studies measuring oxidative stress in depression, there are some reports of opposite results. For example, Herken et al. [29] demonstrated statistically lower activity of SOD1 in the erythrocytes of patients with depression. Srivastava et al. [65] observed similar changes in the activity of SOD1 in the polymorphonuclear leukocytes from depressed patients and control subjects.

The next 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 statistically significant increase in the concentration of MDA in erythrocytes of patients with depression in comparison to that in healthy people. Similar results in plasma levels of MDA were obtained by Bilici et al. [13] and Sarandol et al. [60]. An increase of lipid peroxidation in the plasma of patients with depression was also demonstrated by Selley [64], who measured the concentration of end products of lipid peroxidation, such as 4-hydroxy-2-nonenal (HNE). Moreover, Tsuboi et al.[72] confirmed the above results by estimating of the concentration of lipid hydroperoxide in the serum of women suffering from depression. Increased SOD1 activity and MDA concentrations have also been observed in patients with other diseases such as bipolar affective disorder and schizophrenia [5, 6, 38]. Different results indicating decreased CAT and GPx activities in patients with bipolar affective disorder were obtained by Ozcan et al. [54].

TAS was measured to obtain more information about antioxidant protection in depressed patients. Our results, as well as Sarandol's results [60], imply that TAS is reduced in ill patients before treatment. Reduction in the level of TAS is in agreement with results found by Lesgard et al. [42], who observed such decreases in subjects under physiological stress, which is one of the features characteristic of depression. Additionally, depleted TAS was observed by Manuel y Keenoy et al. [50] in patients with chronic fatigue, which is also correlated with depression. On the other hand, Sofic et al. [68] found no statistically significant differences in TAS between patients before antidepressant treatment and healthy controls.

Due to the lack of information on levels of TAS in patients with depression, we reviewed studies that analyzed the concentration of single non-enzymatic antioxidants, which are components of TAS. However, we believe that the concentration of single non-enzymatic antioxidants does not provide full information about TAS, although it does show the direction of changes in the non-enzymatic antioxidant system [57]. Maes et al. [48] reported a lowered level of vitamin E in the serum of patients suffering from depression. Additionally, Khanzode et al. [33] showed a decrease in the concentration of vitamin C in the plasma of the above patients. Li and Zang [43] found lowered concentrations of antioxidant vitamins in the serum of patients with suicide attempts, which often co-exist with depression. Nevertheless, research carried out by Sarandol et al. [60] demonstrated an increase in vitamin E concentration in the serum of patients with depression. Because evidence suggests that inflammatory components are typical in many people suffering from depression, we reviewed results of TAS in people with rheumatoid arthritis, a disease that often co-exists with depression [76]. Rheumatoid arthritis is also characterized by reduced TAS [2]. Lipopolysaccharide (LPS), which is a component of Gram-negative bacteria, induces behavior typical of depression when injected into animals [19]. The oxidative stress after such an injection appears to decrease TAS in blood, while increasing thiobarbituric acid reactive species in plasma [66].

An increase of the activities in antioxidant enzymes such as CuZnSOD and CAT is probably a response to an increase in production of ROS. Furthermore, there is no decrease in MDA levels. This may be connected with insufficient antioxidant protection, in which the lack of an increase in GPx activity and lowered TAS is involved. Moreover, increased lipid peroxidation might be connected to an increased vulnerability of the brain to ROS. The brain tissue contains large amounts of polyunsaturated fatty acids (PUFAs), which are vulnerable to free radical attack yielding MDA as one of the end products. The brain also has small amounts of CAT, and large amounts of ferric trace element that participate in the production of ROS [26]. Lowered TAS results in poor protection of

the erythrocyte membrane from oxidative stress. Decreases in vitamin E concentrations may be responsible for the reduction in TAS. This component of TAS prevents reactions that cause lipid peroxidation. Lowered concentrations of vitamin E are observed when leukocyte levels increase. This suggests that reduced levels of vitamin E are a result of immune cell activation. In addition, depression is characterized by low levels of zinc, which is a factor involved in immune system activation and reduced absorption of vitamin E from the gastrointestinal tract [48]. However, we cannot conclusively confirm this finding because the FRAP method is based mainly on the reaction of hydrophilic antioxidants, which do not include vitamin E [11, 12]. A high concentration of MDA in depressed patients may also be associated with enzymatic peroxidation, especially because depression is characterized by a high level of PGE_2 that is synthesized from arachidonic acid [4]. Malondialdehyde is an end product of PUFA peroxidation. An increase in MDA concentration stimulates PLA_2, which induces immune cells to produce ROS [7].

The aim of our study was to measure changes of CuZnSOD, CAT, GPx, MDA, and TAS in patients that were in remission after a three-month therapy regime with 20 mg of fluoxetine. Our results indicate no statistically significant changes in the activities of antioxidant enzymes after treatment compared to before treatment. The activities of antioxidant enzymes did not decrease. Similarly, Sarandol et al. [60] did not observe any changes of antioxidant enzyme activities after 6-weeks of therapy with sertraline, venlafaxine, or reboxetin. On the other hand, Bilici et al. [13] had different findings. They report that three months of treatment with an SSRI significantly reduced the activities of SOD1 and GPx in depressed patients. However, their study did not explain if and how fluoxetine influences the activities of antioxidant enzymes and lipid peroxidation. Out of 30 patients participating in the study, only seven were treated with 20 mg of fluoxetine, while the other patients were treated with sertraline, fluvoxamine, or citalopram. Atmaca et al. [4] also reported the impact of citalopram on the activities of antioxidant enzymes and concentration of MDA. Their experiments demonstrated citalopram's ability to significantly reduce the activities of SOD1, GPx, CAT and MDA in erythrocytes. Reduction of SOD1 activity and reduction of MDA concentration was found by Khanzode et al. [33] after treating 62 patients with fluoxetine or citalopram for eight weeks. Ozgocmen et al. [55] showed that sertraline, an antidepressant, reduced the activity of SOD1 in patients suffering from fibromyalgia. However, no changes in MDA concentration were observed in this study. Ozcan et al. [54] observed that of treatment of bipolar affective disorder affects antioxidant enzyme activities and MDA concentration. Herken et al. [29] had results about different changes. According to his report, 8-week therapy with an SSRI induces an increase in SOD1 activity. However, this study does not provide complete information about the influence of fluoxetine on SOD1 activity because only 11 of the 36 depressed subjects were treated with fluoxetine. Important results that differed from our findings were obtained by Zafir and Banu [75]. Their study measured the activities of SOD1, CAT, GPx, and non-enzymatic antioxidants in depressed rats. Treatment with fluoxetine for 21 days protected against oxidative stress by increasing the activities of antioxidant enzymes that were depleted after physical stress, a common feature of depression. Moreover, this study confirmed the ability of fluoxetine to reduce the level of end products of lipid peroxidation, such as MDA. All of the above parameters were compared to those in rats that were not under stress. An increase in the activities of antioxidant enzymes was observed by Kolla et al. [35]. This study looked at the ability of fluoxetine to protect PC12 cells from oxidative stress induced by hydrogen peroxide. Their results showed that fluoxetine in a concentration of 50 μmol/L (as compared to saline solution) increases the activity of SOD1. However, concentrations higher than 200 μmol /L reduced viability. The above results suggest that fluoxetine protects from oxidative damage at only certain concentrations, but the mechanism of this protection is not known. A study by Kim et al. [34] confirmed that fluoxetine influences the activities of antioxidant enzymes. This study explored how fluoxetine influences CuZnSOD, CAT, and GPx activities in the CA1 region of the gerbil hippocampus. Their results showed that treatment with 10 mg or 20 mg of fluoxetine before ischemia does not cause changes in the activities of the above enzymes as compared to saline-treated controls. Nevertheless, a dose increase to 40 mg induced an increase in the activities of the above enzymes.

The next parameter measured in our study was plasma TAS. Our results indicate an increase of TAS after three months of fluoxetine therapy, but this increase was not statistically significant. Sarandol et al. [60] also did not observe an increase in serum TAS af-

ter 6 weeks of therapy with other antidepressants, such as reboxetine, venlafaxine and sertraline. An increase in the level of antioxidants, such as vitamin C, was obtained by Khanzode et al. [33]. Zafir and Banu [75] provide more proof that treatment with antidepressants increases levels of non-enzymatic antioxidants. They found higher concentrations of glutathione and uric acid after treatment with fluoxetine.

Three months of therapy with 20 mg of fluoxetine does not influence the activities of SOD1 and CAT. In the present study, fluoxetine did not modify pro-oxidative processes or antioxidative systems. Such lack of change might be related to the short duration of treatment, or the dosage level used. Studies carried out by Belowski et al. [10] have shown the ability of fluoxetine to reduce the cytotoxic activity of macrophages, which are the sources of ROS. This effect appeared after 2 weeks of treatment with 10 mg fluoxetine. Four-week treatment with the same dosage did not enhance the above process, nor did a lower dose of 2 mg/kg of fluoxetine. This demonstrates that the effect of fluoxetine is dependent on treatment duration and dosage. Different interactions of various concentrations of fluoxetine with antioxidants were also shown by Kolla et al. [35].

Further investigation is needed to explore the effects of different treatment duration and dosage of fluoxetine, especially because we only looked at antioxidant parameters after treatment with 20 mg of fluoxetine daily, whereas the maximum dosage of fluoxetine is 60 mg per day [69]. The mechanism by which fluoxetine could reduce oxidative stress through suppression or stimulation of some specific factor should also be studied further. Some drugs, such as valproic acid or lithium, are capable of increasing the level of mRNA transcription for Bcl-2 (decreased expression of Bcl-2 is associated with oxidative stress). On the other hand, the use of glucocorticoid synthesis inhibitors, such as ketoconazole or metyrapone, or corticotropic hormone antagonists, can also reduce oxidative stress resulting from increased HPA activity. Similarly, the overproduction of ROS in depression could be reduced by factors suppressing glutaminergic transmission [49].

There were some limitations in this study. We did not investigate pro-oxidant processes such as production of superoxide radicals or nitric oxide. However, a study carried out by Ha et al. [25] provided information that fluoxetine is able to stimulate production of nitric oxide in BV_2 murine microglial cells.

Conclusions

Our results indicate a disturbance in pro-/antioxidative balance, manifested as an increase in SOD1 and CAT activities, and MDA concentration, is present in MDD patients during their first episode of depression. A reduction in plasma TAS in depressed patients also demonstrates this imbalance. Three months of treatment with 20 mg/day of fluoxetine did not significantly influence the above parameters. We suggest more studies exploring oxidative stress in depression be carried out using different medication dosages and treatment duration times.

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