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8-Iso-prostaglandin F2α as a potential biomarker in patients with unipolar and bipolar depression

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Abstract. – OBJECTIVE: Previous studies have shown that the disturbance of redox homeostasis plays a role in the pathogenesis of mood disorders. It is currently unclear whether oxidative stress parameters can be used as biomarkers (state vs. trait). The aim of the present study was to investigate oxidative stress markers in patients with major depressive disorder (MDD) and bipolar disorder (BP) in acute depressive episodes and remission, and healthy individuals.

PATIENTS AND METHODS: Thirty-two patients with a diagnosis of MDD, 32 patients with a diagnosis of BP and 32 matched healthy controls were included in the study. We measured the serum levels of markers of oxidative damage, including 8-hydroxy-2'-deoxyguanosine (8-OHdG), 8-Iso-prostaglandin F2α (8-iso-PGF2α; 8-isoprostane), and malondialdehyde (MDA), and also serum activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR) in both acute and remission phase, and in control group.

RESULTS: After controlling for the effects of age, sex, body mass index, and smoking status, serum 8-iso-PGF2α levels were significantly higher in both patient groups compared to controls, regardless of disease phase. The activities of GPX and GR were significantly lower in the acute phase in MDD patients compared to controls. Serum GR activity was lower in both acute and remission phase in MDD compared to BP.

CONCLUSIONS: Our results suggest that both MDD and BP are associated with a disturbed redox balance with a particularly pronounced increase in serum 8-iso-PGF2α levels in both groups and the presence of glutathione metabolism disorders in MDD patients. Further research is needed to confirm the importance of oxidative stress parameters as potential biomarkers of MDD and BP.

Key Words:
Oxidative stress, Depression, Bipolar depression, Glutathione, 8-Iso-PGF2α.

Introduction

Mood disorders, also called affective disorders, are collectively one of the most prevalent mental disorders. Of these, the most common are major depressive disorder (MDD) and bipolar disorder (BP), which affect people across all regions of the world with slightly higher prevalence among women[1]. Substantial evidence suggests that mood disorders often co-occur with various somatic diseases and other mental disorders, including anxiety and substance-related disorders as the most frequent psychiatric comorbidities[2-4]. In addition, a recent systematic review and meta-analysis showed that individuals with preexisting mood disorders are at higher risk of COVID-19 hospitalization and death and should be categorized as an at-risk group[5].

A contemporary model for the etiopathogenesis of depressive and bipolar disorder implies multifaceted gene-environmental interactions. Current evidence confirms that individuals with certain genetic predispositions and multi-

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ple adverse lifetime experiences, like childhood trauma, are prone to undergoing structural and functional changes in the critical brain regions associated with the development of mood disorders[6,7]. Recent studies support the role of inflammation-related mechanisms and the involvement of the immune system in the pathogenesis, course and treatment of affective disorders[8-10]. Patients with depression and bipolar disorder exhibit raised circulating levels of inflammatory biomarkers, including proinflammatory cytokines[11-14], acute phase proteins, for instance, high sensitivity C-reactive protein (hsCRP)[15], adhesion molecules, chemokines and other inflammatory mediators. Those molecules have been shown to affect almost every known neurobiological process related to mood disorders, including brain monoamine metabolism, neuroendocrine function, and synaptic plasticity[16].

Redox homeostasis is an essential and dynamic process that ensures the balance between reducing and oxidizing reactions within cells and regulates diverse biological processes, including metabolism, cell death, differentiation and development, immune responses, circadian rhythm, and others[17]. The disturbance in this delicate prooxidant-antioxidant balance in favor of the former is known as oxidative stress, which can lead to oxidative damage. The brain is particularly vulnerable to oxidative damage due to its high demand for oxygen, high content of lipids, including unsaturated fatty acids, and relatively sparse antioxidant defense systems[18]. Multiple studies[19,20] have shown that the inflammatory processes underlying the development of depression co-occur with a certain degree of oxidative stress. For instance, chronic low-grade inflammation triggers activation of the hypothalamic-pituitary axis and also activates the tryptophan-kynurenine pathway with the consequent synthesis of neurotoxic metabolites, such as quinolinic acid and 3-hydroxykynurenine, and disruption of redox homeostasis[21].

Numerous scientific reports indicate the presence of oxidative stress markers in the course of mood disorders, including the damage to DNA, lipids and proteins caused by reactive species[22,23]. In addition, altered levels of antioxidants are repeatedly reported both in the brain and body fluids of patients with depression[24,25]. However, the data are somewhat inconsistent, making the usage of oxidative stress parameters as biomarkers still distant from clinical practice. An additional issue that should be addressed when considering these parameters as potential biomarkers of depression is the distinction between state and trait biomarkers in psychiatric disorders. The trait marker represents the properties of biological processes that play a causal role in the pathophysiology of mental illness and allows early diagnosis, prognosis, and hopefully early intervention in these disorders, while the state marker represents the status of clinical manifestations in patients and reflects dynamic changes in a disease course and response to treatment[26].

The objective of this study was to investigate the serum levels/activites of oxidative stress parameters, including 8-hydroxy-2'-deoxyguanosine (8-OHdG), 8-Iso-prostaglandin F2α (8-iso-PGF2α; 8-isoprostane), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR), in patients with MDD and BP in an acute phase of the disease and after treatment during remission, compared to healthy subjects. In addition, we focused on evaluating the potential differences in these parameters related to the phase of the disease (acute *vs*. remission) and to the type of mood disorder (MDD or unipolar *vs*. BP or bipolar depression). We also assessed the relationship between these markers and selected clinical features, including duration of illness, number of episodes, and psychometric assessments such as Hamilton Depression Rating Scale (HDRS)[27].

Patients and Methods

Study Participants

The sample consisted of 32 subjects per group. All patients involved in this study were hospitalized at the Department for Affective Disorders at the Clinic for Psychiatry, Clinical Centre of Serbia in Belgrade. The patients were selected to participate in the study based on the following inclusion criteria: (i) diagnosis of major depressive disorder or bipolar disorder type I, and (ii) age of 18-65 years. Acutely ill patients were treated as inpatients after admission, and only those who achieved remission after the follow-up period of ten weeks were included in the study. Diagnostic assessments and clinical evaluations were made by two experienced clinicians according to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition[28]. The control subjects were recruited from healthy individuals with no psychi-

atric disease and were age and gender-matched to the patient groups. Exclusion criteria for all participants in the study consisted of the presence of chronic inflammatory conditions, cardiovascular disorders, diabetes, malignancy, chronic terminal diseases, obesity (body mass index ≥ 30), pregnancy, mental retardation, and alcohol or substance abuse.

This study was approved by the Ethics Committee of the School of Medicine, University of Belgrade (acceptance number 29/X-29) and conducted according to the Declaration of Helsinki. Each participant signed the written informed consent before entering the study.

Clinical Assessment

All clinical data concerning the course of illness and other disease features were gathered by means of direct interviews with the patients and/or their significant others and by an inspection of their medical records. The psychometric ratings were performed in the acute disease phase and after achieving remission. Monitoring of global illness severity and therapy response was assessed by the Clinical Global Impression Scale[29]. The Clinical Global Impressions scale (CGI) consists of three different scores: Severity of illness (CGI-S), Global improvement (CGI-I), and Efficacy index (CGI-E). The assessment of patients' depressive symptoms was carried out using Hamilton Depression Rating Scale[27]. The severity of manic symptoms was evaluated with the Young Mania Rating Scale (YMRS)[30].

Blood Sampling and Biochemical Analysis

To assess the parameters of oxidative stress 6 ml of venous peripheral blood was collected in serum separator tubes from each study participant. Blood samples from patients were obtained within 24 h after admission for the acute disease phase and again after remission was achieved. Blood samples from healthy controls were taken once in the morning after an overnight fast. Serum samples were stored at −80°C until measurements were made.

Levels of 8-isoprostane PGF2α (8-iso-PGF2α) were measured by commercially available enzyme-linked immunosorbent assays (ELISA) kit (ab175819, Abcam, United Kingdom) according to the manufacturer's instructions. Quantitation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was done using an ELISA kit from Abcam (ab101245, Abcam, United Kingdom). MDA concentration is determined in a reaction with thiobarbituric acid (TBA) at high temperature (90-100°C) and in acidic conditions in which a colored MDA-TBA conjugate is formed, the amount of which is measured spectrophotometrically at 535 nm[31]. The activity of SOD was determined by the spectrophotometric assay as a percentage of the inhibition of autoxidation of adrenaline in the basic conditions[32]. Determination of CAT activity was performed according to the method[33] in which enzyme activity is proportional to the amount of depleted hydrogen peroxide measured spectrophotometrically in reaction with ammonium molybdate at 535 nm. GPX activity was measured by a method based on the oxidation of reduced glutathione using NADPH in a reaction catalyzed by the enzyme GR[34]. GR activity was determined according to the method based on monitoring the change in absorbance of NADPH, which is depleted during glutathione reduction catalyzed by GR[35].

Statistical Analysis

Statistical analysis was performed using the SPSS 20.0 package for Windows (IBM Corp., Armonk, NY, USA). All data gathered in the research were described using standard methods of descriptive statistics (i.e., frequencies, means with standard deviation, etc.). Shapiro-Wilk test was used to test the normality of the data. Group differences regarding demographic data for all participants and clinical characteristics among patients were assessed by Chi-square test, *t*-test, and ANOVA, or their nonparametric counterparts Mann-Whitney U-test or Kruskal-Wallis H test, as appropriate. To compare the levels of measured parameters of oxidative stress among participants, we used two statistical approaches. First, paired samples *t*-test was performed for each biochemical parameter across the two phases (acute phase or remission) for patients with depression and bipolar disorder separately. Second, differences between the patients in each phase and healthy controls were analyzed using analysis of covariance (ANCOVA) with adjustment for potential confounding factors (age, gender, BMI, and smoking). Adjustment for multiple comparisons was carried out by post-hoc Bonferroni test. Finally, Pearson product-moment correlation coefficients were calculated for all patients separately for each disease phase to determine the influence of demographic and clinical parameters. A p-value of <0.05 was regarded as statistically significant for all comparisons.

Results

The study enrolled a total of 96 participants, equally distributed in three groups: healthy controls and two groups of patients (with depression and bipolar disorder). All demographic data of the participants are summarized in Table I. There was no statistically significant difference between the percentages of men and women in the control and patient groups (p=0.155) and no difference in mean age between groups (p=0.069). We observed no significant differences between groups regarding marital status (p=0.735) and education level (p=0.114) of participants. Similarly, smoking habits (p=0.881) and mean BMI (p=0.106) were similar in all groups in the research.

The clinical characteristics of the patients in the study are shown in Table II. Patients with bipolar disorder were significantly younger at the onset of disease (p<0.001) and accordingly had a longer duration of illness (p=0.002), total number of episodes (p=0.001), and more hospitalizations (p=0.005) compared to patients with depression. However, there was no difference in the number of depressive episodes (p=0.079) between groups. Psychotic episodes were more common in the bipolar group (p=0.011), as well as suicide attempts (p=0.024). No significant difference was found regarding the family history (p=0.076) between two groups of patients.

As summarized in Table II, no significant differences were observed in mean scores on CGI-S among patients in both the acute phase (p=0.529) and remission (p=0.527). However, patients with depression had significantly lower scores on CGI-I in remission (p<0.001). Mean CGI-E scores were similar among both groups of patients in remission (p=0.328). No difference in the severity of clinical symptoms measured by HDRS between groups was shown in the acute phase (p=0.531); however, after remission, patients with depression had a higher mean HDRS score (p=0.002) compared to bipolar patients. As expected, a higher mean YMRS score in the acute phase (p<0.001) was present in the group of patients with bipolar disorder.

As presented in Figures 1 and 2, none of the measured parameters of oxidative stress were significantly different in the acute phase and remission in both groups of patients. The mean serum concentrations of 8-OHdG (t=–1.376, p=0.179 for MDD; t=–1.703, p=0.099 for BP), 8-iso-PGF2α (t=1.029, p=0.311 for MDD; t=0.747, p=0.461 for BP), and MDA (t=0.789, p=0.440 for MDD; t=–0.051, p=0.960 for BP), along with the mean serum activity of SOD (t=–1.394, p=0.168 for MDD; t=–0.254, p=0.801 for BP), CAT (t=1.879, p=0.070 for MDD; t=–1.169, p=0.251 for BP), GPX (t=–1.730, p=0.094 for MDD; t=–0.820, p=0.419 for BP), and GR (t=–1.269, p=0.214 for MDD; t=0.316, p=0.754 for BP) were at the similar levels after repeated measurement in patients. However, when compared to healthy controls, the levels of 8-iso-PGF2α (F=6.366, p<0.001) were significantly higher in both groups of patients regardless of the disease phase (Figure 1). The difference was not observed with two other investigated markers of oxidative damage, 8-OHdG (F=0.537, p=0.779) and MDA (F=0.897, p=0.501). In addition, patients with depression in the acute phase had significantly lower GPX activity (F=2.849, p=0.014) than controls. Also, the GR activity (F=4.941, p<0.001) was lower in these

Table I. Demographic data of the study participants.

	Healthy controls (n=32)	Patients with MDD (n=32)	Patients with BP (n=32)	Statistics[a]
Sex (female), n (%)	19 (59.4)	23 (71.9)	26 (81.2)	χ^2=3.731 p=0.155
Age (years), mean (SD)	45.44 (11.40)	51.06 (9.60)	50.56 (10.75)	F=2.754 p=0.069
Education (years), mean (SD)	12.69 (1.80)	11.56 (2.47)	11.94 (2.18)	F=2.226 p=0.114
Marital status (with partner), n (%)	20 (62.5)	21 (65.6)	18 (56.2)	χ^2=0.616 p=0.735
Tobacco use, n (%)	17 (53.1)	18 (56.2)	19 (59.4)	χ^2=0.254 p=0.881
BMI (kg/m^2), mean (SD)	24.78 (3.72)	23.11 (1.16)	24.43 (4.16)	F=2.301 p=0.106

[a]Statistical values were derived from the Chi-squared test and ANOVA. MDD major depressive disorder, BP bipolar disorder, SD standard deviation, BMI body mass index.

Table II. Clinical characteristics of patients in the study.

	Patients with MDD (n=32)	Patient with BP (n=32)	Statistics[a]
Duration of illness (years), mean (SD)	11.44 (9.22)	19.41 (10.51)	t=–3.224 p=0.002*
Age at onset (years), mean (SD)	40.25 (10.45)	30.63 (8.62)	t=4.019 p<0.001*
Number of depressive episodes, median (Q1, Q3)	4.0 (3.0, 8.5)	6.5 (4.0, 10.5)	U=382.000 p=0.079
Number of manic episodes, median (Q1, Q3)	/	2.5 (1.0, 4.0)	/
Total number of episodes, median (Q1, Q3)	4.0 (3.0, 8.5)	9.5 (6.0, 13.0)	U=259.000 p=0.001*
Previous psychotic episode, n (%)	8 (25.0)	18 (56.2)	χ^2=6.478 p=0.011*
Number of hospitalization, median (Q1, Q3)	3.5 (1.0, 8.5)	8.0 (4.0, 12.0)	U=303.000 p=0.005*
Positive family history, n (%)	15 (46.9)	22 (68.8)	χ^2=3.139 p=0.076
Suicid attempt, n (%)	11 (34.4)	20 (62.5)	χ^2=5.067 p=0.024*
Psychometric properties			
CGI-S score in acute phase, median (Q1, Q3)	4.00 (4.00, 5.00)	4.00 (4.00, 5.00)	U=455.500 p=0.529
CGI-S score in remission phase, median (Q1, Q3)	1.00 (1.00, 2.00)	1.00 (1.00, 1.75)	U=460.000 p=0.527
CGI-I score in remission phase, median (Q1, Q3)	1.00 (1.00, 1.00)	2.00 (1.00, 2.00)	U=256.000 p<0.001*
CGI-E score in remission phase, median (Q1, Q3)	4.00 (2.00, 5.00)	5.00 (2.00, 6.00)	U=426.500 p=0.328
HDRS total score in acute phase, median (Q1, Q3)	28.00 (23.00, 32.00)	27.00 (19.25, 33.25)	U=450.500 p=0.531
HDRS total score in remission phase, median (Q1, Q3)	8.00 (7.00, 10.00)	6.00 (3.00, 8.00)	U=271.500 p=0.002*
YMRS total score in acute phase, median (Q1, Q3)	1.00 (1.00, 2.00)	5.50 (2.25, 10.00)	U=85.500 p<0.001*
YMRS total score in remission phase, median (Q1, Q3)	0.00 (0.00, 1.00)	0.50 (0.00, 3.00)	U=387.000 p=0.096

[a]Statistical values were derived from Chi-squared test, t-test and Mann-Whitney U-test; significant results (p<0.05) are marked with *. MDD, major depressive disorder; BP, bipolar disorder; SD, standard deviation; Q1 – first quartile; Q3 – third quartile; CGI-S, Clinical Global Impression Scale – Severity of Illness; CGI-I, Clinical Global Impression Scale – Global Improvement; CGI-E, Clinical Global Impression Scale – Efficacy Index; HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale.

patients compared to controls but also compared to bipolar patients both during an acute episode and after achieved remission, as demonstrated by Bonferroni post-hoc test (Figure 2). The serum enzyme activity of SOD (F=1.900, p=0.090) and CAT (F=0.972, p=0.449) did not significantly differ between controls and patients in any phase.

Results of correlation analysis between measured oxidative parameters and selected clinical features in patients with MDD and BP are shown in Tables III and IV. There was a moderate positive correlation between HDRS score and 8-iso-PGF2α levels (r=0.437, p=0.014) in MDD patients in remission. Also, negative correlations were demonstrated between CGI-I scores and 8-iso-PGF2α levels (r=-0.491, p=0.007) and duration of illness and MDA levels (r=-0.580, p=0.023) in BP patients in remission (Table III). The SOD activity positively correlated with age (r=0.389, p=0.028) of MDD patients in the remission phase, while in BP patients in remission, there was a negative correlation between SOD activity and duration of illness (r=-0.404, p=0.022) and CAT activity and BMI (r=-0.533, p=0.002) (Table IV).

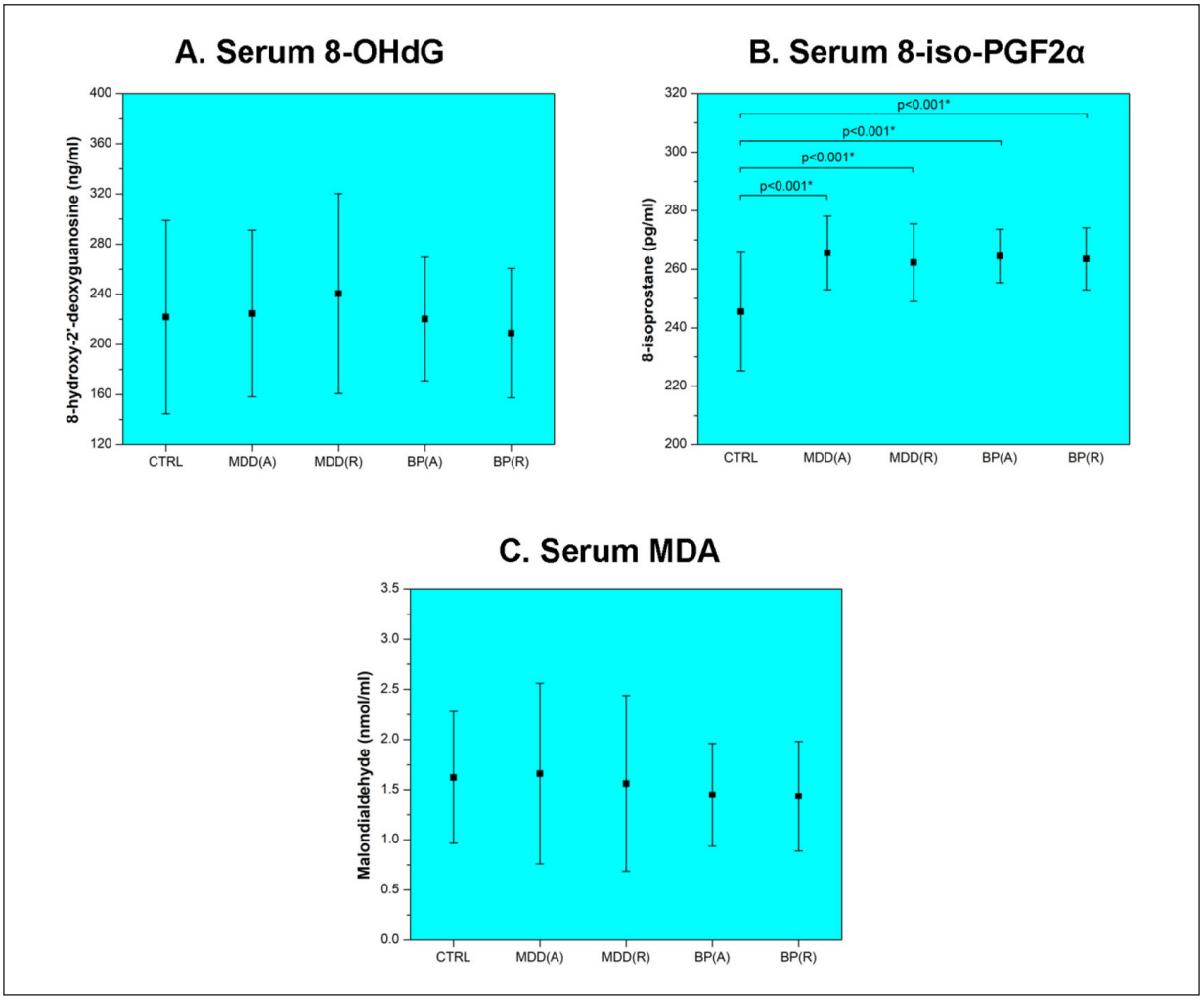

Figure 1. Serum levels of oxidative stress markers in patients and matched controls: (**A**), 8-hydroxy-2'-deoxyguanosine (8-OHdG), (**B**), 8-isoprostane (8-iso-PGF2α), and (**C**), malondialdehyde (MDA). CTRL, healthy control, MDD, major depressive disorder, BP, bipolar disorder, A, acute phase, R, remission phase; *$p<0.05$.

Discussion

In the last few decades, as interest in the role of oxidative stress in various brain diseases has grown, a body of evidence from animal and human studies[20,23,36-41] supporting the importance of this pathophysiological phenomenon in depression has emerged. However, the exact mechanisms underlying the link between oxidative stress and depression are not fully understood, nor are the roles of peripheral markers of oxidative stress in the diagnostic evaluation of these patients. An additional issue is whether the same inherent redox changes accompany the depressed mood that occurs in a spectrum of mood disorders, such as major depressive disorder and bipolar disorder, as the most prominent. In an attempt to answer this question, our study was designed to determine possible alterations in plasma levels of oxidative stress parameters in patients with mood disorders in the acute and remission phase, and then to compare these changes in patients with MDD and BP. The main findings of our study are an increase in plasma levels 8-iso-PGF2α in all patients with mood disorders regardless of disease phase and no significant difference in the pattern of oxidative stress parameters changes between MDD and BP, except for GR activity that was lower in a group of MDD patients.

We have found increased levels of 8-iso-PGF2α in patients with both unipolar and bipolar depression, which is in line with previous reports[20,36] of increased levels of markers of oxidative stress damage in depression. 8-iso-PGF2α is one of many isomers of F2-isoprostanes, prostaglan-

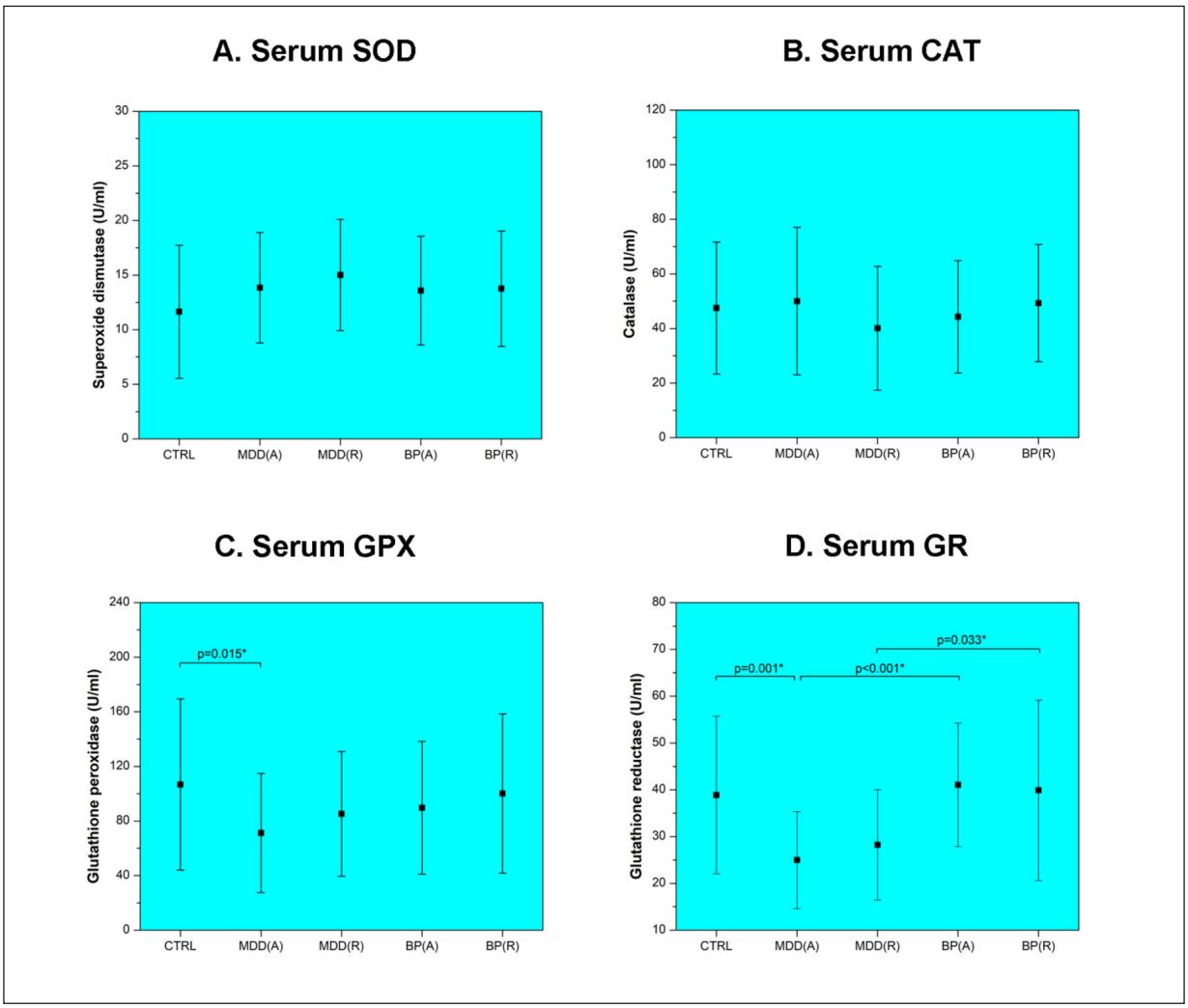

Figure 2. Serum activity of antioxidant enzymes in patients and matched controls: (**A**), superoxide dismutase (SOD), (**B**), catalase (CAT), (**C**), glutathione peroxidase (GPX), and (**D**), glutathione reductase (GR). CTRL, healthy control, MDD, major depressive disorder, BP, bipolar disorder, A, acute phase, R, remission phase; **p*<0.05.

din-like compounds formed by the peroxidation of arachidonic acid esterified to phospholipids in plasma membranes and lipoproteins. They are generated in the context of undergoing oxidative stress, as observed in multiple human diseases[37]. Apart from being potential biomarkers of oxidative damage, F2-isoprostanes possess intrinsic biological effects, including stimulation of vasoconstriction, bone resorption, platelet aggregation and fibrogenesis in the liver and lungs[38-40] which makes these biomarkers important not only for potential diagnostic purposes in psychiatric diseases but also for monitoring possible negative consequences of oxidative stress on somatic health and side effects of treatment. In their meta-analysis, Black et al[22] found increased levels of F2-isoprostanes in depression and reported that this result did not differ by type of depression, biological specimen, or laboratory method used. Likewise, there are reports of increased levels of other commonly measured markers of lipid peroxidation, notably MDA and 4-hydroxy-2-nonenal (HNE)[23,41,42]. However, our results do not support these reports of increased MDA in serum. These discrepancies of different markers of lipid peroxidation in our study can be attributed to methodological issues since the measurement of isoprostanes was carried out using an immunoassay as a more sensitive method compared to the spectrophotometric TBA assay for MDA. Also, many pre-analytical factors, such as sample preparation and storage conditions, can affect MDA concentration due to its low stability in biological samples[43].

Table III. Correlations between levels of oxidative stress markers and selected clinical features in MDD and BP patients.

	8-OHdG		8-iso-PGF2α		MDA	
	MDD	BP	MDD	BP	MDD	BP
Age	A: ra=.071	A: r=.001	A: r=-.080	A: r=.109	A: r=-.132	A: r=-.164
	R: r=.221	R: r=.139	R: r=.201	R: r=.173	R: r=-.184	R: r=-.371
BMI	A: r=.060	A: r=-.121	A: r=-.329	A: r=-.206	A: r=.326	A: r=-.470
	R: r=.263	R: r=.191	R: r=.276	R: r=.119	R: r=.306	R: r=-.481
Duration of illness	A: r=-0.065	A: r=-0.136	A: r=-.292	A: r=.052	A: r=.144	A: r=-.256
	R: r=0.108	R: r=0.253	R: r=-.033	R: r=.301	R: r=.269	R: r=**-.580***
N of depressive episodes	A: r=.044	A: r=-.204	A: r=-.183	A: r=.159	A: r=.355	A: r=-.217
	R: r=-.018	R: r=.103	R: r=.192	R: r=.027	R: r=.247	R: r=-.389
Total N of episodes	A: r=.044	A: r=-.180	A: r=-.183	A: r=.168	A: r=.355	A: r=-.302
	R: r=-.018	R: r=.127	R: r=.192	R: r=.078	R: r=.247	R: r=-.496
CGI-S score	A: r=-.121	A: r=.320	A: r=.089	A: r=-.132	A: r=-.102	A: r=.156
	R: r=.022	R: r=.108	R: r=.210	R: r=-.001	R: r=-.016	R: r=-.226
CGI-I score	R: r=-.161	R: r=-.109	R: r=0.249	R: r=**-.491***	R: r=-.098	R: r=-.231
CGI-E score	R: r=.082	R: r=-.046	R: r=.254	R: r=-.270	R: r=.133	R: r=-.169
HDRS score	A: r=-.053	A: r=.109	A: r=-.042	A: r=-.053	A: r=-.047	A: r=-.070
	R: r=-.226	R: r=-.002	R: r=**.437***	R: r=-.293	R: r=-.180	R: r=-.281
YMRS score	A: r=-.333	A: r=-.058	A: r=-.029	A: r=-.198	A: r=.034	A: r=-.005
	R: r=-.254	R: r=.062	R: r=.024	R: r=.284	R: r=-.120	R: r=-.384

[a]Value r represents Pearson correlation coefficient; significant results ($p<0.05$) are written in bold and marked with*. 8-OHdG, 8-hydroxy-2'-deoxyguanosine; 8-iso-PGF2α, 8-isoprostane; MDA, malondialdehyde; MDD, major depressive disorder; BP, bipolar disorder; A, acute phase; R, remission phase; BMI, body mass index; N, number; CGI-S, Clinical Global Impression Scale - Severity of Illness; CGI-I, Clinical Global Impression Scale - Global Improvement; CGI-E, Clinical Global Impression Scale - Efficacy Index; HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale.

Table IV. Correlations between the activity of antioxidant enzymes and selected clinical features in MDD and BP patients.

	SOD		CAT		GPX		GR	
	MDD	BP	MDD	BP	MDD	BP	MDD	BP
Age	A: ra=.235	A: r=.280	A: r=.057	A: r=-.195	A: r=-.110	A: r=-.130	A: r=.005	A: r=.070
	R: r=**.389***	R: r=.014	R: r=-.008	R: r=.002	R: r=-.193	R: r=-.174	R: r=-.140	R: r=.050
BMI	A: r=.139	A: r=.049	A: r=-.031	A: r=**-.533***	A: r=-.247	A: r=-.056	A: r=.072	A: r=-.137
	R: r=.245	R: r=-.179	R: r=-.151	R: r=-.262	R: r=.059	R: r=-.108	R: r=-.027	R: r=.260
Duration of illness	A: r=.248	A: r=.015	A: r=.076	A: r=-.110	A: r=.013	A: r=-.007	A: r=-.122	A: r=-.013
	R: r=.267	R: r=**-.404***	R: r=.174	R: r=-.208	R: r=-.185	R: r=-.164	R: r=.111	R: r=.085
N of depress. episodes	A: r=-.115	A: r=.092	A: r=.242	A: r=.092	A: r=.185	A: r=-.074	A: r=-.194	A: r=.252
	R: r=.036	R: r=-.235	R: r=.204	R: r=-.110	R: r=-.157	R: r=-.043	R: r=-.026	R: r=.087
Total N of episodes	A: r=-.115	A: r=.091	A: r=.242	A: r=.083	A: r=.185	A: r=-.066	A: r=-.194	A: r=.223
	R: r=.036	R: r=-.276	R: r=.204	R: r=-.154	R: r=-.157	R: r=-.034	R: r=-.026	R: r=.072
CGI-S score	A: r=.323	A: r=-.104	A: r=-.005	A: r=.164	A: r=-.082	A: r=-.234	A: r=.098	A: r=-.298
	R: r=.100	R: r=.036	R: r=.081	R: r=-.007	R: r=.050	R: r=-.111	R: r=.004	R: r=.024
CGI-I score	R: r=-.154	R: r=.012	R: r=-.006	R: r=.278	R: r=.165	R: r=.030	R: r=.029	R: r=.007
CGI-E score	R: r=-.253	R: r=.080	R: r=.034	R: r=.314	R: r=.236	R: r=.022	R: r=-.218	R: r=-.016
HDRS score	A: r=.284	A: r=-.260	A: r=.279	A: r=.327	A: r=.007	A: r=-.203	A: r=.230	A: r=-.125
	R: r=-.178	R: r=-.105	R: r=-.013	R: r=.306	R: r=-.150	R: r=.253	R: r=-.098	R: r=.102
YMRS score	A: r=.028	A: r=-.276	A: r=-.004	A: r=-.068	A: r=.178	A: r=.065	A: r=-.104	A: r=-.079
	R: r=-.024	R: r=-.349	R: r=-.080	R: r=-.034	R: r=.044	R: r=-.281	R: r=-.246	R: r=.031

[a]Value r represents Pearson correlation coefficient; significant results ($p<0.05$) are written in bold and marked with*. 8-OHdG, 8-hydroxy-2'-deoxyguanosine; 8-iso-PGF2α, 8-isoprostane; MDA, malondialdehyde; MDD, major depressive disorder; BP, bipolar disorder; A, acute phase; R, remission phase; BMI, body mass index; N, number; CGI-S, Clinical Global Impression Scale - Severity of Illness; CGI-I, Clinical Global Impression Scale - Global Improvement; CGI-E, Clinical Global Impression Scale - Efficacy Index; HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale.

Another important group of oxidative parameters that many researchers use as an index of oxidative stress consists of the antioxidant defense enzymes. In the present study, we have investigated plasma enzyme activity levels of the key antioxidant enzymes SOD, CAT and GPX. The goal of our study was to primarily focus on the potential difference between enzyme activity in the acute phase and remission in patients with unipolar or bipolar depression. Our results showed no significant difference in enzyme activity among different patient groups and related to the phase of the disease. This is largely consistent with a recently published systematic review and meta-analysis[44] showing that compared with healthy controls, patients with unipolar and bipolar depression did not differ regarding the activity of the CAT, SOD and GPX enzyme. However, a previous meta-analysis[45] from the same authors reported higher CAT activity in bipolar disorder without differences in SOD and GPX compared to healthy controls, and after stratification by polarity and medication status, the study showed that medication-free patients in mania had higher SOD and lower GPX. All patients with bipolar disorders in our study had an acute depressive episode at the time of blood sampling and analysis, and our results showed no difference in SOD, CAT, and GPX activity compared to controls. However, we have found that patients with depression had lower GPX activity levels during the acute phase when compared to healthy subjects, while the activity levels of SOD and CAT remained unchanged. These results are inconsistent with the results from a meta-analysis[41] demonstrating that depression was associated with higher SOD levels, while differences in CAT and GPX were not significant compared to healthy people. However, the same meta-analysis showed no effect of medication on the antioxidant enzyme levels, which is similar to our results.

One of the major findings of our study, and at the same time, the only parameter that differed among the two study groups of patients, was plasma GR activity. We found that patients suffering from major depressive disorder have lower GR activity compared to bipolar patients in both acute phase and remission. Moreover, GR activity was lower in the acute phase of depression compared to healthy subjects. GR is a flavoprotein and homodimeric enzyme that catalyzes the production of glutathione (GSH) from glutathione disulfide (GSSG) at the expense of NADPH. It has a central role in the glutathione redox cycle and, consequently, in the regulation, modulation and maintenance of cellular redox homoeostasis[46]. Its inherited deficiency is clinically associated with hemolytic anemia due to the reduced lifespan of red blood cells and cataracts as a result of primarily UV-induced oxidative damage in the eye lens[47]. Under conditions of oxidative stress in cells, GR would be expected to increase its activity and there is strong evidence from cell culture studies[48] to support this hypothesis. The research[49] on dermal fibroblasts isolated from MDD patients demonstrated an increase in *GR* expression after oxidative stress induction with no change in total glutathione amount. Using a different methodological approach to this issue, Gawryluk et al[50] showed reduced levels of total glutathione and no difference in GR activity in the prefrontal cortex of post-mortem brain tissue from both MDD and BP patients[50]. Finally, results from animal studies[51] provide additional information about brain region-specific changes in glutathione redox homeostasis. Based on the rodent depression model[51], it was demonstrated lower GR expression in the hippocampus, but not in the prefrontal cortex and amygdala of socially defeated rats, when compared to control rats[51]. In the animal model of mania induced by ouabain, *GR* activity was increased in the frontal cortex and hippocampus, along with markers of oxidative damage and SOD and GPX activity[52]. Results from previously published works based on blood levels of GR activity, such as our paper, are heterogeneous. There are reports of no change in GR activity in siblings of BP patients[53], but also increased activity among patients in the late stage of illness[54].

Correlational analysis of our data did not show unequivocal trends in oxidative stress parameters and clinical characteristics of patients with MDD and BP, but some comments can be made. We demonstrated that MDD patients in remission with higher levels of 8-iso-PGF2α tend to have higher HDRS scores, which was not observed in the group of patients with bipolar depression, indicating a possible difference in these conditions. Interestingly, Chung et al[36] found that increases in F2-isoprostane excretion and improvement in the Hamilton depression score between baseline and after the treatment visit were inversely correlated, suggesting that antidepressants may increase urinary F2-isoprostane excretion in depression. Also, our findings of a negative correlation of MDA levels and SOD activity in the remission phase with BP duration suggest that these markers change over time, which may be attributed to the natural course of the disease, treatment effect, or both.

Limitations

Our study has several limitations. Besides being a unicentric study with a relatively small sample size, the major methodological issue is related to various treatment regimens used for patients who participated in the research. This is the reason why we cannot say that part of the results was not confounded by medication status in our analyses. Also, although we controlled for age, gender, BMI, and smoking, there is a possibility that factors like physical activity levels, dietary habits, family history, etc. can have a confounding effect on the results. Finally, our approach to measurement of oxidative stress was "indirect", relying completely on peripheral oxidative stress markers. These molecules in plasma are dominantly originated from blood cells and tissues other than the brain, so every interpretation of the pathophysiological relationships between oxidative stress and mood disorders based on our findings should be interpreted with caution.

Conclusions

In conclusion, we found that the serum levels of 8-iso-PGF2α as an index of lipid peroxidation is increased in both MDD and BP patients regardless of the phase, suggesting its potential role as a state biomarker of depression. Compared to healthy controls, the levels of other investigated biomarkers of oxidative stress damage (8-OHdG and MDA) and serum activities of key antioxidant enzymes (SOD, CAT, and GPX) did not differ in MDD and BP during the acute phase or remission, except for lower serum GPX activity in the acute phase of MDD. The serum activity of GR, a major enzyme in glutathione recycling, was also decreased in the acute phase in MDD compared to healthy subjects and showed overall lower activities in MDD compared to BP patients regardless of disease phase.

Conflict of Interest

The authors declare that they have no conflict of interest to declare.

Ethics Approval

This study was conducted in accordance with the Declaration of Helsinki of 1975 (as revised in 2013), and the protocol was reviewed and approved by the Ethics Committee of the School of Medicine, University of Belgrade (acceptance number 29/X-29).

Informed Consent

All subjects provided written informed consent for inclusion before they participated in the study.

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Authors' Contributions

NP contributed to the conception and design of the study, as well as drafting the manuscript. TN and MV performed immunoassays. TS performed spectrophotometric assays. MV and TS organized the database and performed statistical analysis of the data. TS provided the figures and tables. ML, NP, and MP conceptualization; MT, MN methodology and statistical analysis. JDM performed critical reading of the article and final preparation for publishing.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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