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Lipid peroxidation and antioxidant enzyme levels in patients with schizophrenia and bipolar disorder

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Recent data from several reports indicate that free radicals are involved in aetiopathogenesis of many human pathologies including neuropsychiatric disorders such as schizophrenia, bipolar disorder etc. In the present study, we aimed at determining and evaluating levels of malondialdehyde (MDA), a product of lipid peroxidation, and antioxidant enzyme (superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity levels in patients diagnosed with schizophrenia ($n = 25$) and bipolar disorder ($n = 23$). The control group was composed of 20 healthy subjects. There was a significant increase in MDA levels of patients with schizophrenia and bipolar disorder compared with controls. SOD and GSH-Px activity levels were significantly higher in the schizophrenic group compared with controls. SOD activity levels in bipolar the group were significantly higher than controls whereas there were no significant changes in GSH-Px activity levels in the bipolar group and controls. Significant differences between lipid peroxidation product and antioxidant enzyme (SOD and GSH-Px) activity levels in schizophrenic and bipolar disorder patients compared with controls leads us to believe that these differences are related to the heterogenities in aetiologies of these disorders. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS — MDA; SOD; GSH-Px; schizophrenia; bipolar disorder

INTRODUCTION

There is an enormous amount of convincing data indicating that reactive free radical species are involved in initiation and development of many different forms of human pathologies. Predominantly superoxide, hydroxyl ion and nitric oxide are generated under physiological conditions during aerobic metabolism.[1] A small portion of free radicals have roles in physiological processes, but the remaining are inactivated by antioxidant enzyme systems.[2,3] When free radicals are produced in excessive amounts or antioxidant defence systems are inefficient, some chain reactions causing cellular dysfunction, or even death of cells, are activated.[4,5] Effects of free radicals on pathogenesis of disorders related to brain aging and neurodegenerative diseases have also been investigated. Free radicals have been considered important primarily in the pathogenesis of neuroleptic treatment complications, such as tardive dyskinesia in psychiatric disorders.[1]

Free radicals are produced in many different ways, such as activation of phagocytes and the general immune system, lipid peroxidation, electron transport system in mitochondria, ischaemia and trauma.[6] External resources including anti-neoplastic drugs, anaesthetics, alcohol, radiation, some antibiotics and solvents can cause increased free radicals in the body.[7] Free radicals have a relatively short half-life, and thus the determination of their levels is difficult. Therefore, they can be evaluated indirectly by measurement of some antioxidant enzyme levels such as superoxide dismutase (SOD), catalase (CAT) or glutathione peroxidase (GSH-Px), by products of lipid peroxidation such as malondialdehyde (MDA) or by some transition metal levels such as copper, zinc and iron.[8]

There are some recent studies focused on the role(s) of free radicals in pathogenesis of schizophrenia.

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There are numerous studies indicating that free radical-mediated neuronal dysfunction has roles in pathophysiology of schizophrenia.[1] Increased SOD activity levels in schizophrenia have been reported.[9] Buckman and co-workers reported that chronic schizophrenics had GSH-Px activity similar to healthy controls.[10] In another study where schizophrenics were compared with normals, increased levels of MDA, an indicator of lipid peroxidation, in patients with schizophrenia were reported.[11] It has been reported that the most important source of free radicals is glial cells and free radicals produced by these cells are associated with neuropsychiatric disorders such as Alzheimer's disease and Parkinson disease etc.[9]

In the present study, we aimed at determining and comparing oxidative damage (via determination of MDA) and antioxidant enzyme (SOD and GSH-Px) activity levels in patients with schizophrenia, bipolar disorder and healthy controls.

MATERIALS AND METHODS

Collection of samples

The patient group consisted of 48 patients who had applied to the Department of Psychiatry, College of Medicine at Firat University between June 1999 and February 2000 and had been diagnosed with schizophrenia ($n = 25$) and bipolar disorder ($n = 23$) according to the *Diagnostic and Statistical Manual of Mental Disorders–IV.*[12] The control group was composed of healthy subjects ($n = 20$) who applied to the Department of Psychiatry, College of Medicine at Firat University for driving licence examination and were evaluated to be normal. In addition, controls did not have a history of major mood disorder, dementia, mental retardation or psychosis in their first degree relatives.

Physical and neurological examination was performed for each of the patients and controls. Liver and kidney function tests were evaluated. Subjects having normal results and without any exclusion criteria were admitted to the study. Exclusion criteria were as follows: alcohol and substance abuse or dependence, tardive dyskinesia related to neuroleptics, presence of severe organic conditions, users of any antioxidant agent (i.e. E and C vitamins), presence of epilepsy and severe neurological disorder, presence of infectious disease, excessive obesity.

Determination of plasma MDA levels

Levels of plasma MDA were measured by the thiobarbituric acid (TBA) method which was modified from the methods of Satoh[13] and Yagi[14] as we have reported recently.[15–18] Peroxidation was measured as the production of MDA which in combination with TBA forms a pink chromogen i.e. compound whose absorbance at 532 nm was measured. MDA results were expressed as nmol ml^{-1}.

Measurement of GSH-Px activity levels

GSH-Px activity levels in haemolysates of erythrocytes were measured using the method of Paglia and Valentine[19] in which GSH-Px activity was coupled to the oxidation of NADPH by glutathione reductase as we reported recently.[16–18] The oxidation of NADPH was followed spectrophotometrically at 340 nM and at 37°C. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH = 7), 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 mM glutathione, and 1U ml^{-1} of glutathione reductase. The absorbance at 340 nm was recorded for 5 min. The activity was the slope of the lines expressed as μmol of NADPH oxidized per min. Results were expressed as U g^{-1} Hb.

Measurement of SOD activity

Haemolysates of erythrocytes were used for measurement of SOD activity levels by the method of Sun *et al.*[20] as we reported recently.[15,16] This method is based on the reduction of superoxide, which is produced by the xanthine oxidase enzyme system, by nitroblue tetrazolium. One unit of SOD was defined as the amount required decrease nitroblue tetrazolium reduction by 50%. Results were expressed as U g^{-1} Hb.

Statistical analysis

Statistical evaluations were performed by using the ANOVA test (Kruskal–Wallis). Differences at $p < 0.05$ level were considered to be statistically significant.

RESULTS

To evaluate levels of lipid peroxidation, initially MDA levels were determined in controls and all patients (Figure 1). MDA levels in patients with schizophrenia or bipolar disorder were 4.76 ± 0.79 nmol ml^{-1} and 4.26 ± 0.46 nmol ml^{-1}, respectively, whereas controls had lower MDA levels (2.71 ± 0.50 nmol ml^{-1}). MDA levels of schizophrenic and bipolar groups were significantly higher ($p < 0.001$) than

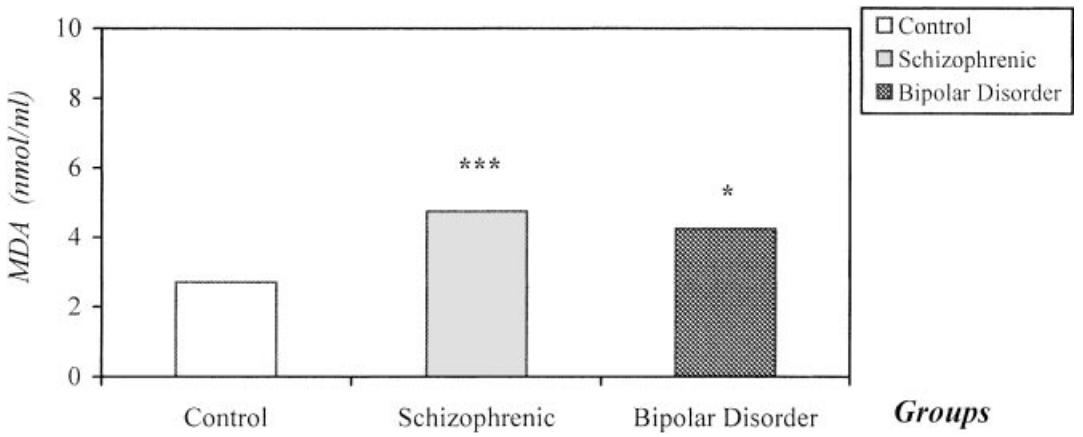

Figure 1. MDA levels in patients with schizophrenia, bipolar disorder and controls. $*p < 0.05$ (control versus bipolar disorder group); $***p < 0.001$ (control versus schizophrenic group). (ANOVA, Kruskal–Wallis)

controls. In addition, the difference in MDA levels between patients with bipolar disorder and schizophrenia was statistically significant ($p < 0.05$) (Figure 1).

SOD activity levels in the schizophrenic group, in patients with bipolar disorder and controls were 2052.10 ± 248.87 U g^{-1} Hb, 1723.44 ± 127.86 U g^{-1} Hb and 1686.63 ± 103.01 U g^{-1} Hb, respectively (Figure 2). SOD activity levels were significantly higher in the schizophrenic group compared to the control group ($p < 0.001$) and bipolar group ($p < 0.01$). Although the schizophrenic group had slightly higher SOD activity levels compared to the bipolar disorder group, the difference was not statistically significant ($p > 0.05$).

In the schizophrenic group, GSH-Px activity levels were 34.55 ± 3.13 U g^{-1} Hb whereas they were 32.82 ± 4.02 U g^{-1} Hb and 30.53 ± 4.72 U g^{-1} Hb in patients with bipolar disorder and controls respectively (Figure 3). GSH-Px activity levels were statistically significantly higher in the schizophrenic group than in the controls ($p < 0.01$). There was no significant difference in GSH-Px activity levels between the bipolar group and the control group or between the bipolar group and the schizophrenic group ($p > 0.05$).

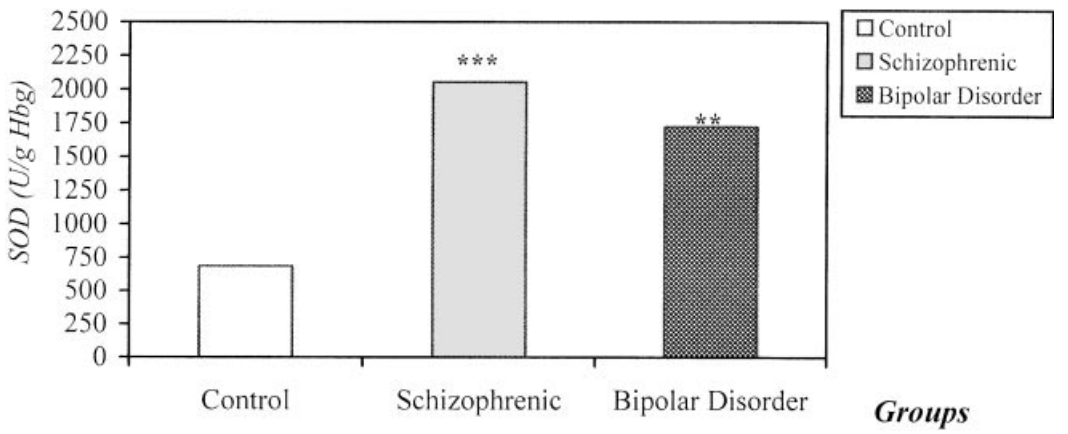

Figure 2. SOD activity levels in erythrocytes of in patients with schizophrenia, bipolar disorder and controls. $**p < 0.01$ (control versus bipolar disorder group); $***p < 0.001$ (control versus schizophrenic groups)

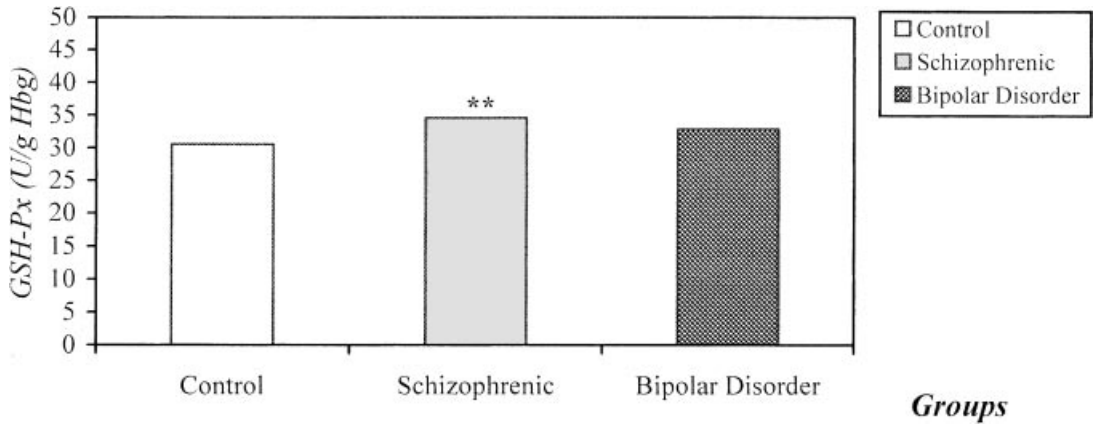

Figure 3. GSH-Px activity levels in erythrocytes of patients with schizophrenia, bipolar disorder and controls. $^{**}p < 0.01$ (control versus schizophrenic groups)

DISCUSSION

The brain is especially vulnerable to free radical damage because it is a highly oxygenated organ that uses one-fifth of the total oxygen required by the body.[21] In addition, the brain contains large amounts of iron and polyunsaturated fatty acids and it is relatively poor with respect to antioxidants such as catalase.[22,23] Probably, catecholamines including dopamine and norepinephrine are associated with the production of free radicals and conditions causing increased catecholamine metabolism may increase the free radical burden.[24,25] Likewise, trauma and ischaemia of the brain may cause increased free radical burden. Free radical damage resulting from ischaemia is related to reperfusion or reoxygenation of the tissue.[26] Increased transition metal (i.e. iron, copper and manganese) concentrations lead to the production of excessive amounts of free radicals.[27]

In the present study, we examined the levels of oxidative damage and antioxidant system responses in patients with schizophrenia and bipolar disorder. We determined significant differences of MDA, GSH-Px and SOD levels between patients with schizophrenia and controls. Previous studies have reported controversial results. Abdalla and co-workers reported that schizophrenic patients had higher erythrocyte SOD activity levels than healthy controls.[28] Cohen *et al.* compared schizophrenic patients and healthy controls with respect to SOD activity and toxic oxygen metabolites and did not find any significant difference.[29,30] GSH-Px activity levels were reported to be decreased in two other studies.[28,31] GSH-Px provides an effective protection mechanism against cytosolic injury because it eliminates H_2O_2 and lipid peroxides. Hence, it is regarded to be critical for maintaining low levels of cellular H_2O_2 and lipid peroxides. GSH-Px levels are high in brain areas prone to oxidative injury.[32] In a study reported by Buckman *et al.*, low peripheral GSH-Px activity was found to be associated with both cortical sulcal prominence on CT and prominent negative symptoms.[33] Güzelhan *et al.* who investigated the importance of the free radicals and the non-enzymic antioxidant defence systems in schizophrenic patients by comparing them with healthy controls reported that patients with schizophrenia had lower erythrocyte GSH levels than healthy controls but there was no significant differences in MDA levels.[34]

In the present study, the bipolar group had stastistically higher MDA levels but there was no statistically significant difference for SOD and GSH-Px levels. In the literature, we did not find any data regarding MDA and antioxidant enzyme levels in patients with bipolar disorder. Increased MDA levels disable cellular membrane functions by stimulating phospholipase A_2 and thus release interleukins by stimulating the immune system.[35] Free radicals can also react with membrane-associated proteins, altering enzyme and neurotransmitter receptor function.[36] We suggest that these effects may be associated with the aetiology of bipolar disorder because changes of neurotransmitter-receptor functions are considered to be responsible for the aetiopathogenesis of bipolar disorder. On the other hand, studies related to free radicals in schizophrenia have produced controversial results. We believe that the heterogenity of clinical and aetiopathogenesis in schizophrenia plays the most important role. More

comprehensive and detailed studies are needed in order to decipher the exact roles of free radicals in different psychological disorders.

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