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Oxidative stress in bipolar and schizophrenia patients

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ABSTRACT

Oxidative stress has an important place in studies investigating the pathophysiology of psychiatric diseases. In spite of this fact, longitudinal studies are required to clarify the subject. Therefore, in this study, we examined lipid peroxidation, protein oxidation, total oxidized guanine species, superoxide dismutase (SOD) and total glutathione (GSH) levels in blood collected from adult bipolar patients ($n=18$) during manic and euthymic episodes, schizophrenic patients ($n=18$) during acute psychotic attack and remission phases and the control group ($n=18$). There was a significant increase in the level of lipid peroxidation in the bipolar disorder manic episode group (BD-ME) compared to control group. The level of protein oxidation was significantly higher in the schizophrenia acute psychotic attack group (SZ-APA) compared to the control group. The level of total oxidized guanine species was statistically higher in all psychiatric groups compared to the control group. There was no significant difference among the groups with regard to SOD and GSH. Consequently, we believe that lipid peroxidation may be effective in the pathogenesis of bipolar patients; that protein oxidation may be of importance in the pathogenesis of schizophrenia and that total oxidized guanine species may be crucial in the pathogeneses of both psychiatric disorders.

1. Introduction

Bipolar disorder (BD) and schizophrenia (SZ) are highly common psychiatric disorders (Merikangas et al., 2007; Schultz et al., 2007). The molecular causes underlying these two disorders are still unclear. A part of the research on these disorders blame the decrease in the energy metabolism of the brain (Volz et al., 2000; Kato, 2005) and the abnormality in mitochondrial genes (Kato and Kato, 2000; Rollins et al., 2009) while another part of the research argue that the culprit is the oxidative stress (Andreazza et al., 2008a; Yao and Keshavan, 2011) which springs from the problems in mitochondrial activity (Andreazza et al., 2010; Gubert et al., 2013) and dopaminergic system (Grima et al., 2003; Kim et al., 2014).

Oxidant agents may lead to oxidative damage in proteins, lipids and nucleic acids. This damage can be detected by measuring the protein oxidation, lipid peroxidation and 8-hydroxy deoxy guanosine. SOD and glutathione are the primary antioxidant defense systems combating the oxidative damage. Oxidative stress occurs when the balance between the oxidant and antioxidant systems is disrupted (Halliwell, 2007). The increased oxidative stress may play a role in the emergence of psychiatric disorders by affecting the neuronal plasticity, signal transduction and neurotransmitter intake which depends on the oxidation of the membrane proteins (Mahadik et al., 2001; Manji et al., 2012).

Recently, the number of studies investigating the association between oxidative stress and psychiatric disorders has increased. However, there is no consensus on the association between the oxidative stress and these disorders (Andreazza et al., 2008a; Flatow et al., 2013). Studies on the oxidative stress generally evaluate its parameters within a single phase of the disease (Savas et al., 2006; Micó et al., 2011; Raffa et al., 2012). The studies comparing different phases of the disease (bipolar manic, euthymic and depressive episode, schizoprenia acute psychotic attack and remission phases) have used different phases of the disease in different patients (Arvindakshan et al., 2003; Andreazza et al., 2007a; Kunz et al., 2008). The number of studies measuring the oxidative stress parameters of the same patient at different phases is relatively limited (Gergerlioglu et al., 2007; Selek et al., 2008). Longitudinal studies are required in order to reveal the underlying causes of the bipolar disorder and schizophrenia (Kapczinski et al., 2011). Thus, the effect of individual differences (lifestyle, nutrition, socioeconomic status, etc.) which may lead to the oxidative stress can be alleviated, which, in turn, may enable more definite results (Raffa et al., 2012). Other counfounding factors used in studies conducted until today were obesity and smoking. Obesity and

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smoking are frequently observed in these patients and increase the oxidative stress significantly (Kalman et al., 2005; McIntyre et al., 2010). Therefore, studies on the subject should be careful about not including obese or smoker patients.

In the light of these, we studied the levels of oxidative stress parameters in non-smoking bipolar and şchizophrenia patients with a body mass index of ≤ 25 kg/m^2. We also studied how these parameters changed with treatment. To this end, we compared the lipid peroxidation, protein oxidation, total oxidized guanine species, SOD and total glutathione levels of bipolar patients in manic and euthymic episodes and of schizophrenia patients in acute psychotic attack and remission phases in this study.

2. Materials and methods

2.1. Subjects

This study was approved by Ondokuz Mayıs University, Medical Research Ethical Committee (No: 2010/147 Issue: 315). All procedure was arranged in accordance with World Medical Association Declaration of Helsinki. All participants were informed orally and in writing. All subjects gave written informed consent.

This study, which was conducted in Ondokuz Mayıs University, Psychiatry Clinic, involved adult bipolar ($n=18$) and schizophrenic patients ($n=18$) and a control group ($n=18$) consisting of subjects who did not differ from these patients with regard to age and sex. The age range for the subjects was 18–65 years. All subjects were evaluated by a psychiatrist prior to the study. Bipolar patients were evaluated in manic and euthymic episodes while schizophrenic patients were evaluated in acute psychotic attack and remission phases. Bipolar disorder and schizophrenia were diagnosed using The Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV). Bipolar patients diagnosed in manic episode Type 1 were considered euthymic if they had a Young Mania Rating Scale (YMRS) score less than 5 for 2 months following their treatment (Young et al., 1978). Of the patients diagnosed with schizophrenia in acute psychotic attack, those who had scored 3 or less in each of the 8 steps set in Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) for 6 months after their treatment were considered in the remission phase and were included in the study. All subjects had a body mass index of ≤ 25 kg/m^2 and were non-smokers. They also had no substance abuse or chronic disease (infection, inflammatory disease, diabetes, hypertension, cancer, mental retardation, neurological disorder, etc.). The control group consisted of subjects who had never suffered from a psychiatric disease. None of the first-degree family members of the control group had a history of a major psychiatric disorder. The subjects of the study had no acute medical condition (e.g. infection) at the time of blood collection and received no medication. Moreover, female subjects were not pregnant. They did not have menstrual bleeding, either.

2.2. Collection of blood

At 8 o'clock in the morning, blood was drawn from each subject on an empty stomach to a tube with no anticoagulant (8 ml) and to a tube with EDTA (2 ml). Blood samples from bipolar patients were drawn in the manic and euthymic episodes and from schizophrenic patients in acute psychotic attack and remission phases. The blood collected was centrifuged at 3000g for 5 min at $+4$ °C. The serum and plasma were separated and stored at -80 °C until the beginning of the study.

2.3. Lipid peroxidation

Lipid peroxidation level was measured in serum with a pre-defined method (Varshney and Kale., 1990). 0.2 ml standard solution which was prepared from malondialdehyde stock solution (10 μmol/L, 5 μmol/L, 2.5 μmol/L, 1.75 μmol/L, 0.875 μmol/L), serum and distilled water (for blank) were put into glass tubes. Then, 0.8 ml buffer solution (KH_2PO_4–K_2HPO_4, 100 mM, pH: 7,4) was added and mixed by vortex. 0.25 ml tricholorocarboxylic acid (TCA, %30) and 0.25 ml thiobarbituric acid (TBA, 52 mM, pH: 2,1; dissolved in 1 M glacial acetic acid) were added into all tubes. After mixing by vortex, the tubes were incubated at 80 °C for 45 min. The pink color produced was measured at 532 nm. Sample concentrations were calculated using the equation obtained from the standard linear curve ($R^2=0.99$, SPSS for Windows 15.0). Results were expressed in μmol/L.

2.4. Protein oxidation

Protein oxidation level was measured in plasma with a commercially available AOPP kit (Immundiagnostik, Bensheim, Germany, Lot: K7811w-111031). This method is based on the spectroscopic analysis of AOPP (advanced oxidation protein product) levels, which emerge as a result of protein oxidative damage. Before assaying, samples were 1:6 diluted with Sample Dilution Buffer. Standards (100 μmol/L, 50 μmol/L, 25 μmol/L, 12.5 μmol/L, 6.25 μmol/L) and all samples were placed in the proper wells on a microtiter plate, the absorbance of the samples was read at 340 nm. Diluted sample concentrations were calculated using the equation obtained from the standard linear curve ($R^2=0.98$, SPSS for Windows 15.0). The obtained AOPP values were multiplied by 6. AOPP concentrates were expressed as CT (chloramine-T) equivalents. Results were expressed in μmol/L.

2.5. Total oxidized guanine species

This measurement was conducted in serum with a DNA/RNA Oxidative Damage EIA kit (Cayman, Ann Arbor, USA, Lot no: 0451239). Several diseases are associated with nucleic acids oxidation. During the repair process of this damage, multiple oxidized guanine species including the ribose-free base (8-oxo-guanine or 8-hydroxyguanine), the nucleoside from RNA (8-oxoguanosine or 8 hydroxyguanosine) and the deoxynucleoside from DNA (8-oxo-deoxyguanosine or 8-hydroxy-2′-deoxyguanosine) are released into blood. In this method, oxidatively damaged guanine species (8-hydroxy-2′-deoxyguanosine, 8-hydroxyguanosine and 8-hydroxyguanine) and 8-OH-dG-acetylcholinesterase conjugate (DNA/RNA Oxidative Damage Tracer) compete in order to bind to a limited number of DNA/RNA Oxidative Damage Monoclonal antibodies. Afterwards, this antibody-oxidatively damaged guanine complex binds to polyclonal anti-mouse IgG, which is previously attached to the wells. After the wells are washed, Ellman's reagent is added and the yellow color formed is read at 412 nm. Absorbance values are inversely proportional to the amount of free 8-OH-dG. Before assaying, all samples were diluted (1:25) with EIA Buffer Solution. Standards (3000 pg/ml, 1333 pg/ml, 592.6 pg/ml, 263.4 pg/ml, 117.1 pg/ml, 52.0 pg/ml) were prepared from DNA/RNA Oxidative Damage EIA Standard solution. Diluted sample concentrations were calculated using the equation obtained from the standard logarithmic curve (Microsoft Office Excel 2007). The obtained total oxidized guanine species values were multiplied by 25. Results were expressed in pg/ml

2.6. SOD

SOD was spectrophotometrically measured in serum with Superoxide Dismutase Assay Kit (Cayman, Ann Arbor, USA, Lot no: 0447900). This kit uses tetrazolium salt to detect superoxide radicals formed by xanhine oxidase. As the superoxide radical converts into O_2, tetrazolium salt converts into formazan dye. The color formed is measured at 440–460 nm. The SOD found in the sample reduces the level of superoxide radical and the formation of formazan dye. SOD activity in the sample is measured as the percent inhibition of the rate of formazan dye formation. Before assaying, samples were diluted 1:5 with Sample Buffer Solution (50 mM Tris–HCl, pH 8.0, containing 0.1 mM diethylenetriaminepentaacetic acid and 0.1 mM hypoxanthine). Standards (0.25 U/ml, 0.2 U/ml, 0.15 U/ml, 0.1 U/ml, 0.05 U/ml, 0.025 U/ml) were prepared with Sample Buffer Solution. Diluted sample concentrations were calculated using the equation obtained from the standard linear curve ($R^2=0.93$, SPSS for Windows 15.0) The obtained SOD values were multiplied by 5. Results were expressed in U/ml.

2.7. GSH

GSH level was spectrophotometrically measured in plasma with a Glutathione Assay Kit (Cayman, Ann Arbor, USA, Lot no: 0450302). In this method, the sulfhydryl group of GSH react with DTNB (5.5′-dithio-bis-2-(nitrobenzoic acid), Ellman's reagent) and 5-thio-2-nitrobenzoic acid (TNB) in yellow is formed. GSTNB, which is formed simultaneously, is converted into GSH and TNB by glutathione reductase. The TNB absorbance value measured at 405–414 nm is directly proportional to the amount of GSH. Before assaying, MPA reagent (%10, metaphosphoric acid, 500 μl), was added to the samples (500 μl) in equal volume for deproteination. After this, 50 μl of TEAM reagent (4 M, triethanolamine) was added to increase the pH of the sample. Standards (32 μmol/L, 16 μmol/L, 8 μmol/L, 4 μmol/L, 2 μmol/L, 1 μmol/L) were diluted with MES Buffer (0.4 M 2-(N-morpholino)ethanesulphonic acid, 0.1 M phosphate and 2 mM EDTA, pH 6.0). Diluted sample concentrations were calculated using the equation obtained from the standard linear curve ($R^2=0.99$, SPSS for Windows 15.0). The obtained GSH values are multiplied by 2.1. Results were expressed in μmol/L.

2.8. Statistical analysis

SPSS for Windows 15.0. was used for statistical analysis. The normal distribution of the data was tested using Shapiro–Wilk Normality Test. The grouped data was compared with Chi square test while the measured values were compared with One-Way ANOVA (Post-Hoc Tukey HSD) and Independent Samples t test. Paired-Samples t test was used for dependent groups. Correlation analysis was conducted with Pearson Correlation and Spermann Correlation methods. A value of

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