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ORIGINAL PAPER

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Free radicals in patients with post-traumatic stress disorder

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Abstract There is mounting evidence indicating that reactive free radical species (FRs) are involved in initiation and development of many different forms of human pathologies including psychiatric disorders. In the present study, we aimed to determine whether antioxidant enzyme (glutathione peroxidase, GSH-Px; superoxide dismutase, SOD and catalase, CAT) activities and malondialdehyde (MDA) levels, a product of lipid peroxidation, were associated with post-traumatic stress disorder (PTSD). The study comprised 14 patients who had been diagnosed with PTSD according to DSM-IV criteria and met the admission criteria and 14 healthy controls. The activities of GSH-Px SOD, CAT and MDA were measured in both the patients and controls. In addition, all patients were assessed using the Clinician Administered PTSD Scale (CAPS). The mean GSH-Px, SOD, CAT activities and MDA levels of the patient group did not differ from those of the controls. However, in patients, the GSH-Px and SOD activities were significantly and positively correlated with CAPS scores, while there was a trend toward positive correlations between CAPS scores and MDA or CAT. In conclusion, our results suggest that the production of FRs does not seem to be related to PTSD.

Key words PTSD · antioxidant enzyme · MDA · lipid peroxidation

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Introduction

There is mounting evidence indicating that reactive free radical species (FRs) are involved in initiation and development of many different forms of human pathologies. Predominantly superoxide, hydroxyl ion and nitric oxide are produced under physiological conditions during aerobic metabolism (Mahadik and Mukherjee 1996). A small portion of free radicals have roles in physiological processes, the remaining are inactivated by antioxidant enzyme systems (Burton and Ingold 1989; Halliwell and Gutteridge 1990). FRs are produced by many different routes, such as activation of phagocytes and the general immune system, lipid peroxidation, the electron transport system in mitochondria, ischemia and trauma (Gutteridge 1995). FRs can be evaluated indirectly by the 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 (Leff 1994). When FRs are produced in excessive amounts or the enzymatic and nonenzymatic antioxidant defense systems are inefficient, some chain reactions causing cellular injury or even death of cells are activated (Stadtman 1992).

There are numerous studies indicating that free radical-mediated neuronal damage plays a role in the pathophysiology of schizophrenia and depression (Mahadik and Mukherjee 1996; Bilici et al. 2001). On the other hand, we have previously investigated FRs in obsessive-compulsive disorder (OCD) and found that OCD may be related to FRs (Kuloglu et al. 2002, in press). Post-traumatic stress disorder (PTSD) is classified under the anxiety disorders in DSM-IV. The oxidation of catecholamines such as dopamine and norepinephrine (NE) by MAO may result in an increased radical burden. In addition, because of determining an association, although controversial, between free radical and schizophrenia, and depression which have similar neuro-

anatomical areas and neurotransmitters (dopamine, serotonin and norepinephrine) in the etiopathogenesis (Coplan and Lydiard 1998), we aimed to examine the association between FRs and PTSD. Moreover, to the best of our knowledge, there has been no study evaluating the association between FRs and PTSD in the literature.

Patients and methods

The study comprised 14 patients (8 females, 6 males; age range, 18–49 years) who had applied to Firat University School of Medicine Department of Psychiatry and diagnosed with PTSD according to DSM-IV criteria and met the admission criteria. Written consent to participate in the study was obtained from the subjects after they were thoroughly informed about the research details. The research protocol was approved by the Local Ethics Committee of the Firat University School of Medicine.

All subjects were free of all medications at least in the previous two weeks. Each patient underwent diagnostic evaluation by one trained psychiatrist on the basis of a semistructured interview to determine DSM-IV diagnoses. The patients with any kind of axis I comorbidity were excluded. All subjects were evaluated by a semistructured questionnaire form which was arranged in accordance with clinical experience and available information sources and included gender, age, marital status, educational condition, socioeconomic status, duration of illness. In addition, the Clinician Administered PTSD Scale (CAPS) (Blake et al. 1995) was used.

Available 14 healthy control subjects according to exclusion criteria were chosen among the hospital staff. Controls were interviewed with the non-patient version of the SCID (SCID-NP) to exclude any axis I disorder (Spitzer et al. 1990).

Exclusion criteria included alcohol and substance abuse or dependence, presence of severe organic condition, users of any antioxidant agent (i. e. E and C vitamins), presence of epilepsy or a severe neurologic disorder, presence of an infectious disease, or excessive obesity.

Venous blood samples from a left forearm vein were collected into 5 ml vacutainer tubes containing potassium EDTA between 7 and 8 am after overnight fasting. Some hematological parameters (hematocrit, Hct; hemoglobine, Hb; mean corpuscular volume, MCV; mean corpuscular hemoglobine concentration, MCHC) were measured by using an autoanalyzer (Coulter Max M, Coulter Electronics Ltd, Luton, UK). The data on smoking were obtained from each patient using a questionnaire on the day before blood drawing. Smoking was not permitted after 23:00 h, one day before blood drawing.

The blood samples were centrifuged at 4000 rpm for 10 min at 4 °C to remove plasma. The buffy coat on the erythrocytes sediment was separated carefully after plasma was removed and was used in the assay of MDA levels. The erythrocyte sediment was washed three times with 10-fold isotonic NaCl solution to remove plasma remnants.

Hemolysates of erythrocytes were used to measure total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity levels by the method of Sun et al. (1988). This method is based on reduction of superoxide, which is produced by the xanthine oxidase enzyme system, by nitroblue tetrazolium. GSH-Px (EC 1.6.4.2) activity levels in hemolysates of erythrocytes were measured using the method of Paglia and Valentine (1967) in which GSH-Px activity was coupled to the oxidation of NADPH by glutathione reductase. CAT (EC 1.11.1.6) activity was determined by the method Aebi (1974). The principle of the assay is based on the determination of the rate constant k (units: s^{-1}) of the hydrogen peroxide decomposition. Levels of plasma MDA were measured by the thiobarbituric acid (TBA) method which was modified using the methods of Satoh (1978) and Yagi (1984). Peroxidation was measured as the production of MDA which in combination with TBA forms a pink chromogen compound whose absorbance at 532 nm was measured.

Obtained data were evaluated by SPSS Windows program 9.05 (SPSS, 1998) using Student-t test, chi-square, analysis of covariance (ANCOVA) and Pearson correlation tests. The confidence interval was accepted as $P < 0.05$.

Results

A total of 14 patients (8 females, 6 males), with a mean age of 32.48 ± 5.34 years (range, 18–41 years) were enrolled in this study. Control group (n = 14) had 7 females and 7 males, with a mean age of 29.88 ± 6.04 years; range 21–44). There were no significant differences in age or female/male ratio between the patients and controls ($P > 0.05$). The mean duration of illness for the patient group was 3.62 ± 2.55 years.

Table 1 shows the antioxidant enzymes and MDA measurements in patients with PTSD and healthy controls. No significant differences in any of the antioxidant enzyme activities or MDA levels were detected between the two groups ($P > 0.05$). Moreover, the ANCOVA did not reveal any significant difference in these variables between males and females ($P > 0.05$). However, in patients with PTSD, the GSH-Px and SOD activities were significantly and positively correlated with CAPS scores ($r = 0.52$, $P < 0.05$ for GSH-Px and $r = 0.55$, $P < 0.05$ for SOD) while there was a trend toward positive correlations between CAPS scores and MDA ($r = 0.41$, $P > 0.05$) levels or CAT activities ($r = 0.44$, $P > 0.05$).

There were no statistically significant differences between hematological parameters (Hct, Hb, MCV, and MCHC) of groups ($P > 0.05$).

There were also significant relationships between the duration of illness and SOD ($r = 0.52$, $P < 0.01$), CAT ($r = 0.39$, $P < 0.05$) activities and MDA ($r = 0.32$, $P < 0.05$) levels for the patient group.

Discussion

The present study shows that patients with PTSD did not differ from healthy controls in the measurements of antioxidant enzymes *activities* and MDA levels. However, in patients with PTSD, the GSH-Px and SOD *activities*

Table 1 Antioxidant enzyme and MDA levels in the patient and control groups*

Groups	GSH-Px (U g^{-1} Hb)	SOD (U g^{-1} Hb)	CAT (k g^{-1} Hb)	MDA (nmol^{-1} ml)
I. PTSD group (n = 14)	26.1±5.6	1003.2±182.1	265.9±34.9	2.9±1.6
II. Control group (n = 14)	24.9±4.3	988.9±91.1	272.3±40.4	2.7±1.1
I-II*	P > 0.05	P > 0.05	P > 0.05	P > 0.05

* used Student-t test

were significantly and positively correlated with CAPS scores, while there was a trend toward to positive correlation between CAPS scores and MDA.

In the previous studies, controversial results have been found in patients with various psychiatric disorders. Herken et al. (2001) who investigated the importance of the FRs in schizophrenia subtypes reported that oxidative stress might have a pathophysiological role in all subtypes of schizophrenia. In our previous study (2002), significant differences between lipid peroxidation product (MDA) and antioxidant enzyme (SOD and GSH-Px) activity levels in patients with schizophrenia and bipolar disorder compared with controls. In another study (Bilici et al. 2001), it has been suggested that patients with major depression, especially melancholic, were associated with the elevated antioxidant enzyme levels and lipid peroxidation. On the other hand, we have previously investigated FRs in another anxiety disorder, OCD, and found that OCD may be related to FRs (Kuloglu et al. 2002, in press). In addition, in our unpublished data, we detected that the patients with social phobia had considerably higher antioxidant enzymes and MDA levels compared with healthy controls (Atmaca et al. unpublished data). Thus, the findings we found in the present study add to the literature on biological differences between PTSD and other anxiety disorders, although they cannot exclude the possibility of free radical damage at least in a group of PTSD patients. In fact, we found that the GSH-Px and SOD activities were significantly and positively correlated with CAPS scores, while there was a trend toward positive correlations between CAPS scores and MDA. So, it may be suggested that a subgroup of patients with PTSD may be affected by FRs. Recently, the importance of oxidant/antioxidant imbalance was emphasized in free radical damage (Akyol et al. 2002). In this study that investigates both oxidant and antioxidant systems in the same plasma samples from schizophrenic patients, Akyol et al. suggested that oxidant/antioxidant imbalance might have a pathophysiological role in schizophrenia. Therefore, in future PTSD studies, we suggest that both oxidant and antioxidant systems in the same plasma samples should be dealt with, especially when taking into consideration the aforementioned correlative relations.

In conclusion, our results suggest that antioxidant enzymes and MDA values in patients with PTSD did not differ from those in healthy volunteers. Our results need to be confirmed by more comprehensive and detailed further studies to establish the findings we found in PTSD.

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