Oxidative Stress Markers in Patients with Post-Traumatic Stress Disorder

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ABSTRACT

Recent study data support the role of oxidative stress in diverse psychiatric disorders. Oxidative stress results from an oxidant/antioxidant imbalance, an excess of oxidants and/or a depletion of antioxidants. There are numerous studies that indicate that free radicals (FRs) damage neurons, and then play an important role in the pathophysiology of schizophrenia and depression. Active oxygen can cause considerable damage and disrupt the important physiological functions of proteins, lipids, enzymes and DNA. The aim of our study was to investigate the possible differences in the concentration of tromboxane B2, 8-OHdG and protein carbonyls, as significant markers of oxidative damage, and urate, albumin and total protein concentrations as antioxidative molecules in PTSD patients in comparison to the healthy control group. The study included 74 male participants who were active soldiers in the Croatian armed forces from 1991 to 1995. 46 subjects with chronic and current PTSD were recruited from the Department of Psychiatry of Dubrava University Hospital during 2010, 28 healthy subjects were recruited in the same period during the regular medical examination at the Dubrava University Hospital. Study results have shown that there is no statistically significant difference in urinary concentrations of 8-OHdG, serum thromboxane B2, and serum urates between two studied groups. Statistically significant difference of the protein carbonyl concentrations was examined. Concentrations were significantly lower in the PTSD group than in the control group. The clinical significance of these results was examined using ROC analysis. The obtained ROC curves did not separate the groups in a satisfactory manner.

Key words: *free radicals, 8-hydroxy-2'-deoxyguanosine, oxidative stress, post-traumatic stress disorder, protein carbonyls, thromboxane B2*

Introduction

Posttraumatic stress disorder (PTSD) is an anxiety disorder that occurs after exposure to a traumatic event, such as life-threatening experience, military experience, combat or natural disasters, terrorist attacks, serious accidents as well as physical or sexual abuse[1]. The highest prevalence of PTSD is among combat-exposed military personnel[2].

Epidemiological and clinical studies have shown that PTSD commonly occurs with other psychiatric disorders. The rate of comorbidity is especially high in combat-related PTSD[2–4]. The survey study of War Veterans includes Croatian war veterans with combat-related PTSD, showed that the 57 to 62% of patients met criteria for co-morbid diagnoses, approximately 2 to 4 years after the combat experiences. The most prevalent diagnoses were alcohol abuse, major depressive disorder, anxiety disorders, panic disorders and phobias, psychosomatic disorders, psychotic disorders, drug abuse, and dementia. Psychotic symptoms have been reported (to occur) for 30 to 40% of patients with combat-related PTSD2.

Because the diagnostic procedure of PTSD rely mostly on the subjective reporting of symptoms, clinical history, interview and mental condition, PTSD is most hard to malinger[5,6].

Clinicians need to have additional tools not only to enhance their ability to identify the patient at risk for PTSD, but also to dynamically monitor the clinical condition of individual PTSD patient during treatment. The

blood biomarker test has been considered as one of the potential convent tools to aid in PTSD diagnosis. Complexity of the molecular mechanisms of the disease and the undefined validation process makes this progress of the search for PTSD biomarkers slow and often frustrating, but advances in molecular biology, material science and coating technology create a most supportive environment for the investigation of the molecular mechanisms of PTSD and development of a biomarker test for PTSD. Biomarkers may indicate PTSD or PTSD characteristics, including the level or type response resulting from exposure to a traumatic stress, genetic susceptibility, genetic responses to traumatic stress exposure, markers of subclinical or clinical state, or indicators of response to therapy. These markers may dynamically alter during the course of PTSD development or differentially change after single or multiple traumatic stresses, respectively[7].

Stress results from an interaction between the mind (brain) and the body. The brain determines reaction to stress as it decides on what is stressful. It controls biological and physiological responses to stress. Stress causes neuroanatomical and neurochemical changes in the brain. Early traumatic experience in childhood may affect the brain structures and functions which may make a person prone to later development of PTSD or other anxiety-related disorder[8].

Vulnerability or resilience to stress may be a part of genetic profile. A long-term deregulation of the main stress mediators (cortisol and noradrenalin), favors the development of different anxiety disorders, including PTSD. Stress-induced atrophy (changes in hippocampus), amygdales (volume reduction) and prefrontal cortex are also frequent findings in patients with these disorders.

Dysregulation of noradrenergic, serotoninergic, dopaminergic, and other neurotransmitter systems cause the neurobiological changes in the brain, which provide the basis for psychopharmacologic treatment of patients with PTSD[8].

Recent study data support the role of oxidative stress in diverse psychiatric disorders. Oxidative pathophysiology in anxiety disorders is strongly supported by animal models, and also by human biochemical data. Most data demonstrating oxidative disturbances have examined indirect measures of oxidative status, such as peripheral and brain levels of antioxidants, oxidative enzymes and products[9].

Oxidative stress results from an oxidant/antioxidant imbalance, an excess of oxidants and/or a depletion of antioxidants. There are numerous studies that indicate that free radicals (FRs) damage neurons, and then play an important role in the pathophysiology of schizophrenia and depression[10].

In addition, there are studies that have attempted to find a correlation between free radical damage, schizophrenia and depression[11].

Under physiological conditions during aerobic metabolism, mainly superoxide, hydroxyl ions and nitric oxide are produced[12].

Small proportion of FR plays a role in physiological processes, while the remaining proportion is inactivated by antioxidant enzymes[13].

The imbalance between production of FRs and antioxidant capacity of the enzymes leads to oxidative stress[9,14]. Thus, oxidative stress is the consequence of the excessive production of FRs or the inefficiency of enzymatic and no enzymatic antioxidant defense system. Direct measurement of free radicals is hindered by their short half-lives and low levels in plasma (or biological fluids)[9].

Oxidative stress effects on cells are various: mutagenicity, cytotoxicity and stimulation of changes in gene expression. Active oxygen, though vital for human organism, can cause considerable damage and disrupt the important physiological functions of proteins, lipids, enzymes and DNA[10]. Thus, the FRs attacks the DNA by attacking deoxyribosyl backbone and form DNA lesions. The most important biomarker of oxidative DNA damage is oxidized DNA, 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is genotoxic and mutagenic[14]. Thromboxane B2 (TXB2) is often used as a marker of oxidative stress effect on lipids. It is a metabolic product of arachidonic acid which is formed by thromboxane A2 and is without biological activity[15]. Protein carbonyls are the most commonly used biomarkers of protein oxidation and their accumulation is associated with aging and the presence of various neurological diseases like Alzheimer's, Parkinson's and Huntington's disease[16].

Considering that FRs mediate neuronal damage in several psychiatric disorders, we wanted to investigate the possible difference in the concentration of tromboxane B2, 8-OHdG and protein carbonyls as significant markers of oxidative damage, and urate, albumin and total protein concentration as antioxidative molecules in PTSD patients in comparison to the healthy control group.

Materials and Methods

Subjects

The study included 74 male participants. They were active soldiers in the Croatian armed forces from 1991 to 1995. 46 subjects with chronic and current PTSD were recruited from the Department of Psychiatry of Dubrava University Hospital during 2010, 28 healthy subjects were recruited in the same period during the regular medical examination at the Dubrava University Hospital.

The diagnosis of PTSD and other psychiatric disorders was done by psychiatrists during comprehensive diagnostic evaluation using the Mini International Neuropsychiatric Interview (MINI)[17], Croatian version, based on DSM-IV criteria. Furthemore, healthy subjects were also assessed by the MINI.

Because the PTSD symptomatology changes during the course of the PTSD in our study we included veterans

with comorbid depression and anxious-depressive disorders, while veterans with other comorbid disorders were excluded.

The procedure was fully explained and all participants provided written informed consent. The study was approved by the Ethics Committee of the University Hospital Dubrava. Patients and healthy control group had body index mass (BMI) inside referral value.

The exclusion criterion was a history of any psychiatric disorders, comorbid substance abuse, and traumatic experience other than combat related experience in the PTSD and healthy control group.

The PTSD patients were treated with Selective serotonin reuptake inhibitors (SSRI drugs).

Blood samples were collected at the Clinical Department of Laboratory Diagnostics at University Hospital Dubrava, Croatia.

Materials

The levels of 8-OHdG were measured in the urine samples collected from patients and healthy controls. Tromboxane B2, protein carbonyl, total protein, albumin and urate levels were measured in sera samples collected from patients and controls.

Urine samples were frozen immediately after the delivery at the Clinical Department for Laboratory Diagnostics and sera was obtained following centrifugation at 1006xg for 15 minutes in a 35 R Rotina Hettich centrifuge (Tuttlingen, Germany), and stored at –80°C until analysis.

Methods

Total protein, albumin and urate concentrations were measured on Olympus AU2700 analyzer (Beckman Coulter, Tokyo, Japan).

The concentration of thromboxane B2 was determined using an enzyme immunoassay kit (Cayman Chemical Company, Ann Arbor, USA) according to the manufacturer's instructions.

The sera level of protein carbonyl was determined using an EIA kit (Cayman Chemical Company, Ann Arbor, USA) according to the manufacturer's instructions.

The level of 8-OHdG was estimated in urine samples using ELISA kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland) according to the manufacturer's instructions.

TABLE 1
BASIC CHARACTERISTIC FOR CONTROL GROUP AND PTSD GROUP

	PTSD group (n=46)	Control group (n=28)	P
Age (years)	43±6	39±7	0.001
8-OHdG urine	75.0 (29.0–320.0)	81.8±33.66	0.726
Tromboxane B2	80.37±16.05	82.55(42.80–330.90)	0.329
Protein carbonyls	15.32±5.45	36.28±23.4	<0.0001
Albumine	42.00 (10.00–49.0)	47.54±2.60	<0.0001
Urate	341.74±71.61	337.18±57.90	0.457
Total proteins	64.61±6.31	70.68±4.63	0.0001

Data that followed normal distribution are shown as mean ± SD, while data that did not follow normal distribution are shown as median (range)

Statistical methods

Normality of data distribution was tested by Kolmogorov-Smirnov test. Differences between groups were analyzed by Mann-Whitney U test. P values lower than 0.05 were considered statistically significant. Correlation between age, urinary concentrations of 8-OHdG, serum thromboxane B2 and protein carbonyl was tested using Spearman correlation coefficient. Chi-square test was used to test the difference in alcohol consumption and smoking between two tested groups. The clinical significance of differences was checked by the ROC curve. All statistical calculations were performed using the MedCalc 9.2.0.0. software (Mariakerke, Belgium).

Results

A total of 46 patients (males), with a median age of 42 years (range, 32–62 years) were enrolled in this study. Control group (n=28) included males, with a median age of 39 years (range 31–58).

Basic characteristic of the studied groups as well as the descriptive statistics for each studied marker are shown in Table 1.

χ^2-test showed no significant difference for smoking status between the groups (p=0.255). Sera concentra-

TABLE 2
ROC CURVE VALUES FOR DETERMINING THE DIAGNOSTIC SIGNIFICANCE OF THE OBTAINED PROTEIN CARBONYLS, TOTAL PROTEINS AND ALBUMINS RESULTS

Analyte	Area under ROC curve	Standard error	95% CI	Optimal cutoff value	Sensitivity %	Specificity %
Protein carbonyls	0.873	0.0415	0.775–0.939	23.86	93.48	67.86
Total proteins	0.777	0.0536	0.664–0.866	69	82.22	60.71
Albumin	0.887	0.0450	0.792–0.949	44	77.78	92.86

tions of protein carbonyls, albumins and total proteins were significantly higher in the control group as compared with a group of PTSD patients. Considering that we found statistically significant differences between the PTSD group and control group, we performed ROC curve analysis to assess the clinical significance of these differences (Figure 1, 2, 3). ROC curve values for protein carbonyls, total proteins and albumins are shown in Table 2.
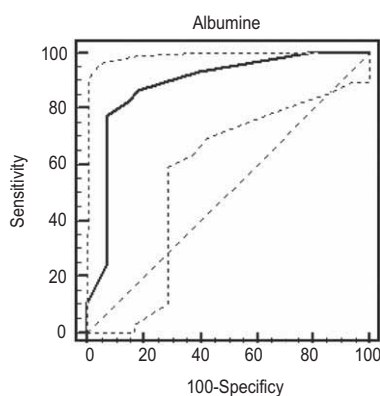

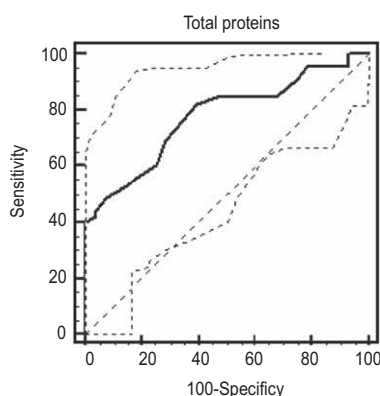*Fig. 1. ROC curves for albumin separating PTSD patients from control group.*

Fig. 2. ROC curve for total protein separating PTSD patients from control group.
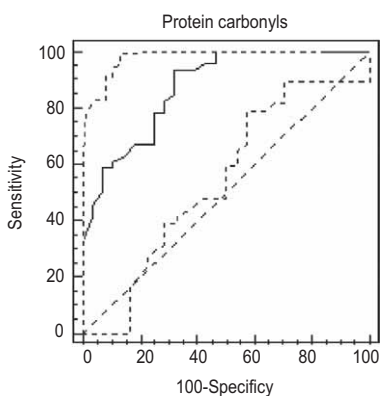

Fig. 3. ROC curve for protein carbonyls separating PTSD patients from control group.

Curves that divide the values of protein carbonyls, total proteins and albumin (Figures 1, 2, 3) PTSD group and control group with its optimal cutoff value (Table 2) determined sensitivity and specificity coordinates did not fully divided the groups as two separate entities.

Mann-Whitney U-test did not show statistically significant difference between values of urine 8-OHdG (p= 0.726) and tromboxane B2 (p=0.329) concentration among studied groups.

Discussion

Our study was focused on investigating the oxidative status in PTSD patients with multiple markers of oxidative stress. We wanted to assess the oxidative damage of proteins, lipids and DNA in PTSD patients. Study results have shown that there is no statistically significant difference in urinary concentrations of 8-OHdG, serum thromboxane B2, and serum urates between two studied groups (Results, Table 1). These results correspond to previously published data that showed no difference in antioxidant enzymes and MDA concentration[18]. However, statistically significant difference of the protein carbonyl concentrations was examined. Concentrations were significantly lower in the PTSD group than in the control group. The clinical significance of these results was examined using ROC analysis. The obtained ROC curves did not separate the groups in a satisfactory manner. The optimal cutoff value between the groups (Results, Table 2), according to the sensitivity and specificity data, would result in 6.52% of false negative and even 32.14% of false positive results for protein carbonyls, 17.78% of false negative results and 39.29% false positive results for total proteins, and 22:22 % false negatives and false positives 7.14% results for albumin. It is important to notice that the levels of total proteins and albumins in both groups were ranged within the reference ranges, so obtained difference has no clinical significance. Given that the proportion of protein carbonyls binds to the protein concentration, it is understandable that a statistically significant difference was obtained. Obtained sensitivity and specificity for protein canbonyls in ROC analysis, despite the higher values of area under the curve, supports the hypothesis that statistically significant differences do not imply clinical significance.

However, some studies showed that antioxidants as albumin, and uric acid were lower in patients with schizophrenia than in controls. According to those studies albumin, and uric acid were also significantly lower in neuroleptic-naive patients with first episode of schizophrenia[9].

The imbalance between FRs production and antioxidant capacity leads to the oxidative damage of proteins, lipids and DNA, and plays an important role in the pathogenesis of many diseases including psychiatric diseases[15].

The attack of free radicals on lipids leads to thromboxane B2 production and protein carbonyls are formed as a consequence of the FRs attack on proteins. As an at-

tack on nucleic acids, 8-OHdG is formed. DNA damage is considered an important pathogenic mechanism in cognitive impairments and dementia[10,14–16]. Literature data suggest that patients with anxiety disorders have increased levels of markers of oxidative stress, and that the regulation of free radicals metabolism and antioxidant capacity were altered in patients with schizophrenia when compared to healthy population[19–22]. It should be noted that the latest findings suggest that schizophrenia should be treated as autoimmune disease and that the altered oxidative and antioxidative status should be considered in relation to the etiopathogenesis of the disease[23].

It is well known that oxidants and antioxidants imbalance occurs in smokers, and alcohol consumptions[24]. The alcohol, cigarette smoke and aldehydes proved to be involved in the oxidation mechanisms of plasma proteins and raise 8-OHdG level[18,24,25]. For this reason the difference between the PTSD group and control group for smoking status was tested. For the same reason, patients and controls with a diagnosis of alcoholism were not included in this study. There was not statistically significant difference in smoking habbit beetwen PTSD patients and healthy control group.

Considering that first-generation antipsychotics treatment may affect lipid metabolism and exhibit prooxidant effect[26–28], while second-generation antipsychotics do not show such an effect and may have antioxidant effects[29], it would be useful to determine whether the type of therapy effects the results.

Our study did not include patients with different psychopharmacotherapy to make such an analysis, so it would be useful to determine the effect of the treatment type on oxidative stress markers in the future studies with a larger study group[30]. The markers of oxidative stress of PTSP patients were not significantly elevated in compare with control group, unlike the case in patients suffering of schizophrenia[13–15].

Thus, our data support the thesis that PTSD, as an acquired disorder, has a different ethiopatogenesis than other anxiety disorders that were not preceded by a traumatic event[27].

In conclusion, our results show that the markers of oxidative stress does not seem to be related to PTSD.

However, it is necessary to expand this research to a larger scale of patients, following different treatment outcome, in order to be able to define the clinical value of oxidative stress markers, especially protein carbonyl, as a possible PTSD diagnostic marker.

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MARKERI OKSIDATIVNOG STRESA U BOLESNIKA S POSTTRAUMATSKIM STRESNIM POREMEĆAJEM (PTSP)

S A Ž E T A K

Brojne studije podupiru hipotezu o utjecaju oksidativnog stresa u različitim psihijatrijskim poremećajima. Oksidativni stres rezultat je neravnoteže oksidansa i antioksidansa u organizmu, odnosno suvišak oksidansa i / ili nedostatak antioksidansa. Brojna istraživanja pokazuju da slobodni radikali oštećuju neurone i imaju važnu ulogu u patofiziologiji shizofrenije i depresije. Aktivni kisik može uzrokovati velike štete i poremetiti važne fiziološke funkcije proteina, lipida, enzima i DNK. Cilj našeg istraživanja bio je istražiti moguće razlike u koncentraciji tromboksana B2, 8-OHdG i proteinskih karbonila, kao značajnih markera oksidativnog oštećenja, te urata, albumina i ukupnih proteina kao antioksidativnih molekula, u PTSP bolesnika u odnosu na zdravu kontrolnu skupinu. U ispitivanju je sudjelovalo 74 muškaraca koji su bili aktivni vojnici u hrvatskim oružanim snagama od 1991 do 1995. Rezultati istraživanja su pokazali da ne postoji statistički značajna razlika u koncentracijama urinskog 8-OHdG, serumskog tromboksana B2 i serumskih urata među dvije ispitivane skupine. Utvrđena je statistički značajna razlika među ispitivanim skupinama u koncentracijama proteinskih karbonila. Koncentracije proteinskih karbonila su statistički značajno niže u PTSP nego li u kontrolnoj skupini. Klinička značajnost dobivenih rezultata ispitana je pomoću ROC analize, rezultati su pokazali da dobivena statistička značajnost nema dijagnostičku vrijednost.