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Serum selenium and plasma malondialdehyde levels and antioxidant enzyme activities in patients with obsessive–compulsive disorder

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ABSTRACT

There is mounting evidence indicating that reactive free radical species 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 serum selenium (Se), antioxidant enzyme (glutathione peroxidase, GSH-Px, superoxide dismutase, SOD, and catalase, CAT) activities, and plasma malondialdehyde (MDA) levels, a product of lipid peroxidation, were associated with obsessive–compulsive disorder (OCD). The participants were 28 patients with OCD that were drug-free at least for a month and a control group (n=28) of healthy subjects, matched with respect to age and sex. In both groups, the levels of the erythrocyte MDA, GSH-Px, SOD, Se, and the CAT were measured. The levels of MDA and SOD were statistically significantly higher ($p<0.01$, $p<0.05$ respectively) in patients than controls. The activities of CAT, GSH-Px, and serum Se levels were statistically significantly lower ($p<0.0001$, $p<0.001$, and $p<0.001$ respectively) in patients than controls. There was a positive correlation in patients between plasma GSH-Px activity and Se concentration (r=52, p=0.001). However, in patients with OCD, CAT and SOD activities were significantly and negatively correlated with MDA levels ($r=-0.45$, p=0.017 for CAT and $r=-0.54$, p=0.020 for SOD). The study shows the presence of a significant relationship of OCD and oxidative stress, and consequently, an involvement of free radicals and of the antioxidant defence.

1. Introduction

Free radicals and disorders of the antioxidant defence system have a pathogenic impact on human neuronal tissues and hence are seen as important factors in the development of various brain disorders (Dubinina and Prustygina, 2007; Kuloglu et al., 2002; Mahadik and Mukharjee, 1996). Free radicals are atoms or molecules with one or more unpaired electrons in their outer orbits and therefore have an extremely high reactivity. The main free radicals in human tissues are superoxide, hydroxyl, hydrogen peroxide, singlet oxygen, and nitric oxide (Gutteridge, 1995). Free radicals are produced in the normal cell metabolism, in biochemical reactions involving oxygen, for the purpose of destroying bacteria and other living organisms taken into the cell by phagocytosis. However, they may also be produced excessively by exposure to radiation, tobacco, and other pollutants and with hyperoxia, excessive exercise, and ischemia. Excessive concentration of free radicals in the cell environment may lead to cell damage and death. This damage may be prevented or alleviated by the presence of antioxidant molecules (Cheeseman and Slater, 1993).

Malondialdeyde (MDA) is one of the final decomposition of lipid peroxidation and it is also formed as a product of the cyclooxygenase reaction in prostaglandin metabolism. Free radicals have a relatively short half-life, and, as a consequence, the measurement of their levels is difficult. Free radicals can be evaluated indirectly by products of lipid peroxidation such as MDA or by the measurement of some antioxidant enzyme levels such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) or by some transition metal levels such as selenium (Tezcan et al., 2003).

Selenium (Se) is present in biological systems as selenoproteins, which characteristically are oxidoreductases. These selenoenzymes have a variety of activities (Kryukov et al., 2003) and many of them, including the glutathione peroxidases and the thioredoxin reductases, have oxidant defense functions. Selenoproteins have been recognized as modulators of brain and signaling (Rayman, 2000). Under conditions of selenium deficiency, tissue levels of these enzymes fall and oxidative stress conditions develop (Yang et al., 1989; Burk, 2006). This increases the susceptibility of cells to certain types of oxidative

Abbreviations: Se, Selenium; MDA, malondialdehyde; GSH-Px, Glutathione Peroxidase; SOD, superoxide dismutase; CAT, catalase; OCD, obsessive–compulsive disorder; NBT, nitroblue tetrazoluim.

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damage and death (Burk and Lane, 1983; Hill et al., 2004). Therefore, maintenance of the serum selenium level, a reflection of selenoprotein content, is important to brain cell function and survival.

There are numerous studies indicating that free radical-mediated neuronal damage plays a role in the pathophysiology of the schizophrenia and depression (Mahadik and Mukharjee, 1996; Bilici et al., 2001). On the other hand, obsessive–compulsive disorder (OCD) may be related to free radicals (Kuloglu et al., 2002; Ersan et al., 2006). Oxidative stress has a detrimental impact on central nervous system. A variety of factors associated with excessive concentrations of free radicals adversely affect the brain metabolism. Research data suggest that an extensive oxidative stress has a substantial and detrimental effect on the brain due to several reasons (Mahadik et al., 2001; Jesberger, 1991; Weber, 1994). Human brain uses high amounts of oxygen (20% of human body's total oxygen consumption occurs within the brain) and has a high percentage of phospholipids that can easily be peroxidized (they are sensitive to damage via free radicals). Neuronal DNA damage in the adult brain cannot be completely repaired. Furthermore, although there is a large concentration of iron in the brain, there are lower levels of antioxidants (particularly catalase). It has been reported that basal ganglia are particularly vulnerable to damage by free radicals due to the large concentration of catecholamines in this region of the brain. Human brain is also exposed to increased levels of free oxygen radicals from environmental factors and this stress also includes reperfusion secondary to pollution, including cigarettes. According to the degree of oxidative stress and its particular timing, oxidative damage in the brain can be a causative factor in abnormal neuronal development, e.g., in the neuronal degeneration or neuronal membrane thinning (Mahadik et al., 2001).

Obsessive–compulsive disorder is characterised by salient and recurrent obsessions and/or compulsions (American Psychiatric Association, 2000). The role of free radicals, Se, and antioxidant enzymes in the OCD has not yet been adequately explored. In this study, we attempted to demonstrate the status of oxidative stress and antioxidant defense mechanism by investigating serum Se, GSH-Px, SOD, CAT, and MDA levels in patients with obsessive–compulsive disorder.

2. Materials and methods

2.1. Subjects

This study was conducted by the collaboration of the Department of Physiology, Medical Faculty of Cumhuriyet University, the Deparment of Chemistry Engineering Faculty and the State Hospital and approved by the ethical committee of the Cumhuriyet University Medical School. The study group comprised 28 patients (18 females, 10 males) who had applied to the Cumhuriyet University School of Medicine Department of Psychiatry and diagnosed with OCD according to DSM-IV criteria and met the admission criteria. The control group (n=28) was composed of local personnel and students found to be healthy in physical and psychiatric examinations. The controls were matched with the patients with respect to age and sex.

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 fallows: 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, and obesity.

2.2. Detemination of plasma MDA levels

Blood samples (10 ml) were drawn from the patients and normal controls and placed in heparinized and sterile (gel) tubes. To measure the level of malondialdehyde in blood samples, the erythrocyte sedimentation rate was determined. Phosphate buffer (pH=7.4) was used for this test. The blood in the heparinized tubes was mixed slowly by upward and downward rotation. The samples were then placed in the centrifuge at 1000 ×g for 10 min. The method of determining lipid peroxidation was based on the fact that MDA, i.e., the specific product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to form a coloured complex that gives maximum absorption at 532 nm wave length (Jain, 1988).

2.3. Measurement of GSH-Px activity levels

GSH-Px activity levels in haemolysates of erythrocytes were measured using the method of Paglia and Valentine in which GSH-Px activity was coupled to the oxidation of NADPH by glutathione reductase (Paglia and Valentine, 1967). 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 1 U ml^{-1} of glutathione reductase. The absorbance at 340 nm was recorde 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.

2.4. Determination of SOD activity in erythrocyte

Total (Cu–Zn and Mn) superoxide dismutase (SOD, EC 1.15.1.1) activity was determined according to the method of Sun et al. (1988). The principle of the method is based on the inhibition of nitroblue tetrazoluim (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the sample after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. Activity was expressed as units per gram (U g^{-1}) Hb.

2.5. Measurement of CAT activity levels

Catalase (CAT, EC, 1.11.1.6) activity was determined by Aebi's method (Aebi, 1974). The principle of the assay is based on the determination of the rate constant k (dimension: s^{-1}) of the hydrogen peroxide decomposition. By measuring the absorbance changes per minute, the rate constant of the enzyme was determined. Activities were expressed as kg^{-1} Hb.

2.6. Determination of serum selenium

Selenium was determined by atomic absorption spectrometry (AAS) using a 1100 Perkin Elmer atomic absorption spectrometer with a mercury hydride system (Welz et al., 1983).

2.7. Instruments

2.7.1. Sociodemographic information form

All subjects were evaluated by a semi-structured questionnaire form which was arranged in accordance with clinical experience and

Table 1
Demographic and clinical features of the patients and the controls

Variable	Patients	Controls	*p value*
Number	28	28	–
Age, years	28.28±5.41	28.85±5.54	N.S.
Sex (M/F)	10/18	10/18	N.S.
Smoking (±)	5/23	4/24	N.S.
Y-BOCS score:	28 22.47±5.39		
–Mild OCD	5 (17.8%)		
–Moderate OCD	11 (39.2%)		
–Severe OCD	9 (32.1%)		
–Very severe OCD	3 (10.7%)		

N.S.: non significant. Values are given as the means±SD.