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Serum Antioxidant Vitamins and Malondialdehyde Levels in Patients with Obsessive-Compulsive Disorder

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Abstract

Objectives: Free radicals have been implicated in the pathophysiology of some psychiatric disorders. To examine the role of free radicals in the etiopathogenesis of obsessive-compulsive disorder (OCD), we aimed to assess whether the antioxidant vitamins (E, C and A) activity and malondialdehyde (MDA) levels were associated with OCD.
Methods: 48 OCD patients diagnosed according to DSM-IV and 48 healthy volunteers as control group were included in this study. Serum levels of vitamin E and A were determined using RP-HPLC, whereas vitamin C was estimated by phenyl-hydrazine spectrophotometric method. MDA level was measured using UV-VIS spectroscopy.
Results: Significant differences in serum vitamin E and C levels were observed between the OCD and control groups ($p<0.05$). MDA levels were found significantly higher in OCD subjects ($p<0.05$).
Conclusion: Our study found an overall imbalance in antioxidant vitamins level in OCD patients, which may have potential role in etiopathogenesis of the disease process (German J Psychiatry 2012; 15 (1): 10-14).

Keywords: antioxidants, malondialdehyde, OCD, vitamins

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Introduction

Oxidative stress and free radicals induced toxicity have been implicated in the pathogenesis of various neuropsychiatric disorders including obsessive–compulsive disorder (OCD) (Bilici et al., 2001; Ersan et al., 2006; Mahadik et al., 1996). Free radicals are produced in the human body during normal cellular metabolism, lipid peroxidation, activation of phagocytes, electron transport system in mitochondria, and after exposure to ultraviolet (UV) light, cigarette smoke, and pollutants (Gutteridge, 1995). Disturbance of the balance between the production of free radicals and antioxidant defenses may result in tissue damage (Cheeseman and Slater, 1993). Since free radicals have a relatively short half-life with an extremely high reactivity, determination of their levels is difficult. However free radical induced damage may be prevented or alleviated by the presence of antioxidant molecules (Butterfield et al., 2002). Therefore, activity of free radicals in the human body can be examined indirectly by the measurement of some antioxidant enzymes (SOD, catalase, or glutathione peroxidase), antioxidant vitamins (Vitamin E, C, and A), transition metals (copper, zinc, and iron), by-products of lipid peroxidation such as malondialdehyde (MDA) (Leff, 1994; Tezcan et al., 2003).

Malondialdehyde (MDA), a naturally occurring end product of membrane lipid peroxidation, is one of the most frequently used biomarker for free radical mediated damage (Janero et al., 1990; Nielsen et al., 1997). Vitamins E, C and A, non-enzymatic antioxidant structures are essential for neurological antioxidant and neuroprotective function (Bates, 1995; Dennert and Lotan, 1978; Diliberto and Allen, 1981; Stephensen, 2001). Several studies showed that vitamin E, C and A are important for the central nervous system and a

decrease in their concentrations causes structural and functional damage to the cells (Chow, 1991; Harrison and May, 2009; Kaplan et al., 2007; Martin et al., 2000; Packer, 1991).

Obsessive-compulsive disorder (OCD), a chronically debilitating neuropsychiatric disorder with 2%–3% population prevalence, is characterized by recurrent obsessions and/or compulsions (American Psychiatric Association, 2000; Karno et al., 1988). Although the biochemical alterations in the pathogenesis of neuropsychiatric disorders is still not clearly known, changes in the level of antioxidant vitamins may play important roles in the obsessive compulsive disorder (OCD). For example alteration in serum level of vitamin A (retinol), vitamin-E (tocopherol) and vitamin C (ascorbic acid) has been reported in various neuropsychiatric disorders (Maes et al., 2000; Miljevic et al., 1997; Rinaldi et al., 2003; Smith et al., 2000). Since the role of antioxidant vitamins in the pathological conditions of OCD has not yet been adequately explored, therefore in this study we attempted to see the status of oxidative stress and antioxidant defense mechanism by investigating serum levels of antioxidant vitamins (vitamin E, C and A) and MDA levels in OCD patients.

Materials and methods

Selection of cases and controls

This study was conducted by the department of Clinical Pharmacy and Pharmacology, University of Dhaka, Dhaka, Bangladesh in collaboration with the department of Psychiatry, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. The study was approved by the ethical committee of the BSMMU.

The study group comprised of forty-eight OCD patients (42 males and 6 females) who were diagnosed according to Diagnostic and Statistical Manual of Psychiatric Disorders, DSM-IV. These patients were randomly recruited from BSMMU by a specialist psychiatrist who was trained in DSM-IV. The control group (n=48) comprised of sex, age, and socioeconomic status matched healthy volunteers (42 males and 6 females). The study subjects were briefed about the purpose of the study and written consent was obtained from each of them.

All subjects had to go through physical and neurological examination to find out existence of other diseases. These subjects did not have liver or kidney failure or other diseases nor had they been treated with any antioxidant agent (i.e. vitamin E, C and A), which can interfere with concentration of antioxidant vitamins. Patients who were mentally retarded and suffered from co-morbid psychiatric disorders were also excluded from the study. Other exclusion criteria were as follows: alcohol and substance abuse or dependence, tardive dyskinesia related to neuroleptics, presence of severe organic condition, presence of epilepsy and severe neurologic disorder, presence of infectious disease and excessive obesity.

All subjects were evaluated by a semi-structured 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 and severity of the illness. The Yale-Brown Obsessive Compulsive Scale (Y-BOCS) was used to assess the severity of OCD without focusing on the contents of obsession and compulsion (Goodman et al., 1989). It has 10 items and each one is assessed by a clinician on a scale of 1 to 4.

Blood Sampling and storage

In total, 10 ml of venous blood was drawn from each of the OCD patients and the control subjects using a plastic syringe fitted with a stainless steel needle. The blood sample was collected into a sterile tube and allowed to clot at room temperature for half an hour and then centrifuged at 3000 rpm for 15 minutes. Serum samples were stored at −80°C and protected from light until analysis.

Measurement of vitamin A, E and C

Reversed-phase HPLC (LC-10AD, Shimadzu, Japan) with UV-VIS detector (λ_{max} set at 291 nm) was used for simultaneous determination of serum vitamin A (retinol) and E (α-tocopherol) as described by Bieri (Bieri et al., 1979). The analytes retinol and α-tocopherol were isolated from the serum by liquid-liquid extraction using n-hexane, concentrated by evaporation under nitrogen stream and reconstituted with HPLC-grade ethanol. From the reconstituted sample 20 µl was injected into chromatography on a C18 Nucleosil column (5 µm, 4.6 X 250 mm) with acetonitrile **:** methanol (75 **:** 25) mobile phase flowing at 1ml/min. Standards (retinol, α-tocopherol and α-tocopheryl acetate) were purchased from Sigma Chemical Co., USA and solvents (HPLC grade) were obtained from Merck (Darmstadt, Germany).

The concentration of vitamin C in the serum was estimated by the phenyl-hydrazine spectrophotometry method (Lowry et al., 1945). For the measurement procedure, 0.3 ml of plasma was added to 1.2 ml of 5% trichloroacetic acid and then centrifuged at 3000 rpm for 10 min. Clear supernatant of 0.96 ml was treated with 0.4 ml of dinitrophenylhydrazine-thiourea-copper sulphate (DTC) reagent and heated at 60°C for 1 hr in a water bath. Immediately after incubation, the sample was chilled in ice-cold water and 1.6 ml of 65% sulphuric acid solution was added gradually. The procedure was repeated with 0.3 ml of working standard solution of ascorbic acid as well as with 0.3 ml of reagent blank. Absorbance of sample and standard was read against reagent blank at 520 nm in the spectrophotometer (UV-1201, UV-VIS Spectrophotometer, Shimadzu Corporation, Japan).

Measurement of MDA

Serum MDA levels were measured according to the modified method described by Satoh and this method is based on the principle that MDA, i.e., the specific product of lipid peroxidation reacts with thiobarbituric acid (TBA) to form a

colored complex that gives maximum absorption at 532 nm wave length (Satoh, 1978; Yagi, 1984). 0.5 ml of serum was added to 2.5 ml of 20% trichloroacetic acid, allowed to stand for 10 min at room temperature. After centrifugation at 3,500 rpm for 10 min, the supernatant was decanted and dispersed with 2.5 ml of 0.05 M sulphuric acid. Then 3.0 ml of 2- thiobarbituric acid (0.07% in 1 mol/l Na_2SO_4) were added to this precipitate, and the coupling of lipid peroxide with TBA was carried out by heating in a boiling water bath for 30 min. After cooling in cold water, the resulting chromogen was extracted with 4.0 ml of n-butyl alcohol by vigorous shaking. Separation of the organic phase was facilitated by centrifugation at 3,000 rpm for 10 min, and absorbance of the supernatant was measured spectrophotometrically (UV-1201 Spectrophotometer; Shimadzu Corporation, Japan) at 530 nm using 1,1,3,3-tetraethoxy-propane as standard. Thiobarbituric acid reactive substances (TBARS) were expressed as μmol/l.

Data analysis

The SPSS software package (Version 11.0) was used to analyze the data. Descriptive statistics were used for all variables. Values were expressed as mean ± standard deviation. Comparison of the mean values on the key variables of patient and control groups was performed by independent sample t-test. Pearson correlation coefficients were calculated to evaluate the magnitude of the relationships among the various parameters. p values of <0.05 were considered significant.

Results

Subjects

Table 1. Socio-demographic characteristics and clinical features of the patients and the controls. N.S. = not significant

	Patients	Controls	p value
Number	48	48	-
Age, years, means ± SD	24.7±4.6	21.3±1.0	N.S.
Sex (Male/Female)	42M/6F	42M/6F	N.S.
BMI (kg/m^2), means ± SD	22.4±3.9	22.2±2.5	N.S.
Case:			
New	12 (25%)		
Old	36 (75%)		
Duration of disease:			
< 5years	30 (62.5%)		
(5-10) years	8 (16.7%)		
> 10 years	10 (20.8%)		
Y-BOCS score:			
Mild OCD	16 (33.3%)		
Moderate OCD	18 (37.5%)		
Severe OCD	14 (29.2%)		

In this experiment the sample size was 96 (48 for obsessive compulsive disorder patients and 48 for control healthy volunteers). Socioeconomic data of both OCD patients and controls, duration of the disease and disease severity score (YBOCS) of OCD patients are presented in Table 1. Difference between the mean BMI of the patients and controls was not significant.

Results of MDA and Antioxidant Vitamins Levels

Serum vitamin E levels in the OCD and the control groups were 5.2±2.8 μmol/l and 7.7±1.8 μmol/l respectively. Vitamin E levels were significantly higher in the controls compared to the OCD group ($p<0.01$). In the control group vitamin C levels were 30.1±4.3 μmol/l compared with 23.0±2.2 μmol/l in the OCD group. Vitamin C levels were significantly higher in the controls compared to the OCD group ($p<0.001$). Vitamin A levels in the OCD and the control groups were 3.6±1.4 μmol/l and 4.0±1.6 μmol/l respectively. Although the OCD group had slightly lower vitamin A levels compared with the control group, the difference was not statistically significant ($p>0.05$). To evaluate the levels of lipid peroxidation, MDA levels were determined in the control group and OCD group. MDA levels in the OCD and control group were 5.7±1.7 μmol/l and 4.3±1.2 μmol/l respectively. MDA levels in OCD group were significantly higher than the controls ($p<0.01$) (Table 2). Correlation analysis among the vitamin E, vitamin C, vitamin A, MDA, age, BMI and YBOCS showed no significant correlations between these parameters both in the patient group and control group.

Discussion

Vitamin E and C are essential for neurological functions. Vitamin A increases the antioxidant potential of the tissues (Kartha and Krishnamurthy, 1977; Ricciarelli et al., 2007). This fact, together with a growing body of data indicates that alterations in the levels of antioxidants vitamins are associated with oxidative stress leading to various neuropsychiatric disorders. In the last several years, there has been growing evidence that alterations in the serum antioxidants level may be directly implicated in the pathophysiology of OCD (Ersan et al., 2006; Kuloglu et al., 2002; Maes et al., 2000; Selek et al., 2008).

In the present study, vitamin E, C levels were significantly lower in OCD patients as compared to controls ($p<0.05$). Ersan et al., also found significantly lower levels of vitamin E in OCD patients (Ersan et al., 2006). Oxidative stress is associated with an increased free radical burden, and to protect the body from free radical induced cell damage, antioxidant vitamins E, C and A, glutathione (GSH), certain trace elements, metalloenzymes, such as the selenium containing glutathione peroxidase, the iron-containing catalase, and superoxide dismutase(SOD) exhibit protective role (Machlin and Bendich, 1987). Several studies have found

Table 2. Antioxidant vitamins and MDA levels in controls and OCD patients

	Controls	OCD	p value
Vitamin E (µmol/l)	7.7±1.8	5.2±2.8	0.001
Vitamin C (µmol/l)	30.1±4.3	23.0±2.2	0.000
Vitamin A (µmol/l)	4.0±1.6	3.6±1.4	0.417
MDA (µmol/l)	4.3±1.2	5.7±1.7	0.003

significant relationship between OCD and oxidative stress suggesting an involvement of free radicals and the antioxidant defense system (Behl et al., 2010; Chakraborty et al., 2009; Özdemir et al., 2009; Selek et al., 2008). Vitamin A exerts antioxidant protection to the tissues against oxidative free radical attack (Bates, 1995). No previous studies have examined vitamin A concentrations in patients with OCD. In our study, we found lower levels of vitamin A in the OCD patients than the controls; however the difference was not statistically significant.

In this study, serum MDA levels were significantly higher in OCD patients than controls ($p<0.05$). These results suggest higher free radical metabolism in OCD and therefore indicate some extent of tissue damage due to oxidative stress. These findings were consistent with other studies where higher levels of MDA in OCD patients were reported (Behl et al., 2010; Ersan et al., 2006; Kuloglu et al., 2002; Selek et al., 2008).

In conclusion, our findings indicate significantly lower levels of vitamin E and C, and higher levels of MDA in OCD patients compared to the controls. We found an overall imbalance of antioxidant vitamins that may have important role in etiopathogenesis of the disease process.

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