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Evaluation the Activity of Enzyme Glutathione Peroxidase, Oxidative Stress Index and Some Biochemical Variables in Serum of Individuals with Obsessive-Compulsive Disorder (OCD)

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Abstract

Aim and background: Obsessive-Compulsive Disorder (OCD) is a type of mental disorder that causes discomfort and anxiety among those who suffer from this. The aim of this study was to assess glutathione peroxidase enzyme activity, oxidative stress index, and other biochemical parameters in the serum of persons with OCD.

Material and methods: In this study, the patient group consisted of 26 people with OCD, and the control group consisted of 26 healthy people. The parameters glutathione peroxidase (GSH-Px), Oxidative Stress Index (OSI), Selenium (Se), Catalase (CAT), Total Antioxidant Status (TAS), Superoxide Dismutase (SOD), Malondialdehyde (MDA), and Total Oxidant Status (TOS) were evaluated in both groups.

Results: Se, CAT, GSH-Px, and TAS were significantly higher in the healthy group than the OCD patient group ($P<0.05$), whereas other parameters such as SOD, MDA, TOS, and OSI were significantly higher in OCD patients than the healthy (control) group ($P<0.05$).

Conclusion: Depending on the results of this study, it may be concluded that a psychiatrist can determine the level of oxidative stress in a person's brain and predict suitable treatment by evaluating the amount of MDA in their plasma. Furthermore, assessing overall oxidative and antioxidant status as well as the oxidative stress coefficient is the best way to assess the overall oxidative status among healthy people, and thus this approach can prevent people from entering the OCD stage. Increasing the amount of selenium in food, relaxation, yoga, walking (jogging), and also breathing clean (healthy) air can have a very positive effect on reducing oxidative stress, which can be very effective in preventing healthy people from getting OCD.

Keywords: Psychiatrist • Disorders • Central nervous system

Introduction

Psychiatric disorders have a specific position in today's psychiatry, and over time, the connection between physical disease and the human psyche becomes more obvious, and more focus is required on the psychological difficulties in order to better treat these disorders [1]. Among patients with mental disorders, patients with Obsessive-Compulsive Disorder (OCD) refer to non-psychiatrists more than others [2]. OCD involves persistent, repetitive thoughts, images, or impulses that are disturbing to the person and cause anxiety and distress (obsession), and in response to an obsession, the person performs repetitive behaviors or certain mental actions (compulsion) [2]. These mental or practical obsessions are so severe enough to cause significant distress in the ordinary course of his/her life, including his/her work function, typical social activities, and even his/her personal relationships [3].

In diseases of the Central Nervous System (CNS), oxidative stress is the most important pathophysiological process. The free radicals produced during this process can cause extensive damage to the brain. Biochemically, lipids are very important in the brain and play a very important role in its function [4].

The brain can receive around 20% of the oxygen that enters the body, and since this consumed oxygen has the potential to produce free radicals, the production of these radicals in the brain increases as a result of disease, inducing the peroxidation of important lipids in the nervous system. Also, high amounts of free radicals cause DNA damage in the brain. Studies have shown that low levels of iron in the brain cause a lack of antioxidants in the brain. The basal ganglia have also been confirmed to be sensitive to free radicals due to the presence of catecholamines. Risk factors such as smoking also lead to an increase in free radicals and brain damage [5].

Anxiety and stress are an integral part of the life of some individuals in society and this high anxiety in susceptible people can lead to significant psychological and physical damage; as mentioned above, the presence of anxiety in individuals' jobs can lead to diseases such as depression and obsession [6-9]. Checking antioxidants and oxidative stress markers can predict the physical damage caused by anxiety, and it can be used to identify susceptible individuals and assess their progression if they have the disease. Furthermore, using diagnostic tests to detect a disease or disorder earlier can help to prevent it from progressing. Even the presence of a complementary test along with other psychological symptoms of the disease can assist to treat the person more effectively [10-12].

Based on the above-mentioned outline, the aim of the current study was to evaluate the activity of plasma Glutathione Peroxidase (GSH-Px), Oxidative Stress Index (OSI) and some biochemical parameters including

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Selenium (Se), Catalase (CAT), Total Antioxidant Status (TAS) of plasma, Superoxide Dismutase (SOD), Malondialdehyde (MDA) and Total Oxidant Status (TOS) in people with OCD and healthy individuals.

Materials and Methods

Sampling

In this study, 26 persons with OCD were selected as the patient group and 26 healthy individuals as the control group. Each participant in the study reads and fills a special consent form, confirming their agreement with their decision to participate in this study. A professional psychiatrist assessed each of the control and patient group for neurological, psychological, and physical health issues. Liver and renal function tests were performed for each of the two groups. People with characteristics such as alcohol consumption, antioxidant (e.g. vitamins E and C) usage, the presence of epilepsy and other neurological disorders, the presence of infectious diseases and severe obesity were excluded from the patient.

Malondialdehyde (MDA)

Following exams and receiving each selected individual's agreement, about 10 ml of blood was taken from each patient and healthy person, transferred to a sterile heparinized tube, centrifuged, and the plasma separated. To determine plasma MDA levels, the Erythrocyte Sedimentation Rate (ESR) was also measured. For this experiment, phosphate buffer (pH=7) was used. Rotation was used to completely mix the blood samples, and then the samples were centrifuged for 10 minutes at 1000 rpm. The MDA detection technique is based on the reaction of MDA with Thiobarbituric Acid (TBA) as a specific lipid peroxidation product, with the dye product being detected at 532 nm [13].

Plasma glutathione peroxidase (GSH-Px)

The Valentine method, in which GSH-Px activity is coupled to βNicotinamide Adenine Dinucleotide (NAPDH) oxidation by glutathione reductase, was used to measure GSH-Px activity in erythrocyte hemolysis [14]. Nicotinamide Adenine Dinucleotide Phosphate (NADPAH) oxidation is measured spectrophotometrically at 340 nm at 37°C. 1 mM EDTA, 0.2 mM NADPH, 1 mM NaN3, 1 mM glutathione, and 1 U/mL Reductase were used in the process. Finally, the absorbance was measured for 5 minutes at 340 nm. The activity of the enzyme glutathione peroxidase is equal to the linear slope in millimoles of NAPDH, which is oxidized per minute, and finally the results were expressed as U g-1 Hb.

Measurement of SOD activity in erythrocytes

Total Superoxide Dismutase (SOD) activity was measured based on the method of Sun et al. [15]. The basis of this method is based on resuscitation inhibition of Nitroblue Tetrazolium (NBT) with xanthine-xanthine oxidase system. SOD activity in the ethanol phase of the samples was performed after adding one ml of ethanol/chloroform mixture (V:V=5:3) to the same volume of the centrifuged sample, and finally the amount of SOD activity was expressed as U g-1 Hb.

Measurement of CAT activity in erythrocytes

The Aebi method was used to determine Catalase (CAT). The principle of this method is that by measuring the constant velocity K (with dimension S-1) related to the decomposition of hydrogen peroxide is determined. By measuring the absorption changes per minute, the enzyme velocity constant is determined [16]. Finally, the amount of CAT activity determined will be expressed as U g-1 Hb.

Measurement of selenium

Selenium was measured using an Elmer-Perkin 1100 atomic absorption spectrometer with a mercury hybrid system, and finally the measurement was reported in µg/l [17].

Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) of Plasma

TOS and TAS in plasma were measured using a new colorimetric method developed by Erel [18]. The Fenton reaction produces hydroxyl (the strongest bio-radical), which reacts with the colorless compound o-Dianisidine to form a yellowish-brown dianisyl radical. As the plasma sample increases, the oxidative/initiating reaction with the hydroxyl radicals present in the mixture is inhibited by the plasma antioxidant constituent and prevents discoloration. The total antioxidant is accurately measured with this method. Finally, the results are given in millimoles per liter (mMol/l).

Determination of Oxidative Stress Index (OSI)

The ratio of TOS to TAS is referred to as OSI, which is equal to the equation (1)

$$\mathrm{OSI} = \frac{\mathrm{TOS\ (mMol/L)}}{\mathrm{TAS\ (}\mathit{mM}\ \mathrm{Trolox\ Eq/L)}} \quad equation\ (1)$$

Collection of demographic information

A semi-structured questionnaire was used to collect demographic information about patients and healthy individuals, such as age, sex, marital status, educational status, economic status, social status, and disease duration.

Yale–Brown Obsessive Compulsive Scale (Y-BOCS)

The Yale-Braun scale was used to estimate the severity of OCD regardless of obsessive-compulsive content, which consists of 10 items. In addition, a professional psychologist graded each individual (healthy or diseased) on a scale of one to four.

Statistical analysis

SPSS-ver21 software was used for data analysis. The mean value of key variables was compared between the two groups using two sample T-test at a significant level (α=0.05). In addition, the Pearson correlation coefficient was used to estimate the connection between parameters at a significant level (α=0.05).

Results and Discussion

In this study, about 26 people with OCD and 26 healthy people (without a history of disease) were studied. These two groups are considering age- and sex-matched. There were no statistically significant differences in age, sex, or smoking among these individuals. Table 1 and Figure 1 provide the demographic information and findings of the study population. Table 2 and Figures 2 and 3 also included clinical results for both the patient and control groups.

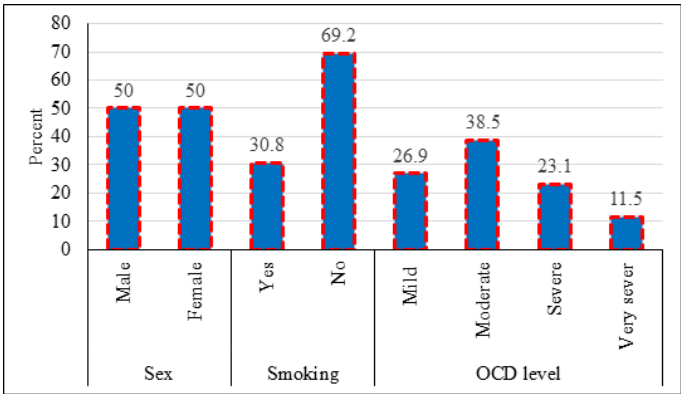

Figure 1. The demographic information related to individuals with OCD.

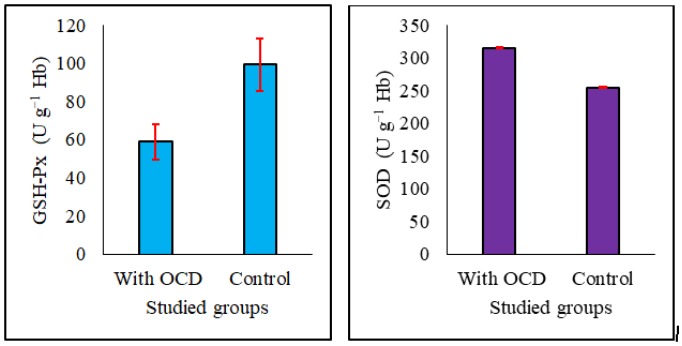

Figure 2. The comparison of Se, CAT, GSH-Px and SOD between OCD group and control group.

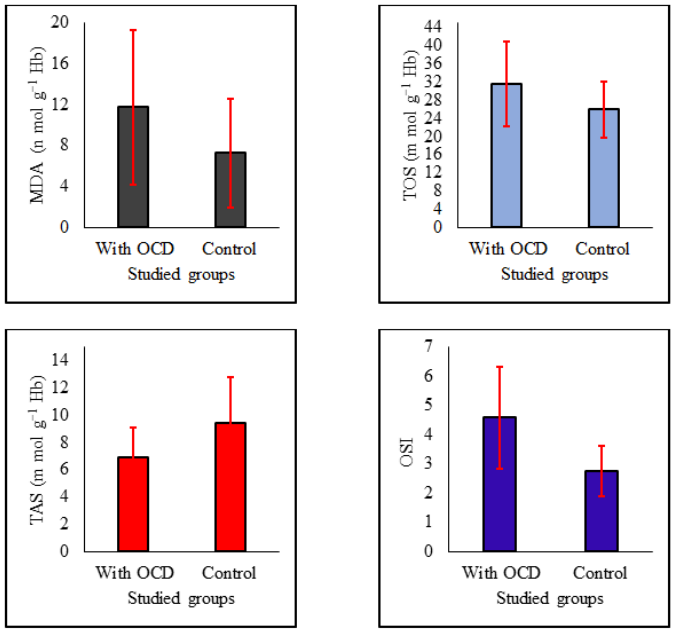

Figure 3. The comparison of MDA, TOS, TAS and OSI between OCD group and control group.

Table 1. Demographic information of studied patient and healthy groups.

Variables	Studied groups	
	Patient with OCD	Control
Number (person)	26	26
Age (year)	35.8 ± 11.5	36.2 ± 12.7
Sex (male/female)	13/26=50%	13/26=50%
Smoking (Yes/No)	8/26=30.8 %	9/26=34.6 %
Y-BOCS score	25.19 ± 5.49	-
Mild OCD (person)	7/26 (26.9%)	-
Moderate OCD (person)	10/26 (38.5%)	-
Severe OCD (person)	6/26 (23.1%)	-
Very severe OCD (person)	3/26 (11.5%)	-

Table 2. The activity of enzyme glutathione peroxidase, oxidative stress index and some biochemical variables in serum of patients with OCD and control groups.

Variables	Studied groups		P
	Patients with OCD	Control	
Number (person)	26	26	-
Se (µg/L)	0.001	92.71 ± 25.74	51.82 ± 16.25
CAT (U g^{-1} Hb)	0.004	83.90 ± 31.35	60.39 ± 23.42
GSH-Px (U g^{-1} Hb)	0.002	99.43 ± 13.76	59.16 ± 9.24
SOD (U g^{-1} Hb)	0.007	254.97 ± 0.47	315.62 ± 0.63
MDA (nmol g^{-1} Hb)	0.005	7.24 ± 5.33	11.72 ± 7.55
TOS (mMol/l)	0.03	25.95 ± 6.14	31.52 ± 9.27
TAS (mMol/l)	0.045	9.40 ± 3.34	6.88 ± 2.24
OSI	0.006	2.76 ± 0.86	4.58 ± 1.75

The results of this study showed that the level of MDA associated with individuals with OCD was significantly higher than the control group (P=0.005). MDA is the end product of lipid peroxidation and can be considered as a coefficient for lipid peroxidation. MDA can generally be produced by the oxidation of unsaturated fatty acids, indicating damage caused by free radicals; therefore, a significant increase in this compound indicates the activation of the lipid peroxidation mechanism in the body of a person with OCD [18-20].

The findings of this study were consistent with the results of similar previous studies. The rate of MDA in OCD patients was significantly higher than the control group, according to Ozdemir [5], Kuloglu [18], Mohammed et al. [19], and Ersan [21]. Given that the patients in this study had no history of metabolic or oxidative stress-related disorders, the increase in MDA may be attributed to active lipid peroxidation in their brain tissue, and the damage to this tissue has left relevant [22].

MDA is the end product of the breakdown of free fatty acids such as arachidonic acid and other long-chain fatty acids. This compound is chemically stable and can easily enter the cell through the membrane. MDA may form bonds with macromolecules and thus damage proteins and other macromolecules, especially MDA [5].

The patient group's level of SOD activity was significantly higher than the control group's (P=0.007), whereas the patient group's levels of GSH-Px and CAT were significantly lower than the control group's (P=0.002 and P=0.004). Looking at the results, it can be said that CAT and SOD levels are significantly related to MDA levels.

One of the most important defense mechanisms against Reactive Oxygen Species (ROS) is antioxidant enzymes, which include superoxide dismutase, catalase, and glutathione peroxidase. Since superoxide is the most abundant Reactive Oxygen Species (ROS) produced. Therefore, it can be said that superoxide dismutase is the most important defense barrier against ROS [5,18,19]. The catalase group is another important enzyme in this regard, since it breaks down H_2O_2 and so aids in the defense against free radicals. Another essential enzyme that regenerates total H_2O_2 and lipid hydro peroxides is glutathione peroxidase. This enzyme belongs to a tetrameric enzyme family that contains the exceptional amino acid selenocysteine and reduces H_2O_2 and lipid peroxidation by using

low-weight thiol molecules like glutathione. SOD, CAT, and GSH-Px are oxidizing enzymes that perform a complimentary role in the defense/antioxidant system. Hydrogen peroxide is the product of a reaction catalyzed by SOD and is itself a substrate for the enzymes CAT and GSH-Px. The amount of SOD, which plays a central role in antioxidant defense, increases in oxidative stress [22-20]. The results of this study demonstrate that the SOD activity of lipid peroxidation is significantly higher in patients with OCD than in the control group (P=0.007). Since oxidative stress increases O_2-measures, therefore SOD activity will increase accordingly, whereas CAT and GSH-Px activity will decrease. The results of the current study in terms of CAT and GSH-Px measures are consistent with the results of Ozdemir [5], and Mohammed et al. [19], but inconsistent with the results of Kuloglu [23].

Based on the current results of this study, selenium levels in the patient group are lower than in the control group. GSH-Px activity and selenium concentration in patients were found to have a positive relationship. No association was found between age, YBOCs, selenium levels, and MDA levels. Human studies have proven the importance of selenium and selenoproteins in the normal functioning of the brain. Selenium deficiency and its deficiencies are associated with impaired motor function and poor cognitive function. Defects in selenoprotein synthesis have been linked to a number of serious brain disorders. Selenium is present in the structure of selenoproteins, which are highly significant compounds. Selenoproteins are present in enzymes like thioredoxin reductase and glutathione peroxidase, which are part of the antioxidant system and have been shown to act as a brain signaling regulator. Moreover, glutathione peroxidase is a type of selenoprotein that plays a very important role in antioxidant defense [5,24,25].

Previous studies have found that selenium deficiency and selenium-free diets can increase oxidative stress sensitivity in numerous parts of the brain, especially dopaminergic parts, and restore dopamine in the substantianigra [26]. In terms of selenium content, the results of this study and those of Ozdemir are consistent [5]. Therefore, it can be concluded that one of the reasons for the decrease in the activity of the antioxidant system in patients with obsessive-compulsive disorder is the reduction of selenium, and by providing the required selenium in people prone to obsessive-compulsive disorder and other related diseases can be prevented.

The level of TAS in the OCD patient group was significantly lower than the control group (P=0.045), according to the findings of this study. Furthermore, the rate of OSI and TOS in the OCD patient group was significantly higher than the control group (P=0.030 and P=0.006, respectively). The study of various neurochemical markers in mental diseases has gotten a lot of interest in recent years. Since detecting these parameters in peripheral blood is relatively simple and can be analyzed using existing methods, the TAS, TOS, and OSI parameters have been routinely employed to measure oxidative and antioxidant status in clinical trials [5]. The oxidative equilibrium, which exists in all organisms, is a balance between the formation of free radicals and their elimination. The number of free radicals produced has an impact on this equilibrium. These balances are disrupted when the formation of free radicals increases and their removal rate declines, resulting in oxidative stress. A change in this balance, without a doubt, causes several of the diseases and tissue damage [20-22].

The current study's results show that TOS had a significant increase in patients compared to the control group (P=0.030). Malondialdehyde and nitric oxide, as specific oxidants, have shown a significant increase in previous studies, including this one [27]. TOS was shown to be considerably decreased in adults in other studies, owing to a decline in compensatory mechanisms with increasing age and disease duration [28]. Furthermore, patients with bipolar disorder had a considerably higher TOS index than healthy individuals [29]. According to some research, the brain is extremely sensitive to oxidants, and that elevated oxidants cause neurodegenerative disease, stroke, fainting, and epilepsy. Furthermore, the reduction in oxidant levels following therapy indicates that oxidants themselves have a role in the development of psychological disorders [30].

The level of TAS in the OCD patient group was significantly lower than the control group (P=0.045), as per the results of this study. Many studies have shown that antioxidants decrease in adult OCD patients [21], whereas some other studies have shown that total antioxidant levels increase in OCD patients [23]. TAS is reduced as a result of the overuse of antioxidants to release oxidative stress, and OCD is caused by deficiencies in antioxidant defense mechanisms and insufficient detoxifying capabilities.

The results of the present study showed that OSI in the patient group was higher than the control group with a significant difference (P=0.006). In general, psychiatric studies have shown that OSI increases in patients with mental disorders [29]. All oxidant and antioxidant factors in oxidative metabolism are compared and evaluated using OSI. The results of this study show that when there is an oxidative imbalance (decrease in antioxidants and increase in OCD), the equilibrium state is in favor of oxidants.

Conclusion

The amounts of four of the eight parameters studied, including Selenium (Se), Catalase (CAT), plasma Glutathione Peroxidase (GSH-Px), and Total Antioxidant Status (TAS), were significantly higher in healthy people compared to patients with OCD, while the other four parameters, including Superoxide Dismutase (SOD), Malondialdehyde (MDA), Total Oxidant Status (TOS), and Oxidative Stress Index (OSI), were significantly higher in OCD patient group compared to control group.

MDA can generally be produced by the oxidation of unsaturated fatty acids, indicating damage caused by free radicals; therefore, a significant increase in this compound indicates the activation of the lipid peroxidation mechanism in the body of a person with OCD. One of the important defense mechanisms against Reactive Oxygen Species (ROS) is antioxidant enzymes that include SOD, CAT and GSH-Px. The results of this study show that the SOD activity of lipid peroxidation is significantly higher in patients with OCD than in the control group, and since oxidative stress increases the amount of O_2-, the amount of SOD activity will be proportionally higher, while the activity of CAT and GSH-Px will be proportionally lower. Based on the results of this study, it can be concluded that selenium deficiency and selenium-free diets can increase the oxidative stress sensitivity of various parts of the brain, especially dopaminergic parts, in patients with OCD. Among the various biochemical parameters, the TAS, TOS, and OSI parameters are commonly used to estimate the oxidative and antioxidant status of clinical trials in patients with mental disorders, including OCD; due to the fact that determining these parameters in peripheral blood is very easy and can be checked by existing methods. According to the results of the present study, it can be said that the cause of decreased TAS in patients with OCD is excessive usage of antioxidants to eliminate oxidative stress and deficiencies in the antioxidant defense mechanism and inadequate detoxification capacity contribute to OCD. Generally, OSI increases in patients with mental disorders, according to psychiatric studies, which confirms the results of this study. Based on the findings of this study, it may be concluded that a psychiatrist may determine the level of oxidative damage in a person's brain and predict suitable treatment by evaluating the amount of MDA in their plasma. Furthermore, assessing overall oxidative and antioxidant status as well as the oxidative stress coefficient is the best way to measure the overall oxidative status among healthy people, and therefore this method can prevent people from entering the OCD stage. Due to the positive relationship between antioxidant enzymes and selenium levels in OCD patients, by increasing the amount of this element in food can prevent healthy people. Relaxation, yoga practices, walking and/or jogging as well as clean (healthy) air breathing can have a very positive effect on reducing oxidative stress, which can be very effective in preventing healthy people from developing OCD.

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