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Serum malondialdehyde and thiol levels in patients with bipolar disorder

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ABSTRACT

Bipolar disorder is a psychiatric diagnosis which shows unusual shifts in mood, energy, activity levels and the ability to carry out day to day works. Studies have suggested possible role of oxidative stress may play an important role in pathophysiology of neuropsychiatric disorders. In the current work, presence of oxidative damage in bipolar disorder is studied. Serum samples from 100 bipolar disorder patients and 52 healthy controls were collected to analyze lipid peroxidation marker malondialdehyde (MDA) and thiol levels using colorimetric methods. There was significant increase in MDA levels in bipolar patients ($p<0.0001$), although there was decrease in thiol levels in bipolar patients but it was not statistically significant. Serum thiol levels correlated negatively with MDA levels ($r = -0.474$, $p<0.0001$). Our study has shown the presence of oxidative membrane damage in bipolar disorder patients, supporting previous similar studies indicating role of oxidative free radicals in development of neuropsychiatric disorders.

KEYWORDS

Bipolar disorder;
Thiols;
Malondialdehyde;
Neuropsychiatric disorders;
Oxidative stress;
Membrane damage.

INTRODUCTION

Bipolar disorder is a psychiatric diagnosis which shows unusual shifts in mood, energy, activity levels and the ability to carry out day to day works[1,2]. It is also referred to as manic depressive disorder or bipolar affective disorder. Symptoms of bipolar disorder are severe and different from the normal ups and downs that everyone goes through from time to time. It is a debilitating mental illness which has recently started to receive the necessary attention from the society and the researchers for further awareness and education[3]. Various research are going on in mental illness, and most scientists believe that mental illness are caused by a combination of several factors working together, and when bipolar disorder is considered, biological and psychological causes are the main ones to play an important role in it[2,4,5]. The prevalence of bipolar disorder ranges from 1% to 1.6% in the United States and from 0.3% to 1.5% worldwide[6].

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TABLE 1 : Serum thiols and MDA levels in patients with bipolar disorder compared to healthy controls. (Values are expressed in mean±SEM)

	Controls (N = 52)	Bipolar disorder (N = 100)
MDA (μM/L)	0.23 ± 0.01	0.83 ± 0.10*
Thiols (μM/L)	355.2 ± 7.91	291.9 ± 6.42
Fasting Plasma Glucose (mg/dL)	75.46±8.93	88.00±1.62
Total Cholesterol (mg/dL)	154.5±6.20	158.05±4.03
Triacylglycerides (mg/dL)	170.02±13.23	184.33±2.79
High Density Lipoprotein (mg/dL)	50.14±4.21	50.16±2.81
Low Density Lipoproetin (mg/dL)	91.8±14.51	103.79±1.42
Serum Urea (mg/dL)	23.19±0.81	27.01±0.87
Serum Creatinine (mg/dL)	0.96±0.19	0.83±0.02
Serum Aspartate transaminase (U/L)	16.03 ± 0.43	14.82±0.60
Serum Alanine transaminase (U/L)	14.05 ± 0.24	18.03±0.61
Total bilirubin (mg/dL)	0.80±0.28	0.75±0.04
Direct bilirubin (mg/dL)	0.34±0.16	0.21±0.02

***p <0.0001, compared to healthy controls**

In patients suffering from bipolar disorder, two major areas of the brain have been found to have 30% more cells which send signal to other brain cells, in turn causing a kind of overstimulation which ultimately results into symptoms observed in patients with bipolar disorder[7]. Oxidants have been found to be related with "membrane associated pathology" and previous studies suggested that oxidative stress may play an important role in pathophysiology of neuropsychiatric disorders. Studies had been done to investigate the status of oxidative metabolism in such patients which determined total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI). TAS was found to be negatively co-related to the number of previous episodes and also the TOS was positively co-related to the severity of the disorder[8].

Malondialdehyde (MDA) is a secondary lipid peroxidation end product and is commonly determined marker of lipid peroxidation of membrane. MDA is the ultimate end product of lipid peroxidation reactions and hence used as measure of oxidative damage to cell membrane[9,10]. Thiols are the major antioxidants in the body and they consists of free sulfhydryl (-SH) groups in plasma and cells, and -SH groups bound to proteins (most abundant when compared to free -SH group). Protein thiols in the plasma include the protein sulfhydryl groups (-SH) and protein mixed disulphides with homocysteine, cysteinylglycine, cysteine and glutathione. Human plasma contains homocysteine (HcySH), cysteinylglycine (CysGlySH), cysteine (CysSH), and Glutathione (GSH) as reduced thiols. These thiols are also found as low-molecular-mass (symmetrical) disulphides, *i.e.*, homocystine [(HcyS) 2], cystinilglycine [(CysGlyS) 2], cystine [(CysS) 2], and glutathione disulphide (GSSG)[11].

In the present study, we have tried to determined levels of oxidative stress in bipolar disorder patients by analyzing MDA, a product of lipid peroxidation, and thiols, a group of antioxidants which are organic compounds containing free sulfhyldryl groups (-SH) groups.

MATERIALS AND METHODS

Subjects and samples

The study was carried out on 100 patients diagnosed with bipolar disorder and 52 healthy controls. The patients were undergoing treatment at Dr A V Baliga Memorial hospital, Udupi. The mean age of patients with bipolar disorder was 63±12 years and that of healthy controls was 52±12 years. The subjects did not suffer from any other pathology that could alter oxidative stress. There were 58 males and 42 females in the patient group. The healthy controls were not on any kind of prescribed medication or dietary restrictions. Informed consent was taken from all subjects involved in the study and was approved by institutional review board.

Blood samples in fasting state, 2ml was drawn into vacutainers containing anticoagulant and sodium fluoride, and 5 ml was drawn into plain vacutainers from the antecubital veins of healthy controls and bipolar disorder patients. The blood samples drawn in vacutainers containing anticoagulant was analyzed within 30 minutes for glucose levels. The blood samples drawn in plain vacutainers was allowed to clot for 30 min and centrifuged at 2000g for 15 min for clear separation of serum.

Biochemical determinations

Special chemicals like 5'5'-dithio-bis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), and standard MDA were obtained from sigma chemi-

cals, St Louis, MO, USA. All other reagents were of chemical grade.

Thiol assay

Reaction mixture contained 900 µL 2 mM Na_2 EDTA in 0.2 M Na_2HPO_4, 20 µL 10 mM DTNB in 0.2 M Na_2HPO_4 and 100 µL of serum. Reaction mixture was incubated at room temperature for 5 min; absorbance read at 412nm. Appropriate sample and reagent blanks were prepared simultaneously and the respective absorbance was noted. Corrected absorbance values were used to calculate serum protein thiols using the molar extinction coefficient 1600 M^{-1} cm^{-1} and values expressed as µM. The calibration curve was produced using GSH dissolved in Phosphate buffered saline (PBS)[12].

MDA assay

Reaction mixture contained 1 mL 0.67% thiobarbituric acid (TBA), 500 µL 20% Tri carboxylic acid (TCA) and 100 µL serum. Incubated at 100°C for 20 minutes; centrifuge at 12,000rpm for 5 minutes. Absorbance of supernatant read at 532 nm. MDA was determined by using molar extinction coefficient 1.56×10^5 M^{-1} cm^{-1} and values expressed as µM[13].

Other biochemical parameters

Fasting plasma glucose (FPG), serum levels of urea and creatinine, aspartate transaminase and alanine transaminase, total bilirubin and direct bilirubin, total cholesterol, triacylglycerol, high density lipoprotein and low density lipoprotein levels were determined by automated analyzer Hitachi 912.

Statistical analysis

The results were expressed as mean±standard error of mean (SEM). A $p<0.05$ was considered statistically significant. Statistical analysis was performed using the statistical package for social sciences (SPSS-16, Chicago, USA). Independent sample t test was used to compare mean values. Pearson correlation was applied to correlate between the parameters.

RESULTS

As depicted in TABLE 1, there was a significant increase in serum MDA levels in patients with bipolar disorder compared to healthy controls ($p<0.001$). Although thiol levels were decreased in bipolar patients compared to healthy controls but the decrease is not statistically significant. As depicted in TABLE 1, other routine biochemical parameters did not show any significant difference between healthy controls and bipolar patients. As shown in figure 1, serum MDA levels correlated negatively with serum thiols ($r = -0.474$, $p<0.0001$).

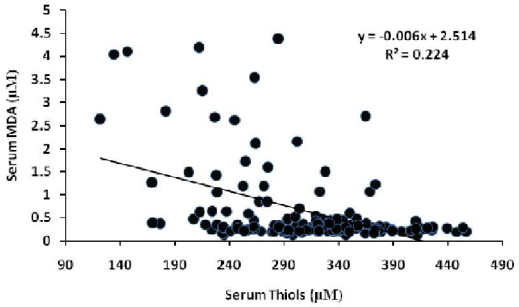

Figure 1 : Correlation between serum thiols and serum MDA

DISCUSSION

A number of studies have been carried out previously which shows the relation between the increased oxidative stress and neurological disorders. Oxidative stress had been attributed to pathology of most of the neurological disorders[14-16]. In the present study, we have found that serum MDA was increased significantly in patients with bipolar disorders compared to healthy controls ($p<0.0001$). We have found increase in MDA levels ranging from 0.23 µM to .83 µM indicating increased presence of oxidant damage to biological membranes including neuronal cells.

Mitochondria being primary site for production of reactive oxygen species (ROS) make them vulnerable to oxidative stress damage with further effect cellular and macromolecular function[17,18]. Mitochondrial permeability transition (mPT), where by the inner mitochondrial membrane suddenly becomes excessively permeable to ions and other solutes, which lead to collapse to inner membrane potential leading to energy failure and cell necrosis This is found in diseases like Alzheimer's diseases (AD), Parkinson's disease, Huntington chorea and amyotrophic lateral sclerosis (ALS)[19]. Studies have also found that altered lipid metabolism may be of particular importance in CNS

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injuries and disorders[20]. These studies support our finding of increased lipid peroxidation due to oxidant stress in certain neuropsychiatric diseases including bipolar disorder.

Products of lipid peroxidation like 4-hydroxyneneol (4-HNE) have been analyzed and also found to increased significantly in patients with bipolar disorder and Schizophrenia[21]. Some studies also report that oxidative stress may be associated with abnormal low levels of brain-derived neurotrophic factor in individuals with bipolar disorder[20]. Supplementation of eicosapentaenoic acid to schizophrenic patients and supplementation of eicosapentaenoic acid and docosahexaenoic acid to patients with bipolar disorder have shown to provide benefits to these patients[22]. It has also been shown that lithium and valproate exert protective effects against oxidative stress induced by amphetamine, a commonly used drug for bipolar disorder[23]. This finding further supports our finding that presence of oxidative stress and damage to biological membrane has some link to bipolar disorder.

Reduced Glutathione (GSH) is the most abundant non-protein thiols and is the preferred substrate for several enzymes in xenobiotic metabolism and antioxidant defense. It plays many important roles in many cellular processes such as cell differentiation, proliferation and apoptosis. GSH is an essential reductant which protects cell and is reduced in wide range of pathologies including neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease[24,25]. Protein bound thiols (-SH) groups are the abundant antioxidants in the body and has been shown to participate in various reductive reactions. They play a major role in neutralizing reactive free radicals and thereby preventing oxidative membrane damage. In the present study, we have found decrease in thiol levels in bipolar patients compared to healthy controls, decrease in thiol levels range from 335.2 μM to 291.9 μM.

In conclusion, our study supports the previous studies on neuropsychiatric disorders that presence of increased oxidative stress and damage to biological membranes including neuronal membrane is related to pathogenesis of bipolar disorder. The results of this study need further investigation to understand the oxidative versus anti-oxidative process in bipolar disorders.

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