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Effect of Lithium Therapy on Non-enzymatic Antioxidants in Patients with Bipolar Affective Disorder

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ABSTRACT

Aim: *Brain is more vulnerable to oxidative free radicals than other tissues. Oxidative stress might primarily or secondarily be involved in the pathogenesis of bipolar affective disorder. Therefore this study was aimed to estimate & compare parameters of oxidative stress and antioxidant levels in bipolar patients and healthy controls.* ***Methods:*** *A total of 32 bipolar patients and 30 healthy subjects were recruited in this study. Serum MDA level was measured as indicator of lipid peroxidation and vitamin C, E & glutathione were determined as a measure of antioxidant status.* ***Results:*** *Significantly ($p<0.001$) elevated MDA level was found in patient before treatment (7.11 ± 1.62 nmol/ml) as compared to control (3.02 ± 1.30 nmol/ml) group. Patients on follow-up also had significantly increased MDA level (5.37 ± 1.36 nmol/ml) as compared to control group and decrease in level was significant ($p<0.001$) in comparison to before treatment patients. Vitamin C (0.82 ± 0.32 mg/dl), E (0.77 ± 0.20 mg/dl) and glutathione (4.44 ± 0.91 µmol/gm Hb) levels were significantly ($p<0.001$) reduced in patient group compared with those of control (1.53 ± 0.56 mg/dl), (1.27 ± 0.37 mg/dl) and (9.66 ± 1.79 µmol/gm Hb) respectively.* ***Conclusion:*** *The findings suggest that there is increased oxidative stress in bipolar patients in comparison to controls.*

KEY WORDS

Antioxidant, Bipolar Affective Disorder, Glutathione, Lithium, Reactive Oxygen Species

INTRODUCTION

Bipolar affective disorder (BPAD) is a chronic, severe, and highly disabling psychiatric disorder which is estimated to affect 1% of the world population [1].The exact neurochemical mechanisms underlying the pathophysiology of BPAD are not completely understood. Several hypotheses have been postulated including a role for monoamines, gamma amino butyric acid (GABA), glutamate and second messenger signaling pathways. More recently, oxidative stress has been implicated and there is evidence accumulating to support its' role [2].

Under physiological conditions there is a balance between oxidative and antioxidative systems in the organism. Oxidative stress is the imbalance between these systems in favor of the former and has been implicated in the pathophysiology of several neuropsychiatric diseases, including BPAD [3]. Oxidative stress, Due to either increased free radical production and/or inefficient antioxidant systems, leads to lipid peroxidation [4]. Malondialdehyde (MDA), an end-product of lipid peroxidation, is one of the most extensively studied indices of lipid peroxidation and thus oxidative stress.

Antioxidant defense system comprises a series of enzymatic and non-enzymatic components. Superoxide dismutase (SOD) and catalase (CAT) are critical antioxidant enzymes that act cooperatively at different sites in the metabolic pathway of free radicals, and altered activity of one of the enzymes without compensatory changes in the other enzymes may result in oxidative stress. The most common non-enzymatic antioxidant molecules are albumin, uric acid, bilirubin, vitamin E, vitamin C and β-carotene [5].

Oxidative stress might primarily or secondarily be involved in the pathogenesis of BPAD [6]. The brain is much more vulnerable to oxidative free radicals than other tissues, since it utilizes 20% of the oxygen consumed by the body [7]. Moreover, the brain contains great amounts of polyunsaturated fatty acids (PUFA) and iron and low concentration of antioxidant enzymes. Psychological stress, which accompanies BPAD, may increase lipid peroxidation [8]. BPAD is also characterized by activation of inflammatory response with increased production of proinflammatory cytokines which may increase lipid peroxidation by inducing free radical production [9].

In the present study we aimed at determining and comparing oxidative damage (via determination of MDA) and antioxidant status (vitamin C, vitamin E, and glutathione levels) in patients with BPAD and healthy controls.

METHODS

Sample collection

This comparative cross-sectional study was conducted at the Biochemistry and Psychiatry Departments of B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal. The study was approved by BPKIHS Ethical Committee. The patient group comprised of 64 patients, Who had visited to the Department of Psychiatry from August 2009 to July 2010 and had been diagnosed with BPAD according to the *Diagnostic and Statistical Manual of Mental Disorders-IV* [10].The control group comprised 30 healthy individuals matched for age, sex, dietary and exercising habits. In addition controls did not have a history of any kind of mental disorders in their first degree relatives. After diagnosis was made, informed and written consent were obtained either from the patient or from close relative when the patient was deemed unable to provide consent.

Venous blood samples were collected into 5ml vacutainer tubes containing potassium EDTA and 5ml vacutainer tubes without anticoagulants. Blood was centrifuged at 1000g for 10 min at 4^0C to remove plasma. The erythrocyte sediment washed three times with normal saline to remove plasma remnant. The sediments were treated with four-fold ice cold deionized water to obtain haemolysate. Serum was separated from another vacutainer tube. All analyses were performed at the same laboratory in the Department of Biochemistry.

Inclusion and exclusion criteria

Patients aged 15-50 years, and who had been diagnosed of BPAD and who were experiencing an acute mood episode, were included in the study. Patients with substance abuse or dependence in the past year or with other psychiatric, neurological disorders and serious medical conditions and not willing to participate in the study were excluded from the study.

Determination of plasma MDA level

Levels of plasma MDA were measured by the thiobarbituric acid (TBA) method which was modified from the methods of Satoh and Yagi [11]. Peroxidation was measured as the production of MDA which in combination with TBA forms a pink chromogen i.e. compound whose absorbance at 532 nm was measured. MDA levels were expressed as nmol/ml.

Determination of Non-enzymatic antioxidants

Vitamin C: Determination of Vitamin C depends on the reduction of ferric ion to ferrous ion by ascorbic acid as red-orange, α, α'-dipyridal complex as developed by Sullivan et al. (1955) [12].

Vitamin E: This is based on Emmerie Engel procedure in which tocopherol is oxidized to tocopheryl quinone by ferric chloride and resultant ferrous ion is complexed with α, α'-dipyridyl to procedure a red colored compound [13].

Glutathione: The method is based on the development of a yellow color complex when 5,5-dithiobis-(2-nitrobenzoic acid) [DTNB] is added to sulfyhydryl compounds as described by Beutler E et al (1963) [14]. The reaction was read at 412nm.

Statistical analysis

Data were analyzed using SPSS (version 17.0) for windows statistical software. Non-parametric statistical methods were used to analyze the data. Statistical evaluations were performed by using the t-test and Pearson correlation coefficient. Differences at $p<0.05$ level were considered to be statistically significant.

RESULTS

The results are summarized in Tables 1-2. Table1 shows the socio-demographic variables of control and patient. The mean age (26.03±7.58 yrs), height (162±6.5cm) and weight (54±6.85 kg) of patients' were compared to that of controls' age (27.80±5.11), height (164±4.6cm) and weight (56±6.1 kg) respectively. There were no significant differences in the above parameters between these two groups ($p>0.05$). There were 20 males and 12 females in patients group and 20 males and 10 females in controls group. The minimum and maximum age (yrs) of patients were 15 and 47 and that of controls were 19 and 38 respectively.Thus this table confirms the matching of cases and controls. Out of 32 BPAD patients, 26 patients had no positive family history where as 6 patients had positive family history of some kind of mental illness. Out of the total BPAD patients 21 were in mania with psychotic symptoms, 8 were in mania without psychotic symptoms and only 3 were in mixed episode.

Table 1: General characteristics of patients and controls[†]

Variables	Control (n=30)	Case (n=32)	P value
Sex			
Male	20 (67%)	20 (63%)	0.11
female	10 (33%)	12 (37%)	
Age (yrs)†	27.80±5.11	26.03±7.58	0.44
Weight (Kg)†	54 ±6.85	56±6.1	0.21
Height (cm)†	164±4.6	162±6.5	0.09

[†]Values are given as mean ± SD

Table 2 shows the comparison of biochemical parameters among the controls and before treatment and after treatment patients group. The increase levels of MDA were observed in BPAD patients as compared to controls in both before treatment and after treatment groups. However there was significant decrease in the level of MDA in after treatment group than in before treatment group. The significant decrease concentration of other biochemical parameters viz; vitamin C, vitamin E, and glutathione were observed in before treatment patients group as compared to controls' vitamin C, vitamin E, and glutathione respectively. Similarly there were significant increase in the levels of vitamin C, vitamin E, glutathione in the after treatment group as compared to before treatment group. However these values were still lower than those of the control group.

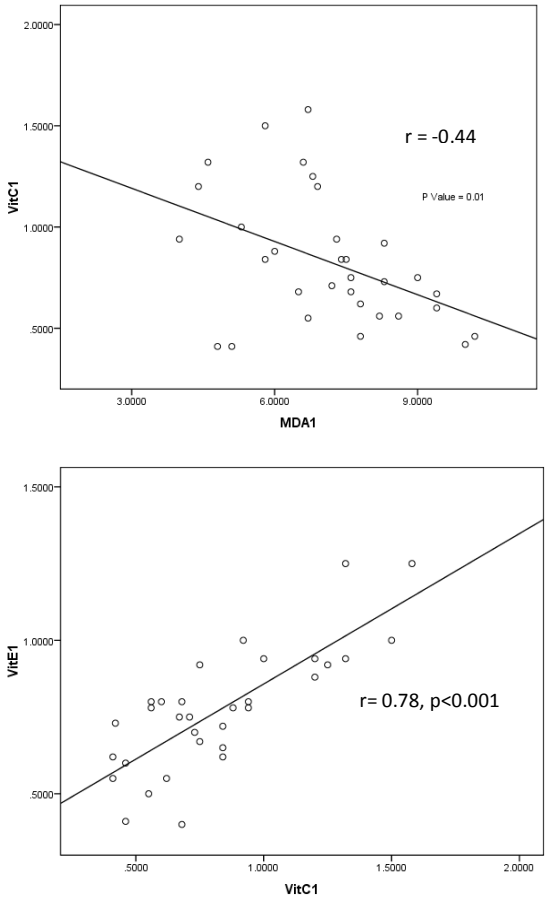
There was significant positive correlation between vitamin C and vitamin E ($r = 0.785$) in the before treatment group. However there was significant Negative correlation between vitamin C and MDA ($r = -0.440$) in the before treatment group as shown in Figure 1. Similarly we found significant negative correlation between vitamin E and MDA ($r = -0.397$) in after treatment group.

Table 2: Comparison of biochemical parameters among the controls and before treatment and after treatment patients group[†]

Parameters	Control (N=30)	Before treatment (N=32)	After treatment (N=32)
MDA (nmol/ml)	3.02 ± 1.30	7.11 ± 1.62[a]	5.37 ± 1.36[a,b]
Vitamin C (mg/dl)	1.53 ± 0.56	0.82 ± 0.32[a]	1.11 ± 0.23[a,b]
Vitamin E (mg/dl)	1.27 ± 0.37	0.77 ± 0.20[a]	0.96 ± 0.17[a,b]
Glutathione (µmol/gm Hb)	9.66 ± 1.79	4.44 ± 0.91[a]	4.82 ± 0.77[a,b,c]

[†]Values are given as mean ± SD; [a]$p<0.01$ vs control, [b]$p<0.01$vs before treatment, [c]$p<0.05$ vs before treatment

Fig 1: Correlation between different biochemical parameters in the before treatment group.



DISCUSSION

Free radicals associated oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders resulting usually from deficient natural antioxidant defenses. Recent studies have consistently reported increased production of lipid peroxidation and alterations of the major antioxidant enzymes in people with bipolar disorders [15, 16,17]. It has been widely demonstrated that the generation of reactive oxygen species (ROS) plays an important role in pathophysiology of several neuropsychiatric disorders [18, 19, 20]. The brain is particularly vulnerable to ROS production because it metabolizes 20% of total body oxygen and has a limited amount of antioxidant capacity [21]. In addition, the brain contains large amounts of iron and PUFA and it is relatively poor with respect to antioxidants [22, 23]. Probably catecholamines including dopamine and norepinephrine are associated with the production of free radicals and conditions causing increased catecholamine metabolism may increase the free radical burden [24,25]. Likewise, trauma and ischemia of the brain may cause increased free radical burden. Free radical damage resulting from ischemia is related to reperfusion or reoxygenation of the tissue [26]. Increase transition metals i.e. iron, copper and manganese concentration lead to the production of excessive amounts of free radicals [27]. The present study has highlighted the alteration of natural antioxidants viz, vitamin C, vitamin E, and glutathione with lipid peroxidation marker MDA in patient with BPAD before and after lithium treatment.

In the present study the bipolar groups had statistically significant higher MDA level. After lithium therapy MDA level decreased significantly but did not get normalized to the level found in the controls. This result is in agreement with the study done by Oczan et al, [17] Kuloglu et al, [15] Machado-Vieira et al [28] and Andreazza et al [29] which also showed the higher value for MDA in patients than controls.

Increased MDA levels disable cellular membrane function by stimulating phospholipase-A_2 and thus release interleukins by stimulating the immune system [30]. The majority of pre-treatment patients (21 out of 32) were experiencing severe acute manic episode with psychotic symptoms. Increased MDA levels in patients compared to control group suggests that there is increased lipid peroxidation and oxidative stress during an acute manic episode in patient of BPAD.

Free radicals may play important roles in membrane function [31]. The impact of lipid peroxidation can markedly alter membrane function and structure, including membrane fluidity, and may result in altered calcium influx [32]. One main piece of evidence for altered membrane function in affective disorders is the significantly decreased level of docosahexaenoic acid (DHA) and omega-3 essential fatty acids in the membranes of erythrocytes in patients with depression [33]. There are significant correlations between both dietary omega-3 intake and erythrocyte membrane omega-3 levels and severity of depression. Previous reports suggesting therapeutic benefit from omega-3 supplementation in patients with unstable bipolar disorder [34] support the hypothesis that omega-3 depletion may be a cause rather than an effect of depression. BPAD is associated with increased activity of one of the phospholipase group of enzymes, which leads to increased formation of free arachidonic acid and DHA and of the eicosanoids formed from arachidonic acid [34]. Lithium inhibits the release of arachidonic acid and the formation of prostaglandins, inhibits the phosphatidyl-inositol cycle and decreases the rate of arachidonate turnover in brain phospholipids [35]. According to our results, the increased MDA level in patients with BPAD may also indirectly demonstrate decreased membrane arachidonic acid and DHA content. Therefore, the present study provides evidence that increased production of ROS might be due to impaired catecholamine metabolism, or to the other possible mechanisms in BPAD. Increased production of ROS might lead to decreased phospholipids amount in the membrane of erythrocytes and to increased MDA levels in erythrocytes and serum.

We found significant lower levels of vitamin C, vitamin E and glutathione in patient group as compared to control group. Increased in the levels of these parameters were statistically significant after treatment in comparison to before treatment patient group but decreased in the levels were such that they did not normalize even after treatment with mood stabilizer agent such as lithium. In the literature, we did not find abundant data regarding these vitamins in BPAD patients. Significantly lower serum vitamin E (an antioxidant agent) concentration in patients with major depression (Maes et al 2000) [6] and enhancement of neurogenesis in dentate gyrus of adult rats by addition of vitamin E (Cuppini et al 2002) [36] warrants a substantial role for the addition of antioxidant agents in the treatment with affective disorders.

A growing body of data shows that many medications and electroconvulsive therapy, which are used for patients with BPAD, have a substantial effect on the molecular targets that improve mood via neuroprotective mechanisms. Lithium has major effects on bcl-2, as well as on GSK-3β (glycogen synthase kinase-3β), which may be responsible, at least in part, for the growing body of data demonstrating that lithium exerts long-term neurotrophic/neuroprotective effects both in vitro and in vivo [37].

The usual medication treatment protocol is probably ineffective for improving the antioxidant defence system of the BPAD patients. Therefore, supplementation of the treatment regimen with antioxidant drugs in patients with affective disorders may be considered to protect membranous structures from lipid peroxidation. Future studies should include larger sample size to investigate whether there is any significant difference in the antioxidant enzyme profile in patients with affective disorders who are treated with mood stabilizers alone versus a combination of mood stabilizers and antioxidant agents (e.g. vitamin E, oestradiol, etc).

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Conflict of Interest: The authors declare that there is no conflict of interest among them and no any compelling interest exists.

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