Original Article

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Biochemistry Section

Oxidative Stress and Major Depression

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ABSTRACT

Background: Major causative factor for major depression is inflammation, autoimmune tissue damage and prolonged psychological stress, which leads to oxidative stress. The aim of this study was to know the association of free radicals and anti-oxidant status in subjects suffering from major depression.

Materials and Methods: Sixty patients diagnosed as a case of unipolar depression as per DSM IV, fulfilling the inclusion and exclusion criteria were compared with 40 healthy age and sex matched controls. The sera of both the groups were collected taking aseptic precautions and were evaluated for the markers of oxidative stress and for the antioxidants. The age group of the sample and the controls was between 18-60 y, both males and females were equally represented in the groups.

Results: A significantly high level of malondialdehyde (MDA) was found in the patients with major depression (1.95 ± 1.04 mmol/L) as compared to healthy controls (0.366 ± 0.175 mmol/L) ($p < 0.0001$). The serum level of nitrite was found to be lower in cases (23.18 ± 12.08 µmol/L) in comparison to controls (26.18 ± 8.68 µmol/L) ($p = 0.1789$). Similarly the serum level of ascorbic acid and superoxide dismutase (SOD) were significantly below as compared to healthy controls (all $p < 0.0001$). Ceruloplasmin levels were also depressed in cases ($p = 0.3943$).

Conclusion: The study concluded that in the absence of known oxidative injury causative agents, the lowered levels of antioxidants and higher levels of MDA implicate the high degree of oxidative stress in unipolar depression.

Keywords: Malondialdehyde, Major depression, Nitric oxide, Reactive oxygen Species, Super oxide dismutase

INTRODUCTION

At global perspective, depression has a lifetime prevalence of 12% [1]. Neuro-psychiatric diseases are responsible for 11% of Disability Adjusted Life Years [2]; depression is one of the most important factors among them [3]. Even, the suicides related to depression are as much as 16 per 100,000 [4]. Only less than two thirds of depressed patients achieve remission when they are prescribed current standard drug therapy which mostly targets 5-HT receptors. The current theories about major depression do not provide sufficient explanations for the exact cause and nature of depression despite extensive research in this area. Now in the current scenario, inflammation and neurodegeneration appear to play an important role in the pathogenesis of depression. Various biomarkers, like biomarkers of neurodegeneration, cytokines, markers of oxidative stress and catabolites of tryptophan have been established in patients with depression and these findings are also supported by animal models of depression [5]. Inflammation and mitochondrial oxidative processes generate free radicals, which are highly reactive species chemically. When these radicals become in excess or when the antioxidant system gets consumed, Reactive Oxygen Species (ROS) may react with macromolecules of the cell like fatty acid, DNA, protein, etc thereby causing damage to these macromolecules. Brain, due to its high metabolic rate, is one of the most vulnerable organs to the damaging effects of ROS. This may explain ROS involvement in several neuropsychiatric diseases [6]. ROS may play an active role in the pathophysiology of depression by various mechanisms such as tissue damage, inflammation [5], neurodegeneration [7], autoimmune mechanisms generated by tissue damage, apoptosis [6].

The present study was undertaken to explore the burden of oxidative stress present in major depression exclusive of some of the well-known confounding factors such as smoking, tobacco intake and ageing. Biochemical markers such as MDA (malondialdehyde), nitric oxide, ascorbic acid, superoxide dismutase (SOD) and ceruloplasmin were compared in the serum of 60 patients of major depression with 40 healthy age and sex matched controls. Malondialdehyde (MDA) and nitric oxide (NO) are oxidant parameters while superoxide dismutase, ascorbic acid and ceruloplasmin are markers of antioxidant defense.

MATERIALS AND METHODS

Selection of Study Population

The study included 60 drug naive and fresh cases of major depressive disorder diagnosed as per the DSM IV, by the consultant psychiatrist. The control group included age and sex matched 40 healthy control subjects taken from the general population. Patients were not using or were dependent on tobacco, alcohol or any other substance. Patients aged greater than 60 y were not included in the study. All control subjects selected for study were healthy, non-diabetics, normotensive, not addicted to tobacco or alcohol and showed no evidence of any acute or chronic infection. The study was approved by the institutional ethical committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi for MD thesis. Under the rule and regulation of institutional ethical committee, signed informed consent was taken from every study subjects.

Specimen Collection

After taking a written informed consent the blood samples were collected from the cases and controls. Taking all aseptic precautions, about 5 ml of blood was drawn by venipuncture from peripheral vein, with disposable syringe. The 5ml blood thus collected in clean dry glass tube was allowed to stand for 30 min at room temperature for the retraction of clot. This was then centrifuged at 3000 rpm. for 10 min to separate the serum. The serum samples were stored at -80^{0}C in the refrigerator for analysis. Care was taken to avoid hemolysis of the sample.

Estimation of serum analytes

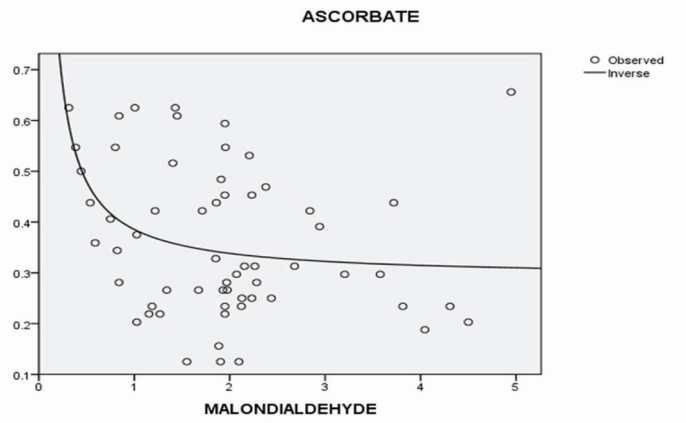
Malondialdehyde: Malondialdehyde was estimated by the thiobarbituric acid (TBA) test.MDA reacts with TBA to generate a pink colored product. In acid solution, the product absorbs light at 530 nm, and is extracted into organic solvents such as butanol [8,9].

Gender	Males (25)	Females (35)
Urban/rural	Urban (15)	Rural (45)
Marital status	Married (50)	Unmarried (10)
Education	Illiterate (9)	Educated (51)
Socioeconomic status	Poor (45)	Affluent (14)
Occupational status	Employed (21)	Unemployed (39)

[Table/Fig-1]: Socio-demographic parameters of cases

Parameters	Cases (n=60)	Control (n=40)	p-value
Ceruloplasmin (mg/dL)	6.27 ± 3.4	7.04 ± 5.6	0.3943#
Ascorbic acid (mg/mL)	0.36 ± 0.14	0.736 ± 0.41	< 0.0001*
SOD (µg/mL)	0.123 ± 0.068	0.177 ± 0.042	< 0.0001*
MDA (mmol/L)	1.95 ± 1.04	0.366 ± 0.175	< 0.0001*
Nitrite (mmol/L)	23.18 ± 12.08	26.18 ± 8.68	0.1789#

[Table/Fig-2]: Status of oxidants and antioxidants in cases and controls,
*Data were represented as mean ± standard deviation. # = non- significant, * = highly significant; SOD= Superoxide Dismutase, MDA= Malondialdehyde, n = Numbers of Subject*



[Table/Fig-3]: Inverse regression analysis of malondialdehyde and ascorbic acid

Nitrite: Determination of the nitrite was carried out by the method described by Griess [10]. In this method a magenta colored azo product is formed due to diazotization of NO radical which can be measured at 540 nm.

Ascorbic acid: Ascorbic acid was measured by a method described by Roe JH et al., Ascorbic acid is oxidized by copper to form dehydroascorbic acid and diketogulonic acid. These products form the derivative bis-2, 4-dinitrophenylhydrazine when treated with 2, 4-dinitrophenylhydrazine. This compound undergoes a rearrange ment to form a product which can be measured at 520 nm [11].

Superoxide dismutase (SOD): Superoxide Dismutase (SOD) was estimated by pyragallol auto-oxidation method described by Marklund and Marklund in 1974 [12]. One unit of enzyme corresponded to 50% of pyragallol auto-oxidation inhibition and was subsequently equal to 100 ng per ml.

Ceruloplasmin: Ceruloplasmin was measured by its oxidase activity. Ceruloplasmin catalyzes the oxidation of a dye p-phenylene diamine to a violet product, the absorbance of which is measured at 546nm [13].

All the chemicals were bought from the HIMEDIA.

Equipment: UV – 1201 (Shimadzu) UV – VIS spectrophotometer was used to measure absorbance for all the markers of oxidative stress.

STATISTICAL ANALYSIS

Statistical analysis between group 1 (controls) and group 2 (patients) was performed by the Independent't' test using the statistical packages (SPSS Software Windows Version 16.0). The data were expressed as mean ± SD. $p < 0.05$ was considered significant. Degree of freedom (df) was 98 for the study population of 100.

RESULTS

The case population consisted of 60 patients out of which there were 25 males and 35 females, 45 patients were from urban background while 15 were from rural background. Considering marital status; 10 were unmarried, 50 were married out of which 1 was widow and 1 was divorced. The socio-demographic profile is depicted in [Table/Fig-1]. The mean plasma levels of MDA was significantly higher in cases (1.95 ± 1.04 µmol/L) as compared to control group (0.366 ± 0.175 µmol/L), ($p < 0.0001$), while the mean plasma level of nitrite in cases (23.18 ± 12.08 µmol/L) was lower than controls (26.18 ± 8.68 µmol/L), although it was not significant ($p = 0.1789$). Mean plasma concentrations of SOD and ascorbic acid were significantly lower in cases (0.123 ± 0.068 µg/mL and 0.36 ± 0.14 mg/mL respectively) than controls (0.177 ± 0.042 µg/mL and 0.736 ± 0.41 mg/mL respectively) (all $p < 0.0001$). Although the mean plasma ceruloplasmin level was lower in cases (6.27 ± 3.4 mg/dL) than controls (7.04± 5.6 mg/dL), the differences was statistically not significant ($p = 0.3943$) [Table/Fig-2].

All parameters were evaluated for the regression analysis of which correlation of malondialdehyde and ascorbic acid was found to be correlated on inverse regression analysis with R^2 value of 0.136 ($p=0.04$) while other parameters were not related on regression analysis [Table/Fig-3].

DISCUSSION

Our study was done to find the oxidative stress among the subjects suffering from major depression, apart from this the antioxidant level in these subjects was also estimated. The comparison was done with normal age sex matched controls. The experimental group consisted of 60 and the control group comprised of 40 subjects. Majority of the subjects were young and middle aged adults. The groups were equally distributed in terms of gender, majority of the subjects were married and came from rural background. MDA level was significantly higher in the patients with major depression than healthy volunteers ($p < 0.0001$). The SOD and ascorbic acid levels were found to be significantly lower in patients with major depression than healthy volunteers (all $p < 0.0001$); while nitrite level was non-significantly decreased ($p = 0.1789$) [Table/Fig-2]. The prolonged psychological stress is a causative factor for major depression, which leads to increase in MDA levels, oxidative stress and depressive symptoms [14]. The levels of different oxidative stress markers (SOD, MDA, and nitrite) are found to be altered in depressive disorders [15,16]. Thus, the oxidant and antioxidant valance system needs to treatment for its homeostasis. The sort-term antidepressant treatments have little effect [17], while fluoxetine and serotonin reuptake inhibitors treatments, trying to stabilize oxidative stress in valance state [18,19].

MDA status is used as biomarker for oxidative stress. It has been reported that elevated MDA was related with different depressive disorder, like auditory-verbal working memory, impairment of visual-spatial, and, short-term and delayed declarative memory [6,20,21]. In several studies, MDA levels have been found to be increased in depression [17,22]. In our study also we found significantly increased levels of MDA in patients of major depressive disorder with a p value < 0.0001 which suggests an extremely significant relationship. Increased MDA levels implicate increased lipid peroxidation products in major depressive disorder.

The relation of nitrite levels in major depression has also been studied [23]. This study hypothesized that reduced nitrite levels could reflect a decreased nitric oxide production in the central nervous system of depressed patients which further can play a role in mediating cardiovascular dysfunction often found in major depressive disorder. A study by Srivastava et al., revealed that nitrite content in the depressive subject decreases than normal one [24]. In our study also we found lowered levels of nitrite in patients of major depression although it was not significant ($p = 0.1789$) however only five of our

patients had significant cardiovascular history which may implicate that nitric oxide production in our patient population was not attenuated to severe degree to be significant. However, nitrite levels also reflect the oxidative stress by RNS (reactive nitrogen species). Many studies implicate the role of RNS in unipolar depression [6].

Ceruloplasmin is a copper containing oxidase. It acts as defensive agent against oxidative stress. The diseased condition leads to altered in its status [25,26]. The role of ceruloplasmin in depression has not yet been established but in diseases where ceruloplasmin is found to be minimal [27], depression is not a predominant presentation [28], however, ceruloplasmin is an antioxidant also. In our study, ceruloplasmin has been found to be decreased but it was not significant. This can be due to consumption of this antioxidant due to increased oxidative stress.

Oxidative damage leads to increased oxidative stress. The increased oxidative stress maintained by different antioxidant system (ascorbic acid, vitamin-E, SOD and so forth) and this antioxidants status altered in different uncontrolled disease condition [29-32]. Ascorbic acid, an antioxidant, has negative correlation with the major depression is a finding well-established [33]. Even in the present scenario ascorbic acid has been found to be effective in the mouse model of depression [34]. In a study done in the paediatric age group also the ascorbic acid treatment as an adjunct to fluoxetine has been found to be effective [35]. In our present study we evaluated the levels of ascorbic acid in major depression as compared to healthy controls and found the levels of ascorbic acid to be decreased significantly ($p < 0.0001$). These findings conclusively point out towards a major role of oxidative stress in the major depression and the role of ascorbic acid as being one of the primary scavengers of free radicals generated during the pathogenesis of major depression.

SOD is a potent oxidative stress marker. It acts as reactive oxygen species scavenger, thus eliminates the reactive oxygen species and maintains the oxidative valance [36]. Its activity altered in numbers of diseases [29,30,37]. SOD activity found to be decreased in different depressive animal model [38,39]. Rybka et al., reported low levels of SOD in the cases of major depression as compared to healthy controls but this was not significant [22]. However, in another study the level of SOD has been found to be significantly decreased as compared to healthy controls [40]. In our study also, we found the levels of SOD to be decreased significantly in the patients with major depression as compared to healthy controls.

CONCLUSION

In the context of the present study and discussion, it can be concluded that oxidative stress may be playing a role in the pathogenesis of depression. This means that the oxidative stress is a well established finding and is present in samples across various groups of population. How far this finding can be translated into clinical benefit remains to be established. The present study was a cross-sectional study; the levels of the biochemical parameters after treatment of the patient group could have been able to make the study more informative.

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