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Original article

Evaluation of Biochemical Markers of Oxidative and Nitrosative Stress Pathways in Major Depression

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ABSTRACT

Background: Major depression is characterized by decreased antioxidant status, an induction of the oxidative and nitrosative pathways. Abnormal levels of antioxidant enzymes and lipid peroxidation further substantiate the role of free radical in major depression. The objective of this study is to evaluate & compare serum levels of oxidative stress markers and peroxidation marker and nitrosative stress pathway markers. **Methodology:** The study included 100 subjects consisting of 50 healthy controls and 50 newly diagnosed patients of Major Depressive Disorder (MDD). Informed consent and institutional ethics committee approval was taken. Serum MDA levels was compared with parameters like SOD, Uric acid, NO, Vitamin E, Vitamin C. Clinical severity was diagnosed by trained psychiatrist using 21-items Hamilton Rating Scale for Depression (HRSD). **Results:** Serum MDA, NO levels were significantly ($p < 0.05$) increased and SOD, Vitamin E, Vitamin C, Uric acid were significantly decreased in MDD patients as compared to healthy controls. There was moderate positive correlation between MDA levels and clinical severity of depression as measured by 21-items Hamilton Rating Scale for Depression (HRSD) score which was found to be statistically significant ($r = 0.317$, p value = 0.025). **Conclusion:** The study concluded that serum MDA, SOD, Vitamin C, Vitamin E, Uric acid and NO combined together provided fairly useful index of oxidative stress and nitrosative stress pathways in MDD. Evaluation of such critical biomarkers would be useful and supportive for early diagnosis and treatment response.

KEYWORDS: Malondialdehyde, Superoxide Dismutase, Nitric oxide, 21- item Hamilton Rating Scale for Major Depression

INTRODUCTION

Depression is the major disorder in present scenario which needs essential attention to prevent significant negative impact on quality of life, mortality/ morbidity and cognitive function. Overproduction of oxygen free radicals results in an imbalance of pro and anti oxidative processes which create a phenomenon known as oxidative stress [1].Evidences have shown nitric oxide plays a prominent role in the pathogenesis of major depression. Nitric oxide modulates norepinephrine, serotonin, dopamine, and glutamate, the major neurotransmitters involved in the neurobiology of major depression [2].

In the literature there are limited and conflicting data on the relationship between lipid peroxidation and antioxidants and nitric oxide in major depressive disorders (MDD), so we took this study to evaluate & compare serum levels of oxidative stress markers and peroxidation marker and nitrosative stress pathway markers (SOD, uric acid, alfa-tocopherol, ascorbic acid, MDA and NO levels) and also correlated with clinical severity diagnosed by trained psychiatrist using 21-item Hamilton Rating scale for depression (HRSD).

Diagnosis of major depressive disorder summarised from Diagnostic and Statistical Manual of Mental Disorders [3]. Assessment of severity of depression done by 21 items Hamilton Rating Scale for Depression (21 item HRSD) proven useful for many years as a way of determining a patient's level of depression before, during, and after treatment [4]. Epidemiologic studies show that roughly 40–

50% of the risk for depression is genetic. This makes depression a highly heritable disorder, at least as heritable as several common complex medical conditions (type II diabetes, hypertension, asthma, certain cancers), which are often thought of as genetic [5]. Free radicals and reactive oxygen species attack lipids, carbohydrates, proteins, and nucleic acids and induce their oxidation may result in oxidative damage such as membrane dysfunction, protein modification, enzyme inactivation and strand breaks and modification of bases in DNA [6]. An antioxidant can be defined as "any substance that when present in low concentration compared to that of an oxidizable substrate significantly delays or inhibits the oxidation of that substrate" [7].

There is evidence that inflammatory, oxidative, nitrosative pathways can cause neuro degeneration which progress to major depression [8].A higher production of oxygen free radicals has been observed in patients with depression and anxiety, allowing a link to be established between oxidative stress and alterations in behaviour [9]. Malondialdehyde (MDA) is an organic compound mainly exists in the enol form, reactive oxygen species degrade polyunsaturated fatty acids, forming malondialdehyde. The production of MDA is used as a biomarker to measure the level of oxidative stress [10].

Nitric oxide, a mediator of inflammation causes vasodilation by relaxing vascular smooth muscle and was therefore called endothelium – derived relaxing factor [11].Superoxide Dismutase (SOD) is the major intracellular antioxidant enzyme which catalytically scavenges the superoxide radical, provides defence against oxygen toxicity [12].Vitamin E acts as antioxidant by scavenging molecular oxygen free radicals and has a role in cellular respiration [7]. Vitamin C is a potent water-soluble antioxidant vitamin in humans [13].Uric acid is formed as the end product of the catabolism of other purine bases and it is a good antioxidant [14]**.** Mohammad Hasanuzzaman Shohag et al have published in the year 2012 that there was significant rise of MDA and imbalance in antioxidants in patients with Obsessive Compulsive Disorder [15].

MATERIALS AND METHODS

After obtaining ethical committee clearance, a total number of 100 subjects were included in the study. Among them 50 were healthy controls and 50 were newly diagnosed cases of MDD. Inclusion criteria was cases diagnosed as major depression by trained psychiatrist using Diagnostic Statistical Manual Text Revision (DSM TR)- IV as inclusion criteria involving both sexes between the age group 18-65 years. They were compared with equal number of age and sex matched controls**.** Subjects who were associated with other psychiatric disorders, metabolic disorders, autoimmune diseases, acute infection, endocrinopathies (Diabetes, Hypothyroidism, Cushing's syndrome), malnutrition, malignancy, chronic inflammatory diseases were excluded.

After taking informed consent, venous blood was collected in a sterile vacutainer under aseptic precaution from selected subjects after overnight fasting. Serum separated and analyzed for various biochemical parameters.The following parameters were estimated, malondialdehyde by Thiobarbituric acid method [16], superoxide dismutase (SOD) by Marklund and Marklund method [17], vitamin E byBaker and Frank method [18], vitamin C by 2, 4 – dinitrophenyl hydrazine method [19], nitric oxide by by Griess method [20] and uric acid by enzymatic (uricase) colorimetric test is according to the recommendation by international federation of clinical chemistry in autoanalyser (ROCHE COBAS INTEGRA 400PLUS) [21].

Statistical analysis: Results are expressed as mean ± SD. Unpaired 't' test will be used. P- value < 0.05 was considered as statistically significant.Correlation between oxidative & nitrosative stress markers with clinical severity was analysed by HSRD score.

RESULTS

Table 1 shows comparative analysis of serum Uric acid, MDA, SOD, Vitamin E, Vitamin C, SOD activity and MDA levels between controls and MDD cases. From the table the mean levels of serum Uric acid, MDA, SOD, Vitamin E, vitamin C & NO in controls were in the range of 4.7 ±1.4mg/dl, 2.3 ±0.9nmoles/ml, 7.8 ±3.5 U/ml and 1.2 ±0.3 mg/dl, 1.1 ±0.1 mg/dl & 29.4 ± 5.6 µmol/L respectively. In MDD cases the mean levels of serum Uric acid, MDA, SOD, Vitamin E, vitamin C & NO were in the range of 3.2 ±1.2 mg/dl, 5.5 ± 0.68 mg/dl, 3.1 ±1.8 U/ml, 0.81 ± 0.2 mg/dl, 0.5 ±0.1 mg/dl, and 34.4±5.5 µmol/L respectively. Statistical analysis by unpaired t-test shows that mean levels of serum uric acid, SOD, vitamin E, vitamin C, were significantly decreased ($p < 0.05$) and mean level of serum MDA and NO were significantly increased in MDD cases when compared to healthy controls and are statistically highly significant ($p < 0.05$).

Table 2 shows there was moderate positive correlation between MDA levels and clinical severity of depression as measured by 21-item Hamilton Rating Scale for Depression (HRSD) score which was found to be statistically significant ($r = 0.317$, $P = 0.025$). There was poor negative correlation between clinical severity and Uric acid and Vitamin C levels. There was no statistically significant correlation between SOD, vitamin E, NO with HSRD score.

Table: 1 Comparison of various parameters between cases and controls

Parameter	Cases	Controls	t*	p-value
Uric acid (mg/dl)	3.2 ±1.2	4.7 ±1.4	1.71	0.001
MDA (nmoles/ml)	5.5 ± 0.68	2.3 ± 0.9	20.04	0.001
SOD(U/ml)	3.1 ±1.8	7.8 ±3.5	8.19	0.001
Vitamin E (mg/dl)	0.81 ± 0.2	1.2 ±0.3	7.48	0.001
Vitamin C (mg/dl)	0.5 ±0.1	1.1 ±0.1	16.8	0.001
NO (µmol/L)	34.4 ±5.5	29.4 ± 5.6	4.48	0.001

Note: *: Independent t test.

Table: 2 Correlation between oxidative & nitrosative stress markers with clinical severity by HSRD score.

Parameter	Correlation coefficient (r)	p-value
Uric acid	-0.127	0.380
MDA	0.317	0.025
SOD	0.046	0.749
Vitamin E	0.057	0.695
Vitamin C	-0.172	0.233
NO	0.191	0.184

DISCUSSION

Major depression is a commonly occurring, seriously impairing, and often-recurrent mental disorder [22, 23]. The World Health Organization (WHO) ranks MDD as the fourth leading cause of disability worldwide and projects that by 2020 it will be the second leading cause owing to currently unexplained increasing prevalence in recent cohorts [24]. MDA, the product of lipid peroxidation reacts with lysine residues in protein to produce immunogenic molecules, which can exacerbate inflammation. The longer chain polyunsaturated fatty acids are especially potent at increasing lipid peroxidation and causing cell damage by oxidative stress which supports the need for studies assessing the therapeutic role of free radical scavengers in MDD. This is in accordance with studies of Khanzode et al and Asli Sarandal et al [25, 26].

CONCLUSION

Major depressive disorder is a common mental disorder associated with a significant negative impact on quality of life, morbidity/mortality. The results of our study demonstrate that the oxidative stress and nitrosative stress pathway markers plays a role in MDD. Major limitation of the study is the sample size. Study with larger group may be required for further evaluation.

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