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Oxidative stress index as a biochemical parameter in major depressive disorder

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ABSTRACT

Background: Oxidative stress is considered to be involved in pathogenesis of different diseases including Major Depressive Disorder (MDD). In laboratory, this can be measured by a biochemical parameter called Oxidative stress Index (OSI), which can be calculated as the ratio percentage of Total peroxide to the total anti-oxidant potential. **Aims and Objectives:** This study was undertaken to determine OSI in MDD and healthy control and to find out whether any significant difference exists among the mean values of OSI in MDD and healthy controls. It was also aimed to find out whether OSI can be correlated with the severity of MDD. The validity of OSI asa biochemical parameter to diagnose MDD was also evaluated. **Materials and Methods:** In this study OSI was determined in 101 cases of Major Depressive Disorder (MDD) along with 106 age and sex matched controls by measuring Total Peroxides (TP) and Total Antioxidant Capacity (TAC). TP was measured by (FOX 2) method and TAC was measured by The Ferric Reducing Ability of Plasma (FRAP) method. The correlation of OSI & severity of depression was assessed by Spearman rank Correlation test. ROC curve was used for determining the validity of OSI for diagnosis of Depression. **Result:** Significant increase in OSI was observed in MDD (2.33 + 0.457) when compared to healthy controls (1.311 + 0.352). The increase was also found to be associated with severity of MDD (Spearman coefficient of rank correlation, rho = 0.289). The diagnostic ability of OSI was evaluated by ROC curve, which showed Area under curve as 0.96. The optimal cut off value of OSI was found to be 1.83, with 87.13% sensitivity and 92.45% specificity. **Conclusion:** Oxidative stress may play a role in pathogenesis of MDD as indicated by measuring OSI. This parameter is found to be significantly associated with severity of disease. The diagnostic ability of OSI for MDD is quite satisfactory. However, further study is needed to validate this finding.

Key words: Oxidative Stress Index, Major Depressive disorder, Total antioxidant capacity, Total peroxide concentration

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INTRODUCTION

Oxidative stress is defined as a dynamic imbalance between the amounts of Reactive oxygen species (ROS) generated in the body and levels of anti-oxidants (AO) to quench and/or scavenge them in order to protect the body against their deleterious effects. When production of the ROS is so much that antioxidant defense of the cells can no more provide the protection, oxidative damage takes place to the macromolecules, namely lipids, proteins, and nucleic acidsof the cells. ROS causes per oxidation of lipids, oxidation of proteins, and damage to DNA. These well-known outcomes of oxygen-derived free radicals, lead to cellular pathology and ultimately to cell death. Finally this leads to various pathological conditions. The role of oxidative stress in pathogenesis of different diseases has been established. Studies have revealed that oxidative stress is involved in causation of depression.[1] An accurate and comprehensive parameter to measure oxidative stress is known as oxidative stress index (OSI), which is calculated as

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the ratio percentage of the total peroxide to the total anti-oxidant potential. Total peroxide concentration reflects the oxidant status whereas measurement of total antioxidant capacity (TAC) is considered as a better alternative to measurement all individual AO parameters separately.[2]

Oxidative stress in depression is comparatively a new approach. It is based on the fact that neurons are vulnerable to free radical attack. Hence insufficient anti-oxidant defense or exposure to excess ROS can lead to dysfunction of neurons leading to neuronal death ultimately. It has been reported that oxidative stress causes destruction of cells and decrease the volume of hippocampus in patients with major depression.[3] ROS is also known to oxidize cellular components like lipids, protein & DNA. The low levels of fatty acid in cell membrane of leucocytes have been reported in patients with depression which is due to oxidative damage.[4] Yanik et al reported that deceased TAC and increased peroxidation in the blood of depressive patients is associated with the severity of disease and is proportional to the oxidative stress levels.[5]

These findings are important as on one hand, the diagnosis of depression depends entirely upon presence of some symptoms and so far, no known biochemical parameter is available to diagnose the condition positively. On the other hand, Major Depressive Disorder (MDD) is a leading cause of disability along with high suicidal rate. The prevalence of depression is quiet alarming globally (1 Year prevalence: 5.8% for men and 9.5% for women) as well as in India.[6] If excessive ROS is involved in pathogenesis of depression, OSI is supposed to be an important biochemical parameter to diagnose MDD.

Hence this study was done to find out:

1. Determination of OSI in MDD and healthy control by measuring
   A) Total Antioxidant capacity by FRAP method
   B) Total peroxide concentration by FOX 2 method.
2. To find out whether any significant difference exists among the mean values of OSI in MDD and healthy controls.
3. Whether OSI can be correlated with the severity of MDD.
4. To find out the validity of OSI asa biochemical parameter to diagnose MDD.

MATERIALS AND METHODS

This Case control study was undertaken inDepartment of Biochemistry, College of Medicine & SagoreDutta Hospital in collaboration with Department of Psychiatry of same Institute. The study period was from June, 2014 to Dec, 2015. The study was approved by Institutional Ethics Committee.

Selection of study subjects

All patients who were newly diagnosed to suffer from Major depressive disorder (MDD) were selected from the Psychiatry outdoor of College of Medicine &SagoreDutta Hospital. Patients were evaluated by detailed history taking and clinical examination through a structured proforma designed for this study. Then they were screened with WHO Five well being index. The raw score was calculated. When raw score was below 13 or if the patient had answered 0 to 1 to any of the 5 items, they were further tested. Patients were diagnosed as having major depressive disorder according to the Structured Clinical Interview for DSM-IV[7] and who scored at least 14 points on Major Depression Inventory (MDI). This inventory was also used to classify the patients according to ICD 10 criteria for depression.[8] The same was also used to find out the severity of depression.A MDI score of 20 – 24 was considered as mild, 25 – 29 as moderate and >30 as severe.

The exclusion criteria were significant psychiatric co-morbidity, organic mental disorder, mental retardation, bipolar disorder, intake of any psychotropic drugs during and at least 1 week before the study, substance abuse, history of endocrine disorders, pregnancy, postpartum depression, lactation, and any sleep disorder other than depression-related insomnia.

Apparently healthy age and sex matched individuals were assessed using General Health Questionnaire (GHQ 12). A score of less than or equal to 15 were considered as not to suffer from major psychiatric illness.[9] Such individuals were selected as control group.

Informed consents were taken from the patients or legal guardians and from the control subjects.

Sample collection, separation & analysis of serum

An amount of 5 ml of fasting blood samples was drawn aseptically from the superficial veins of each of the study subjects (Both cases & controls) using EDTA as anticoagulant.

Plasma was separated and divided into 2 aliquots with proper labeling. One was analyzed to measure TAC was by FRAP assay by Benzie & Strainslightly modified.[10,11] It is based on the principle that at low pH, Ferric TripyridylTriazine (Fe III TPTZ) complex gets reduced to ferrous form developing an intense blue colour. Ascorbic acid standards prepared in concentration of 500, 1000, 1500, 2000 & 2500 µmol/L were used for comparison. The working reagent was prepared by mixing (a) 300 mM acetate

buffer (pH 3.6), (b) 10 mM TPTZ in 40 mMHCl and (c) 20 mM $FeCl_3$ in ratio of 10:1:1 at the time of use. 100 µl sample or standard was mixed with 3 ml of working FRAP reagent, vortexed and incubated at room temperature for 4 minutes. The colour developed was measured at 630nm in a semiautoanalyser.The absorbance of the standards was used to establish the linearity of the test.

The other aliquot was used to measure total plasma peroxide concentrations by FOX2 method with minor modifications.[12] This has advantage over FOX 1 method as FOX 2 assay is more specific. Use of BHT decreases false positivity and use of methanol increases reagent stability in FOX 2 method.The method is based on the oxidation of ferrous iron to ferric iron by the various types of peroxides contained in the plasma samples, in the presence of xylenol orange which produces a coloured ferric-xylenol orange complex whose absorbance was measured at 560 nm.

The TP content of the plasma samples was determined as a function of the difference in absorbance between the test and blank samples using a solution of H_2O_2 as standard. The coefficient of variation for individual plasma samples was less than 5%.

The FOX2 reagent was prepared by dissolving ammonium ferrous sulphate (9.8 mg) in 250 mM H_2SO_4(10 ml) to give a final concentration of 250 µM ferrous iron in acid. This solution was then added to 90 ml HPLC-grade methanol containing 79.2 mg of butylatedhydroxytoluene (BHT). Finally, 7.6 mg of xylenol orange was added, with stirring, to make the working reagent (250 µM ammonium ferrous sulphate, 100 µM xylenol orange, 25 mM H_2SO_4, and 4 nM BHT, in 90% (v/v) methanol in a final volume of 100 ml). The blank reagent contained all the components of the solution except ferrous sulphate.

Aliquots (200 µL) of plasma were mixed with 1.8 ml FOX2 reagent. After incubation at room temperature for 30 min, the vials were centrifuged at 12,000 g for 10 min. The absorbance of the supernatant was then determined at 560 nm.

H_2O_2 standards prepared in concentration of 5, 10, 15, 20, 25 and 30 µmol/L were used for comparison. The absorbance of the standards was used to establish the linearity of the test.Calculation of OSI was done. As it is a ratio & both parameters are expressed in µmole/L, no unit isrequired.

Statistical analysis

The parameters, TAC & TP were expressed in mean + SD. The mean values were compared for significance by student's t test. A p value of <0.05 was considered to be significant. OSI was calculated with the help of the formula: TP*100/TAC.

The correlation of OSI & severity of depression was assessed by Spearman rank Correlation test.

ROC curve was used for determining the validity of OSI for diagnosis of Depression.

The analysis was done using MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2016).

RESULT

A total of 212 subjects (106 cases of depression & 106 age and sex matched controls) were enrolled for the study. Among the 106 patients, 4 patients did not turn up for blood collection and 1 patient did not give consent for the study. Thus a total of 101 cases suffering from depression (Male: 24, female: 77) and 106 (Male: 30, female: 76) controls were studied. Among all depression cases, 19 cases were of mild grade, 37 were of moderate and 45 were suffering from severe depression. Table 1 shows mean value (with 95% confidence interval), standard deviation & standard error of mean of TAC, TOS & OSI in MDD cases along with healthy controls. The difference of mean was found to be significant ($p < 0.0001$) in all cases.

The Spearman coefficient of rank correlation (rho) between OSI and severity of depression was found to be 0.289, which was statistically significant ($p = 0.0035$, Table 2).

To evaluate the diagnostic ability of OSI, ROC curve was used (Figure 1) which was used to find out the optimal cut off values to measure sensitivity and specificity of the test.

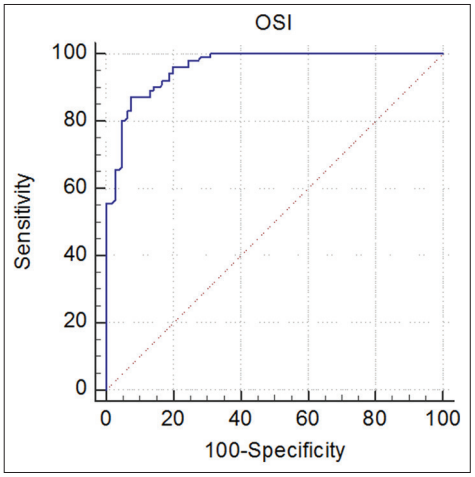

Figure 1: ROC curve of OSI to diagnose MDD

Table 1: TAC, TOS & OSI in study population

Biochemical parameter	Statistical parameter	Control (n=106)	MDD (n=101)
Total antioxidant capacity (TAC)	Mean	1238.9113	1134.1792
	95% CI for the mean	1205.1121 to 1272.7106	1103.7760 to 1164.5824
	Standard deviation	175.5003	154.0087
	Standard error of the mean	17.0461	15.3244
	Two-tailed probability	p<0.0001	
Total peroxide (TP)	Mean	15.7970	26.0266
	95% CI for the mean	15.2458 to 16.3482	25.2193 to 26.8339
	Standard deviation	2.8620	4.0894
	Standard error of the mean	0.2780	0.4069
	Two-tailed probability	p<0.0001	
Oxidative stress index (OSI)	Mean	1.3116	2.3310
	95% CI for the mean	1.2438 to 1.3795	2.2408 to 2.4212
	Standard deviation	0.4570	0.4570
	Standard error of the mean	0.04548	0.04548
	Two-tailed probability	p<0.0001	

Table 2: Estimation of correlation coefficient of OSI with MDD

Statistical parameter	Value
Spearman's coefficient of rank correlation (rho)	0.289
Significance level	p=0 0.0035
95% confidence interval for rho	0.0986 to 0.460

Table 3: Validity of value of OSI as 1.83 to diagnose MDD from ROC curve

Statistical parameter	Value
Area under the ROC curve (AUC)	0.960
Standard error	0.0114
95% confidence interval	0.923 to 0.982
z statistic	40.403
Significance level p (Area=0.5)	<0.0001
Sensitivity	87.13
Specificity	92.45

The area under the curve (AUC) is an effective measure of accuracy, which was found to be 0.96 (Table 3). In this study a value of OSI 1.83 was found to have optimal cut off value where sensitivity was 87.13 (95% Confidence interval: 79 – 93) and specificity was 92.45 (95% Confidence interval: 85.7 – 96.7).

DISCUSSION

The results demonstrate that Oxidative stress is significantly present in patients with MDD, where both components of oxidative stress, i.e. oxidants and antioxidants are found to be significantly altered. This finding is supported by earlier studies. A review article on a meta-analysis study finds that oxidative stress, as measured by 8-OHdG and F2-isoprostanes, is increased in depression.[13] Excess generation of pro oxidants like hydrogen peroxide and other derivatives of peroxide can cause damage of the cell by modifying lipids, proteins, and DNA. Modification of lipids damage cell membranes and modification of proteins alter function of receptors and different enzymes.

The antioxidants get consumed to combat the deleterious effects of prooxidants and are decreased. However, when individual antioxidants were measured, different study groups reported differently. Both increased and decreased activity of SOD has been reported.

Lowered activity of Glutathione peroxidase in comparison to healthy control has been reported whereas no significance difference in GPX activity was observed in MDD by other study groups.

Elevated activity of catalase was observed in patients in acute phase of depression and also in patients with bipolar disorder on lithium medication.On the other hand, other works detected lowered activity of catalase in depression. The contradiction can be caused by small study sample, heterogeneity of patients' statuses, or variability in individual experiments.[14] This contradiction can be overcome by measuring TAC and incorporate the finding to measure OSI.

A significant correlation was found to be present between OSI values and severity of depression in ordinal scale in our study. Similar observation was first reported by Yanik et al in 2004.[4] This finding strengthens the observation that oxidative stress plays an important role in pathogenesis of MDD. Hence OSI can be considered as a biochemical parameter to diagnose MDD. Its diagnostic ability was assessed by ROC curve which gave an optimal cut off value of 1.83 with significant sensitivity &specificity. To best of our knowledge this is the first approach to measure the diagnostic ability of OSI. Further studies are needed to validate this observation.

CONCLUSION

Oxidative stress can be considered to play a role in pathogenesis of MDD as OSI was found to be significantly higher in MDD. OSI was also found to be significantly associated with severity of MDD. The diagnostic ability of OSI for MDD was also found to be quite satisfactory. However, further studiesinvolving larger number of MDD patients are needed to validate this finding.

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Authors Contribution:
SG - Concept of the study, review of literature, data analysis, manuscript preparation; **SK -** Estimated total peroxide concentration, total antioxidant capacity; **SC -** Tabulation of data, review of literature, assisted in manuscript preparation; **SD -** Selection of study subjects; **SN -** Supervised the clinical aspect (Psychiatry part) of the study; **HND -** Supervised the biochemical part of the study.

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