

## Oxidative Stress and Antioxidant Status in Schizophrenia Patients

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### Abstract

**Introduction:** Oxidative stress has been assumed to contribute to the pathophysiology of schizophrenia. Increased oxidative stress is the result of either an increased production of free radicals or a depletion of the endogenous antioxidants.

**Objective:** To assess the levels of oxidative stress and antioxidant status in schizophrenia.

**Materials and Methods:** This observational study was carried out in the department of Pharmacology, Bangabandhu Sheikh Mujib Medical University from September 2013 to January 2015. Ninety three schizophrenia patients were enrolled as study group and 30 healthy individuals were taken as control group. The peripheral levels of several molecules associated with oxidative stress namely malondialdehyde (MDA), glutathione (GSH) and anti-oxidant status like plasma levels of ascorbic acid (vitamin C) and  $\alpha$ -tocopherol (vitamin E) in 93 patients with schizophrenia and 30 healthy participants were assessed.

**Results:** Study found that the schizophrenia group presented substantially higher levels of oxidative stress than the control group, as revealed by elevated quantities of the pro-oxidant MDA ( $6.3 \pm 0.5 \mu\text{mol/L}$  in study group and  $2.1 \pm 0.5 \mu\text{mol/L}$  in control group), decreased levels of the antioxidants GSH ( $0.6 \pm 0.2 \text{mg/gm}$  of Hb in study group and  $2.1 \pm 0.5 \text{mg/gm}$  of Hb in control group), plasma  $\alpha$ -tocopherol and ascorbic acid. Results found were highly significant ( $p=0.001$ ).

**Conclusion:** In schizophrenia there are increased level of oxidative stress and decreased level of the antioxidants.

**Key-words:** Schizophrenia, Malondialdehyde, Glutathione,  $\alpha$ -Tocopherol, Ascorbic acid, Oxidative stress.

### Introduction

Schizophrenia is a major mental disorder expressed in the form of disturbed thought, perception and behavior. It has a life-time prevalence of approximately 1% of the world's population<sup>1</sup>. Schizophrenia is characterized by unknown etiology, complex pathology and long lasting and not having completely successful treatment<sup>2</sup>.

Different neuro-developmental, structural and behavioural abnormalities are associated with schizophrenia<sup>3</sup>. It has been suggested that such abnormalities might be originated from malfunctioning genes. Non-genetic factors such as ethnicity, drug or alcohol abuse, life-style, medications, pre-natal and neonatal infections, maternal malnutrition, complication during birth and many other factors may play an important role in aetio-pathogenesis<sup>2</sup>.

Oxidative stress has been proposed to be common to several psychiatric disorders including schizophrenia<sup>3</sup>. Oxidative stress is a condition where there is imbalance between generation of reactive oxygen species (ROS) with pro-oxidant processes and antioxidant defenses in favor of the former. Most studies that have assessed oxidative stress in schizophrenia populations point to an increase in toxic damage due to both increased pro-oxidants and decreased antioxidants<sup>2</sup>. Several authors have reported elevated levels of the oxidative stress indicators MDA and nitric oxide (NO), along with lower levels of the antioxidant molecule GSH in schizophrenia patients as compared to healthy controls<sup>4,5</sup>.

### Materials and Methods

This observational study was carried out in the department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU) from September 2013 to January 2015. Ninety three schizophrenia patients were recruited in this study and 30 healthy individuals were taken as control.

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Patients with schizophrenia attending the outpatient department or admitted in the inpatient department of psychiatry, BSMMU were selected in the study. Healthy controls (n=30) were included from outpatient department of BSMMU.

Care givers of patient were always present and for treatment, standard protocol according to DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, 2013) was followed by the department of Psychiatry, BSMMU. Apparently healthy attendants of patients and people without any known diseases who came only for routine checkup and investigations were enrolled as control.

The patients with following characteristics were included in the study:

- i) Study Group: Patients with schizophrenia from 18 years above of either sex diagnosed by Psychiatrists of BSMMU.
- ii) Control Group: Apparently healthy persons matched with age and sex of study group.

Patients with following characteristics were excluded from the study:

- i. Patients receiving antipsychotic drugs within last 30 days.
- ii. Patients taking vitamin E, vitamin C for last 3 months.
- iii. Patients having serious cognitive deficit that causes disturbance of communication.
- iv. Patients having serious infection or terminal illness.

The patients who meet above-mentioned inclusion criteria were selected and briefed about the trial. Patient's information sheets were given to each participant. Written informed consent, by explaining the nature of the study and information regarding age, sex, occupation, family status, economic status, marital status, were taken from each patient and/or their care givers before they were enrolled in the study. There were patients with schizophrenia (n=93) as study group and apparently healthy persons of similar age and sex as control group (n=30). Blood collection was done for only once after overnight fasting to measure MDA, GSH, vitamin C and vitamin E levels. With all aseptic precaution 5 ml blood were collected by venepuncture from the antecubital vein and kept it in 1 x 5ml K3EDTA (anticoagulant) containing test tube. Plasma was separated by centrifugation (3,500 rpm for 10 minutes) and stored at labeled Eppendorf by micropore. The plasma and buffy coat were separated from the top and the remaining packed RBCs were washed thrice with five volumes of cold 0.9% saline by

centrifugation at 3,500 rpm for 10 minutes. The packed cells were suspended in five volumes of deionized distilled water at 4°C for 24 hours to lyse RBCs. Then centrifuged it at 3,500 rpm for 10 min to collect RBCs lysate. Plasma and erythrocyte hemolysate were stored at -10°C in BSMMU to estimate parameters of the study.

Plasma MDA level was estimated by UV-180 Spectrophotometer (Shimadzu) by thiobarbituric acid (TBA) reaction method of Yagi<sup>6</sup>. Estimation of erythrocyte GSH level was based on the method of Ellman<sup>7</sup>. Estimation of plasma vitamin C level of Rahman et al<sup>8</sup> and vitamin E level of Baker and Frank<sup>9</sup> were also determined using established method.

Data were collected on variables of interest using a semi-structured questionnaire (research instrument) by interviewing and biochemical examination. Collected data was checked daily and edited into Excel sheet.

Data were processed and analyzed using computer software SPSS (Statistical Package for Social Sciences) version 16.0. The test statistics unpaired sample t-test was used to compare the continuous data between and within groups respectively.

## Results

The mean plasma MDA levels±SD in study group and control were 6.3±0.5 µmol/L and 2.1±0.5 µmol/L respectively. MDA level was higher in study group and difference from control group was significant (p=0.001). The mean erythrocyte GSH levels±SD in study group and control were 0.6±0.2 mg/gm of Hb and 2.1±0.5 mg/gm of Hb respectively. GSH level was lower in study group than control and difference was significant (p=0.001). The mean plasma vitamin E levels±SD in study group and control were 4.3±0.6 mg/L and 7.3±0.7 mg/L respectively. Vitamin E level was lower in study group and difference was significant (p=0.001). The mean plasma vitamin C levels±SD in study group and control were 30.6±8.3µmol/L and 78.7±10.9 µmol/L respectively. Vitamin C level was lower in study group and difference was significant (p=0.001). Results are shown in Table-I.

**Table-I:** Oxidative stress markers in study group and control group

Oxidative Stress Markers	Group		P value
	Study group (n=93)	Control (n=30)	
Plasma MDA (µmol/L)	6.3±0.5	2.1±0.5	0.001
Erythrocyte GSH (mg/gm of Hb)	0.6±0.2	2.1±0.5	0.001
Plasma vitamin E (mg/L)	4.3±0.6	7.3±0.7	0.001
Plasma vitamin C (µmol/L)	30.6±8.3	78.7±10.9	0.001

Data were analyzed using Unpaired t-Test and were presented as mean±SD

## Discussion

A growing body of evidence suggests that oxidative stress is involved in the pathophysiology of schizophrenia and results regarding oxidative stress and antioxidant status in this disease are divergent. In this study, examination of oxidative stress status revealed that the MDA level, an indicator of oxidative stress, was significantly higher in schizophrenia patients as compared to control subjects. We also assessed the antioxidant status by measuring GSH level in erythrocyte and non-enzymatic antioxidants plasma vitamin E and vitamin C levels. We found a significantly lower level of GSH in schizophrenia patients compared to control group. Vitamin E and vitamin C levels were also significantly lower in patients in comparison to control group.

In a similar study, assessment of oxidative stress showed that the MDA level was significantly elevated in schizophrenia patients compared to control subjects ranging in age from 18 to 60 years<sup>5,10</sup>. In contrast, GSH, SOD and vitamin C values show a significant decrease in sera ( $p < 0.005$ ) of schizophrenia patients when compared with those of control group. Previous study also recorded a significant decrease in the RBC levels of total GSH in schizophrenia patients in comparison with controls<sup>5</sup>.

In another study plasma levels of MDA in schizophrenia patient groups were found to be increased three to four fold compared to normal controls<sup>11</sup>. Also elevated levels of MDA have been shown in plasma, erythrocyte, leukocytes and platelets of patients with schizophrenia<sup>12</sup>. The finding of an elevation in plasma levels of MDA is consistent with the majority of studies of schizophrenia with increased lipid peroxides<sup>13,14</sup>. However, some other studies have reported no significant difference in lipid peroxides between patients and control<sup>15</sup>.

It has been anticipated that antioxidant defense mechanisms could be impaired in patients with schizophrenia<sup>16</sup>. Vitamin C and vitamin E act as radical scavenging antioxidants and suppress lipid peroxidation<sup>17</sup>. In this study, a significant reduction ( $p = 0.001$ ) in erythrocyte GSH, plasma vitamin E and vitamin C levels was found in schizophrenia patient compared to control group. Issa et al measured MDA, GSH, SOD and vitamin C levels in sera of seventy schizophrenia patients and thirty healthy individuals, where a significant decrease ( $p < 0.005$ ) in GSH, SOD and vitamin C values in sera of schizophrenia patients was observed in comparison with control group<sup>10</sup>. These results of decreased GSH and vitamin C are consistent with the hypothesis. Basal ganglia, which are rich in dopamine, also contain the highest level of

vitamin C<sup>18</sup>. Vitamin C serves as an essential defense line against dopamine induced neuro-degenerative processes<sup>19</sup>. As most of the vitamin C is utilized for scavenging free radicals and inhibiting lipid peroxidation, levels of vitamin C may be decreased in such patients<sup>20</sup>.

The levels of vitamin E, the most potent lipid bound chain breaking antioxidant in serum was found to be significantly lower in schizophrenia patients as compared to control<sup>21</sup>. The report is consistent with our results of decreased vitamin E level in schizophrenia patients. This decrease is probably contributed to the increased consumption of vitamin E for free radical neutralization and its conversion to  $\alpha$ -tocopheroxyl radical<sup>22</sup>.

There were some limitations of this study. Testing sample (blood, plasma or serum), age, diet and smoking could influence the results. The number of patients studied was not large enough to draw a definitive conclusion. We could not measure the activities of enzymatic antioxidants.

## Conclusion

It is evident from this study that oxidative stress occurs in schizophrenia patients. By increasing the level of antioxidants like vitamin C, vitamin E by supplementation particularly with those vitamins may play an important role in the treatment of schizophrenia within certain subgroups. It might have an influence on improving the symptom status of schizophrenia that needs to be explored through a broad based study.

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