ALTERED OXIDANT AND ANTIOXIDANT STATUS IN DIFFERENT SYMPTOMS OF SCHIZOPHRENIA AND ITS EFFECT ON ACUTE, CHRONIC PATIENTS

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ABSTRACT

Objective: The aim of this study is to investigate the status of antioxidants, nitric oxide and malondialdehyde in the schizophrenia patients with positive, negative and cognitive symptoms and also investigate the status of these oxidants and antioxidants in the acute and chronic stages of schizophrenia. Method: The study was conducted among 60 schizophrenics, and 60 healthy volunteers. All patients were selected from a local mental health care center. The activities of three free radical scavenging enzymes (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), and plasma nitrate levels, an index of in vivo nitric oxide production and the level of thiobarbituric acid-reactive substances (TBARS) as an index of lipid per oxidation were analyzed in patients with positive(n=20), negative(n=20) and cognitive (n=20)symptoms. The study also included the effect acute and chronic phases of schizophrenia on the level of these oxidants and antioxidants in the selected patients. Results: Compared with the control groups, the average values of plasma-Nitrite, erythrocyte SOD, LPO were significantly increased (P<0.01), and of E-CAT and E-GSH-Px decreased (P <0.01) in the patient groups, but statistically more significant increase in the activity of SOD was found for schizophrenics with positive symptoms. Schizophrenics with positive symptomology were found to have pronounced decrease in the activities of GSH-Px
and statistically more significant decrease in CAT was found in negative symptomatic people. Plasma nitrite level was increased more significantly in patients with cognitive and negative symptoms compared with positive symptomatic patients. Further a significant rise in oxidative stress and decreased antioxidant status was observed in the chronic stage of schizophrenics as compared to those in acute condition. The study showed that the level of malondialdehyde was increased in schizophrenics with positive (163%), negative (137%) and cognitive (132%) symptoms compared to control groups. **Conclusion:** The findings of this study reveal that antioxidant defense mechanisms might be impaired in schizophrenic patients and may further support the heterogeneity among positive, negative and cognitive symptoms of schizophrenia.

**Keywords:** Schizophrenia, symptoms, antioxidant enzymes, Nitric Oxide, Malondialdehyde.

**INTRODUCTION**
A major mental disorder that affects young people, schizophrenia (Greek ‘split mind”) is characterized by a variety of symptoms including but not limited to, loss of contact with reality, bizarre behavior, disorganized thinking, speech, decreased emotional expressiveness, loss of contact with reality and society and long-lasting, not completely successful treatment. In general, schizophrenia has symptoms that fall into three categories - positive, negative and cognitive ((Frances R Frankenberg, 2006).

Schizophrenia affects about 24 million people worldwide. Though the incidence is low (3-10,000), the prevalence is high due to chronicity. Various psychologic, social, developmental, environmental, anatomic, genetic, biochemical, and other factors have been involved in the pathogenesis of schizophrenia (Herken et al., 2001). There is an increasing evidence that oxidative injury contributes to pathophysiology of schizophrenia, indicated by the increased lipid per oxidation products and nitric oxide metabolites in plasma and altered levels of enzymatic antioxidants in schizophrenic patients (Mahadik and Scheffer, 1996).

The chemical nature of the schizophrenic brain is still not completely understood. The brain makes up about 2% of body mass but consumes 20% of metabolic oxygen. The vast majority of energy is used by the neurons (Shulman et al., 2004). Due to the lack of glutathione-producing capacity by neurons, the brain has a limited capacity to detoxify ROS. Therefore, neurons are the first cells to be affected by the increase in ROS and shortage of antioxidants and, as a result, are most susceptible to oxidative stress. The radical-induced damage may be
important in schizophrenia, as there is increasing evidence that oxidative injury contributes to the pathophysiology of schizophrenia (Lohr, J.B. 1991). The hypothesis that reactive oxygen species (ROS) play an important role in schizophrenia as well as neurodegenerative disorders remains speculative and there have been no detailed studies to test this hypothesis. Under normal conditions, a dynamic equilibrium exists between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell (Granot and Kohen, 2004).

A growing body of evidence suggests that peripheral activities of antioxidant enzymes and lipid per oxidation are abnormal in schizophrenic subjects (Reddy and Yao, 1996). Mahadik found increased lipid per oxidation products and altered defense system in both chronic and drug-naive first episode schizophrenics (Mahadik and Scheffer, 1996).

The interest in the role of nitric oxide (NO) in psychiatric disorders is currently increasing. Today, the role of NO in the etiology of psychiatric disorders such as schizophrenia, affective disorders, substance abuse and autism is investigated extensively (Sweeten et al., 2004).

Though there is accumulating evidence of altered antioxidant capacity in schizophrenia, studies of antioxidant systems in schizophrenia have given dissimilar results. The free radical mediated oxidative injury in various sub types of Schizophrenia has so far been reported in few literatures. However, the status of antioxidants and the extent of per oxidation in erythrocytes have not been investigated much in schizophrenia patients with different symptoms. In order to examine the antioxidant and oxidant status in the schizophrenics with positive, negative and cognitive symptoms, the activities of three free radical scavenging enzymes (superoxide dismutase (SOD), catalase (CAT)), glutathione peroxidase (GSH-Px) and the level of nitric oxide metabolites and malondialdehyde as an index of lipid per oxidation were investigated. The effects of acute and chronic phase of schizophrenia on the level of these antioxidants were also analyzed. The present study was undertaken during the month of September 2004 to June 2007, in the Postgraduate and Research department of biochemistry, Dr. N.G.P Arts and Science College, with the collaboration of Kovai Medical Centre and Hospital (KMCH), a multispeciality hospital with a separate division for Psychiatry.
MATERIALS AND METHODS

Patients
A total of 60 schizophrenic patients of age group 18-65 years of both sexes from good socio-economic background were selected from Udhayam Mananala kaapagam, a mental Health care center, Coimbatore, Tamilnadu, India. The patients were divided into three groups: (1) schizophrenics with positive symptoms, n= 20, (2) schizophrenics with negative symptoms, n = 20, and (3) schizophrenics with cognitive symptoms, n= 20. They all met DSM-IV (Diagnostic and Statistical Manual of Mental Disorders-IV) criteria (American Psychiatric Association, 2000) for schizophrenia. Informed and written consent was obtained from all subjects prior to examination. Patients with a history of drug abuse or dependence, serious medical conditions, severe head injury or seizure disorders were excluded from the study. With the help of team of psychologists, the participants were interviewed at the time of collection of biological samples and information regarding their age, family background, family medical history and economic status were collected. Information regarding chronic illness, smoking, alcohol consumption and drug intake was obtained by questionnaires.

CONTROL
Sixty age and sex-matched healthy normal control subjects with no individual and familial history of mental illness were recruited to participate in this study. They included 30 males and 30 females with their ages ranged from 15 to 65 years. Both patients and controls were recruited during the same period from Coimbatore district. Matching between the patients and controls was done according to sex and age. Study subjects were currently within normal ranges in their routine blood, urine and feces tests, electrocardiograph and radiographs; disorders associated with heart, brain, lung, liver, kidney and other pivotal organs were excluded.

The design and the layout of this project was carried out with the approval of the Chairman, Kovai Medical Center and Hospitals, and due permission was obtained from the board of institutional review Committee of the Kongu mananala Arakkattalai, before the start of the work. Informed and written consent was obtained from all subjects prior to examination.

Determination of antioxidants and MDA Levels
SOD (EC 1.15.1.1) and CAT (EC 1.11.1.6) activities were determined by the methods of Das et al., (2000) and Aebi (1974). GSH-Px (EC 1.6.4.2) activity was measured by the method of Takahashi (2000). MDA levels was estimated according to the method of Ohkawa
et al (1979) with minor changes adopted by Devasaghayam et al (2003). Plasma nitrite level was estimated by the method of (Zhou et al., 2000). Activities of SOD, CAT, and GSH-Px were expressed as Units per gram hemoglobin. Results of MDA levels were expressed as nanomoles per gram hemoglobin. Concentration of nitric oxide metabolite was expressed as nmol /L.

All reagents used were of analytical reagent grade, obtained from Sigma Chemicals, St. Louis, MO, U.S.A and were used without further purification and samples were done in triplets. Statistical analysis between control and patient groups were performed by students’ t’ test. The results were expressed as a difference between the two values. All the values were presented as a mean value ± SD.

RESULTS
Results in Table 1 shows significant increase in SOD activity and Nitrite, MDA levels in all the study groups compared to the control group. Erythrocyte superoxide dismutase activity was increased more significantly in schizophrenics with positive type (379.69 ± 19.9 U/g Hb, p<0.001) compared to control values (293.27± 66.14), There is also statistically significant difference among schizophrenics with various symptoms (p<0.01).

The estimation of glutathione peroxidase activity suggests a statistically significant decrease in all groups of schizophrenic patients, which was more expressed in schizophrenics with positive symptoms. (35.97 ± 1.24 U/g Hb, p<0.001). The levels of erythrocyte CAT were significantly decreased in schizophrenics, compared to controls, but the decrease is statistically more significant in schizophrenics with negative symptoms.

Results also showed that there was a significant increase in the levels of plasma nitrite and malondialdehyde in all groups compared with control groups, but we observed a more significant increase in the level of nitrite in schizophrenics with cognitive and negative symptoms compared with positive symptoms.

Among all schizophrenics, the Activity of all antioxidant enzymes were more decreased in chronic patients as compared to acute ones (Table-2), and similarly the oxidant status was found higher in chronic cases as compared to acute patients.
Table 1
Mean antioxidant enzyme activities and MDA levels of schizophrenics with different symptoms and P values among the study and control groups. Results were expressed as mean ± standard deviation

<table>
<thead>
<tr>
<th>Schizophrenic Groups</th>
<th>SOD U/g of Hb</th>
<th>CAT U/g of Hb</th>
<th>GSH-Px U/g of Hb</th>
<th>Nitrite nmol/L</th>
<th>Lipid Peroxides nmolMDA*/g of Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Positive Symptoms</td>
<td>379.69± 19.901a*bc</td>
<td>9.88 ± 2.72a</td>
<td>35.97± 1.24a<em>bc</em></td>
<td>471± 21.78a*</td>
<td>10.7± 0.38a*</td>
</tr>
<tr>
<td>II Negative Symptoms</td>
<td>342.09 ± 45.71a</td>
<td>8.73 ± 0.42a*d</td>
<td>51.92 ± 1.02a</td>
<td>562± 35.29a<em>b</em></td>
<td>9.62± 0.22a</td>
</tr>
<tr>
<td>III Cognitive Symptoms</td>
<td>334.18 ±76.36a</td>
<td>10.27 ± 0.25a</td>
<td>49.81 ± 1.19a</td>
<td>589±57.31a*c</td>
<td>9.13± 0.65a</td>
</tr>
<tr>
<td>IV Control</td>
<td>293.27 ±66.14</td>
<td>13.85 ± 1.79</td>
<td>74.68 ±1.32</td>
<td>373±12.06</td>
<td>7.29± 0.73</td>
</tr>
</tbody>
</table>

a b c d  p<0.01 ,a* b*c* p<0.001
a (statistical significance compared to control group).
b (statistical difference between positive and negative group),
c (statistical difference between positive and cognitive group),
d (statistical difference between negative and cognitive group)

Table -2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acute Schizophrenics</th>
<th>Chronic Schizophrenics</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/g of Hb)</td>
<td>375.13 ± 32.43</td>
<td>312.83 ± 20.345*</td>
</tr>
<tr>
<td>CAT (U/g of Hb)</td>
<td>9.92 ± 0.61</td>
<td>7.69 ± 0.49*</td>
</tr>
<tr>
<td>GSH-Px (U/g of Hb)</td>
<td>63.12 ± 1.76</td>
<td>43.88 ± 1.40*</td>
</tr>
<tr>
<td>Nitrite nmol/L</td>
<td>472± 24.15</td>
<td>566±42.09*</td>
</tr>
<tr>
<td>Lipid Peroxides (nmolMDA*/g of Hb)</td>
<td>7.26 ± 0.523</td>
<td>12.92± 0.791**</td>
</tr>
</tbody>
</table>

MDA, Nitrite and Enzymic antioxidant levels of acute and chronic Schizophrenics (Values are mean ± SD)

Statistical comparison was done between acute and chronic Schizophrenics *p < 0.01  
**p<0.001
DISCUSSION

The brain contains both enzymatic and non-enzymatic antioxidants against free radical damage. As the intensity of lipid per oxidation and antioxidative defense in erythrocytes to a certain extent reflects the state of the cell membranes of different tissues, including brain tissue (Vilkov et al., 1991). In the current study, oxidant and antioxidant status in erythrocytes of schizophrenic patients with positive, negative and cognitive symptoms were investigated.

Based on results, a significant increase in the levels of erythrocyte SOD in all schizophrenic patients was observed when compared to controls. No significant differences in SOD activity between negative and cognitive symptomatic groups was found. But schizophrenics with positive psychosis have statistically highly significant elevation in SOD activity. Many research groups, (Lohr JB, 1991; Vaiva et al, 1994 Zhang et al 2006) have demonstrated an increased SOD erythrocyte activity in schizophrenic patients, But Mahadik et al. and Mukherjee et al have shown their contrasting results. This discrepancy may be due to the different duration of the disease in different studies. The changes in erythrocyte SOD activity in positive schizophrenia found in the current study confirm the results of Vaiva et al. and Dusica et al.(2002). Their hypothesis on the relationship between catecholaminergic hyper-metabolism and SOD increase in positive psychosis is supported by the fact that SOD activity could be an adaptive response of this enzyme to increased production of O2 following oxidative decomposition of catecholamines. On the other hand, an increase in SOD activity results in reduction of quantity of O2, which is important for the process of nitric oxide (NO) degradation. Despite important physiological roles of NO, excessive formation or inadequate degradation of this compound (NO) has been suggested an important factor in the etiology of neurological disorders (Heales et al., 1997).

GSH-Px, an oxidative stress-inducible enzyme, plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes. In the current study, GSH-Px activities have been decreased significantly in schizophrenia patients with different symptoms. (Positive (149 %), negative (123%) and cognitive (127%). The noticeable decrease in GSH-Px activity in positive symptomatic patients ,found in the current study also similar to the results of Dusica et al (2002) and Zhang et al.,2006).The decrease in the activity of GSH-Px could be due to its exhausted adoptive response to
counter the effect of increased oxidative stress (Dusica et al, 2002). GSH-Px provides an effective protective mechanism against cytosolic injury, because it eliminates H2O2 and lipid peroxides by reduction, utilizing GSH (Mahadik and Soheffer, 1996). The free radicals produced during the metabolism of catecholamines may result in neurotransmission abnormalities at dopamine terminals. Decreased erythrocyte GSH-Px activity in all types of schizophrenia could be due to fact that the affinity of selenium GSH-Px for glutathione is low (Mukerjee et al., 1996), so GSH-Px is not saturated with glutathione even at high concentrations of this substrate. Decreased glutathione content, found in Dusica et al (2002) study, supports this hypothesis. The obtained decrease in this enzyme activity could be a consequence of exhausted adaptive response to a long-lasting oxidative stress during chronicity of this disease.

In the present study, decrease in catalase activity in schizophrenic patients, as compared with normal healthy subjects, was observed. There was a significant decrease in CAT activity in the schizophrenics with negative symptoms compared to positive and cognitive symptoms, but there is no significant differences between negative and cognitive symptoms were noticed. Catalase is the enzyme that protects the cells from the accumulation of hydrogen peroxide, by dismutating it to form water and oxygen or by using it as an oxidant in which it works as a peroxidase (Lenzi et al., 1993). It is likely that sustained oxidative stress may decrease CAT and GSH-Px activity. Decreased antioxidant defense probably exist later in patients under chronic treatment with neuroleptics (Yao et al., 1999).

Results showed that there was significant increase in the level of plasma nitrite in all types of schizophrenics, but the increase is more significant in patients with cognitive and negative symptoms. In the brain nitric oxide regulates noradrenaline (NA) and dopamine (DA) release, memory, learning, awareness, smelling, food and liquid intake (Savas et al., 2002). Obviously, the level of NO increases in patients and alters with alleviating of psychiatric cognitive symptoms as proposed; which is quite consistent with the work of Herken et al. (2001b) who reported a remarkable increase in nitrite plus nitrate levels in red blood cells of patients with schizophrenia compared with control subjects. It seemed that nitrite may play a critical role in schizophrenia.

Karson et al (1996) showed that the NOS concentration is increased in the cerebellar vermis of postmortem brains of those who had schizophrenia. By means of ex vivo
experiments, Das et al (1995) showed NOS activity to be significantly elevated in the platelets of drug-naive patients with schizophrenia.

Examination of oxidative stress and antioxidant status revealed that the MDA level an indicator of oxidative stress was found to be significantly raised (P<0.001) in schizophrenics with various symptoms especially positive symptoms as compared to control subjects ranging in age from 15 to 65 years. Increased MDA levels in erythrocyte from our schizophrenia patients are consistent with the previous results of Herken et al., 2001, Hui-chun et al, 2006. The raised MDA level reflects the oxidative injury due to schizophrenia, which is attributed to free radical formation that abstracts hydrogen atoms from lipoproteins causing lipid per oxidation, of which MDA is the main product(Frei, 1999) and their delayed neutralization in the presence of low antioxidant concentration and condition is further aggravated by antipsychotic agents (Benedicta and Vivian2003).

Among all schizophrenics the activity of all antioxidant enzymes more decreased in chronic patients as compared to acute ones (Table-2), and similarly the oxidative stress was found higher in chronic cases as compared to acute. Further on comparing the levels of these enzymes in acute and chronic patients, its level depleted significantly in chronic subjects due to the increased oxidative stress levels found in chronic patients. Lipid per oxidation is an autocatalytic process, which ultimately results in cell death (D’Souza and D’Souza,2002) .Because of continuous generation of free radicals by the oxidation of hemoglobin, erythrocytes are exposed to continuous oxidative stress, which ends in insufficient neutralization of free radicals causes oxidation of cellular lipids(Afanas’ev,2005).Therefore it is claimed that the long term chronic complications of schizophrenics are related to the accumulation of increased free radicals and lipid per oxidation. Taken together, the above data reveal that antioxidant defense mechanisms might be highly impaired in schizophrenic patients with positive symptoms and nitric oxide metabolites were high in schizophrenics with cognitive and negative symptoms.

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