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ORIGINAL ARTICLE / RESEARCH

Status of Lipid Peroxidation, Glutathione, Ascorbic Acid, Vitamin E and Antioxidant Enzymes in Schizophrenic Patients

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ABSTRACT

The exact pro- and antioxidant status in schizophrenic patients is still not clear. To add a new insight to the question, changes in the erythrocyte lipid peroxidation products (MDA), levels of glutathione (GSH), ascorbic acid and plasma vitamin E (non-enzymatic antioxidant parameters) and activities of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GP_X) and catalase in erythrocytes were studied in 48 schizophrenic patients and 48 healthy subjects. It was observed that there was a significant increase in erythrocyte MDA levels and activities of SOD and GP_X and a significant decrease in erythrocyte GSH, ascorbic acid, plasma vitamin E levels and catalase activity in patients with schizophrenia, when compared to controls. The results of our study have shown higher oxygen free radical production, evidenced by increased levels of MDA and decreased levels of GSH, ascorbic acid, vitamin E and catalase activity, which supports the oxidative stress in schizophrenic patients. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. The decreased concentrations of the glutathione and antioxidant vitamin status support the hypothesis that lipid peroxidation is an important causative factor in the pathogenesis of schizophrenia. These data reveal that antioxidant defence mechanisms might be impaired in schizophrenic patients. These findings also provide a theoretical basis for the development of novel therapeutic strategies, such as antioxidant supplementation. This may suggest the hope for use of antioxidants in clinical trials to prevent and treat schizophrenic patients.

Key words: Malondialdehyde (MDA), glutathione (GSH), ascorbic acid, vitamin E, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), schizophrenia.

INTRODUCTION

If a homeostasis between rate of formation of free radicals and the rate of their neutralisation of free radicals is not maintained, an oxidative damage, known as oxidative stress, occurs [1].

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There is abundant evidence that free radicals are involved in membrane pathophysiology in the central nervous system and may play a role in neuropsychiatric disorders including schizophrenia. Schizophrenia is a hereditary, major mental disorder of the brain, resulting from abnormalities that arise early in life and disrupt normal development of the brain. The chemical nature of the schizophrenic brain is still not completely understood. The brain and nervous system are

particularly prone to the free radical damage, since the membrane lipids are very rich in polyunsaturated fatty acids and areas of human brain are very rich in iron, which plays an essential role in generating free radicals [2],[3]. The radical-induced damage may be important in schizophrenia, as there is increasing evidence that oxidative injury contributes to the pathophysiology of schizophrenia [4]. Free radicals, primarily the reactive oxygen species, superoxide and hydroxyl radicals, which are highly reactive, having an unpaired electron in an atomic or molecular orbit, are generated under physiological conditions during aerobic metabolism. As free radicals are potentially toxic, they are usually inactivated or scavenged by antioxidants before they can inflict damage to lipids, proteins or nucleic acids. Alteration in the oxidant–antioxidant profile is known to occur in Schizophrenia [5],[6]. Moreover the body's defence mechanisms would play an important role in the form of antioxidants and try to minimise the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals, or oppose their actions [1], and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated [7]. They exist in both the aqueous and the membrane compartment of cells and can be enzymes or non-enzymes. The human body has a complex antioxidant defence system that includes the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GP_X) and catalase. These block the initiation of free-radical chain reactions [4]. The non-enzymatic antioxidant components consist of molecules such as glutathione (GSH), vitamin E, ascorbic acid and beta-carotene that react with activated oxygen species and, thereby, prevent the propagation of free-radical chain reactions.

In the present study, the following parameters were assessed in the erythrocytes and plasma to elucidate the oxidant–antioxidant status in schizophrenic patients. Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid-reacting substances (TBARS), which serve as an index of extent of lipid peroxidation. Erythrocyte GSH, ascorbic acid and plasma vitamin E serve as non-enzymatic antioxidant parameters. The antioxidant enzymes SOD, catalase and GP_X in erythrocytes were estimated. The present study is an attempt to examine oxidative stress and the status of the protective antioxidants under condition of stress due to schizophrenia.

METHODS

The study was conducted in Department of Biochemistry, Dr. Pinnamaneni Siddhartha Institute of Medical Sciences & Research Foundation, Chinoutpally, Gannavaram (Mandal), A.P., INDIA. The study included 48 patients, without any organic illness, of either sex (30 males and 18 non-pregnant, non-lactating females) in the age group ranging from 22 to 65 years, newly diagnosed for schizophrenia, at OPD of Psychiatry, Dr PSIMS & RF General Hospital, Chinoutpally. The results were compared with those of 48 age- and sex-matched normal subjects who comprised the control group. Diagnosis of schizophrenia was made by Diagnostic and Statistical Manual of Mental Disorders (DSM – IV) classification. Subjects who had no other psychiatric disorder and subjects with normal nutritional habits without supplementing any vitamins during the previous 6 months were included. The complete clinical and personal history of the subjects was recorded. Subjects with history of electro-convulsive therapy (ECT) in last 1 year, substance dependence for last 1 month and history or present symptoms of any other stress-induced disorder were excluded. Informed consent was taken form patients before drawing blood. All the blood samples were collected before starting the treatment for schizophrenia. Due permission was obtained from the ethical committee of the Dr. PSIMS & RF General Hospital, Chinoutpally, before the start of the work.

The controls and patients were divided into two groups:

- Group 1: Forty-eight healthy age- and sex-matched subjects as controls.
- Group 2: Forty-eight schizophrenic patients.

The heparinised venous blood samples obtained from these subjects were used for the analysis. Plasma was separated by centrifugation at 1000 g for 15 minutes. Separated plasma was used for the estimation of vitamin E. The buffy coat was removed, and the packed cells were washed three times with physiological saline. The erythrocyte suspension was prepared by the method of Dodge et al [8], modified by Quist [9]. The packed cells were used for the analysis of GSH, ascorbic acid, MDA, SOD, catalyse and GP_X. Erythrocyte GSH was estimated by the method of Beutler et al [10], using dithio-bis-nitrobenzoic acid (DTNB). Ascorbic acid

levels were estimated in plasma by the method of Tietz [11]. Plasma vitamin E levels were estimated by the method of Baker et al [12]. Erythrocyte MDA was determined as the measure of TBARS [13]. SOD (EC 1.15.1.1) activity was determined in the hemolysate, according to the method described by Marklund and Marklund [14], with some modifications as described by 1 and Chatterjea [15]. Catalase (EC 1.11.1.6) activity was measured in the hemolysate by the method of Sinha [16], and the activity of GP_X (EC 1.11.1.9) was measured as described by Paglia and Valentine [17], in erythrocytes. All reagents used were of analytical reagent grade. DTNB and thiobarbituric acid were obtained from Sigma Chemicals, St. Louis, MO, U.S.A. Statistical analysis between group 1 (controls) and group 2 (patients) was performed by the student *t*-test using the stat-view package. The data were expressed as mean ± SD; $P < 0.05$ was considered as significant.

Table/Fig 1: The mean ± SD values of MDA, GSH, ascorbic acid, VITAMIN E, SOD, catalase and GP_X in controls and schizophrenic patients

Parameters	Group1 (controls) (*n* = 48)	Group 2 (patients) (*n* = 48)
Glutathione (mg/gm of Hb)	39.76 ± 3.88	24.94 ± 2.75**
Ascorbic acid (mg/dl)	11.88 ± 0.22	11.36 ± 0.11**
Vitamin E (μmol/L)	10.20 ± 0.41	9.06 ± 0.26*
MDA (nmol/g of Hb)	6.81 ± 0.46	7.43 ± 0.74**
SOD (U/g of Hb)	475.98 ± 28.11	511.04 ± 28.21*
Catalase (U/g of Hb)	9.47 ± 0.41	8.63 ± 0.25***
GP_X (U/g of Hb)	69.96 ± 1.53	71.59 ± 0.86**

*$P < 0.01$ compared to controls.
**$P < 0.001$ compared to controls.
***$P < 0.05$ compared to controls.

RESULTS

The mean ± SD of erythrocyte GSH, ascorbic acid, MDA, SOD, catalase, GP_X and plasma vitamin E was indicated in [Table/Fig 1]. There was a statistically significant increase in the erythrocyte MDA levels in schizophrenic patients, compared to the controls. The activities of erythrocyte antioxidant enzymes SOD and GP_X were significantly increased in group 2, compared to group1. The levels of erythrocyte GSH, ascorbic acid, plasma vitamin E and catalase activity were significantly decreased in patients with schizophrenia, compared to controls.

DISCUSSION

The results indicate that there is increase in free-radical generation and antioxidant defence is impaired in schizophrenic patients. Impaired antioxidant defence and increased lipid peroxidation have been reported in chronic schizophrenic patients [18]. The free radicals play an important role in the genesis of structural and functional changes of neuronal membrane that could be responsible for the beginning or aggravation of the basic disease [19]. The brain and nervous system possess high potentials for the initiation of free-radical reactions, which, relative to the other tissues, can cause more damage in the brain and nervous system, due to insufficient antioxidant protection and existing intensive aerobic metabolism, accompanied with oxygen free-radical production [20]. Of the different brain regions, the basal ganglia may be particularly at risk of radical-induced damage, because they contain large amounts of iron (which can be associated with increased free-radical production through the Fenton reaction). There are several ways by which excess free radicals may be generated in the brain. The metabolism of catecholamines, such as norepinephrine and dopamine, is probably associated with free-radical formation, and conditions associated with increased catecholamine metabolism may increase the free-radical production. Antipsychotic drugs can also cause an increase in metabolic turnover of catecholamines [21].

In the present study, the lipid peroxidation product, i.e. MDA, levels have been increased significantly in erythrocytes of the schizophrenic patients than that in control group. This may show the presence of increased oxidative stress. Rise in MDA could be due to increased generation of reactive oxygen species (ROS), due to the excessive oxidative damage generated in these patients. These oxygen species, in turn, can oxidise many other important biomolecules, including membrane lipids. The lipid peroxides and free radicals may be important in pathogenesis of schizophrenia [22]. 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 peroxidation, of which MDA is the main product [23]. The membrane phospholipids, specifically polyunsaturated fatty acids, are converted to MDA by peroxidation, which can be analysed by reactivity to thiobarbituric acid. Increased levels of thiobarbituric acid reaction products have been found in the cerebrospinal fluid of neuroleptic-treated patients [24] and also in plasma of schizophrenic patients, with or without tardive dyskinesia [25].

We observed a significant decrease in the levels of erythrocyte-reduced GSH, ascorbic acid and plasma vitamin E (non-enzymatic antioxidant defence system) in schizophrenic patients, when compared to controls. GSH, vitamin E and ascorbic acid are important chain-breaking antioxidants, responsible for scavenging the free radicals and suppression of peroxidation in aqueous and lipid region of the cell [26]. The decrease in the levels of these non-enzymatic antioxidant parameters may be due to the increased turnover, for preventing oxidative damage in these patients, suggesting an increased defence against oxidant damage in schizophrenic patients. Similar reports of decreased GSH, ascorbic acid and vitamin E levels in schizophrenic patients were reported by various studies [27].

In our study, the erythrocyte antioxidant enzyme, i.e. SOD and GP_x, activities have been increased significantly in patients with schizophrenia, compared to controls. The increased activity of SOD may be indicative of increased superoxide generation by whichever mechanism, like increased catecholamine metabolism. SOD is the important antioxidant enzyme, having an antitoxic effect against superoxide anion. The over-expression of SOD might be an adaptive response, and it results in increased dismutation of superoxide to hydrogen peroxide. In schizophrenia, increased erythrocyte SOD activities have been found in most of the studies [28],[29], but on the other hand, low erythrocyte SOD activities have also been reported in first episode of drug-naïve patients [30]. This discrepancy may be due to the different duration of the disease in different studies. GP_X, an oxidative stress-inducible enzyme, plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes [31]. The rise in the activity of GP_X could be due to its induction to counter the effect of increased oxidative stress. GP_X provides an effective protective mechanism against cytosolic injury, because it eliminates H_2O_2 and lipid peroxides by reduction, utilising GSH [4]. The free radicals produced during the metabolism of catecholamines may result in neurotransmission abnormalities at dopamine terminals. The brain has certain attributes that make it exceptionally vulnerable to free-radical attack. It has highly oxygenated structures responsible for almost one-fifth of the body's total oxygen. In addition, there is disruption of brain energy metabolism, mediated by antioxidant perturbation [32]. Decrease in antioxidant enzyme status probably exists later in patients under chronic treatment with neuroleptics [22],[33].

In the present study, we have observed a significant decrease in the activity of catalase in patients with schizophrenia, compared to controls. 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 [34]. A decrease in catalase activity in schizophrenic patients, as compared with normal healthy subjects, was also observed [35].

In conclusion, oxidative stress may be involved in schizophrenic patients. The results of our study have shown higher oxygen free-radical production, and decreased catalase activity supports the higher oxidative stress hypothesis in schizophrenic patients. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. Lipid peroxides could be a part of the cytotoxic mechanisms leading to the neural injury. The decreased concentrations of the glutathione and antioxidant vitamin status support the hypothesis that lipid peroxidation is an important causative factor in the pathogenesis of schizophrenia. It is evident from the study that increased oxidative stress in schizophrenics leads to decrease in the levels of antioxidants like GSH, vitamin E and ascorbic acid and disturb their metabolism, which weaken their ability to fight the growing stress. Intense oxidative stress and decreased antioxidants may contribute to neural death and alter the information processing in schizophrenia. So, the treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidative damage and deterioration of the neural tissues in schizophrenic patients. Further studies are needed to use antioxidants such as vitamin E, ascorbic acid and beta-carotene as

secondary therapy, in addition to current drug therapy in schizophrenia.

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