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Original Article

Status of Antioxidant Vitamins and Reduced Glutathione in schizophrenia patients with positive, negative and cognitive symptoms

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ABSTRACT

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. A disturbance in the antioxidant defense system including α-tocopherol, ascorbic acid, vitamin B_{12}, folic acid and reduced glutathione metabolism due to free radical induced oxidative injury has been implicated in various neuro-psychiatric disorders including schizophrenia. The current study was undertaken to assess the non-enzymatic antioxidants status in patients with schizophrenia. A total of 60 schizophrenic patients of age group 18- 65 years of both sexes from good socio-economic background were selected and 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 for schizophrenia. 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. Fresh blood samples were collected and plasma was separated to measure the status of non-enzymatic antioxidants. It was observed from the results that there was a significant decrease in Vitamin B_{12}, folate, ascorbic acid, vitamin E levels and reduced glutathione activity in patients with various symptoms of schizophrenia when compared to controls. The results confirm the higher oxygen-free radical production, evidenced by decreased GSH, ascorbic acid, vitamin E and folic acid, support to the oxidative stress in schizophrenia and suggest that the supplementation of antioxidants may prevent further oxidative injury in schizophrenics.

Keywords: Schizophrenia, antioxidant defense system, ascorbic acid, reduced glutathione, vitamin E, oxidative stress

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 [1]. 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 [2]. There is increasing evidence that oxidative injury contributes to pathophysiology of schizophrenia since the radical-induced damage have been seen in many patients with schizophrenia [3]. 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

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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 [4].

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 [5]. 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 [6]. This protective mechanism functions cooperatively in form of a cascade in which the cellular antioxidants α-tocopherol, ascorbic acid and reduced glutathione act in combination [7]. α - tocopherol is a chain breaking antioxidant that by neutralizing a free radical gets converted to α-tocopheroxyl radical. It can be reduced back to alpha-tocopherol by ascorbic acid. Dehydroascorbic acid formed in this reaction can be reconverted back to ascorbic acid by reduced glutathione [8]. It is important that sufficient amounts of α-tocopherol, reduced ascorbic acid and reduced glutathione be present within the cell so as to provide protection against oxidative injury. The literature suggests that folate, vitamin B_{12}, and vitamin B_6 deficiencies may result in defect in brain pathology, both indirectly, through the influence on homocysteine concentration, and directly, through actions on neuronal and supporting cells that have not yet been well defined [9].

Vitamin C is water soluble acid based and it needs to be consumed every four hours. The body uses Vitamin C quickly and stress increases the body's need four times. There appears to be an inverse correlation between ascorbic acid intake and the risk of schizophrenia, even when the dietary vitamin C intake is adequate for normal, schizophrenics may have decreased plasma vitamin C levels and may demonstrate a greatly reduced urinary excretion of ascorbic acid after an ascorbic acid load [10]. Many neurological and psychiatric disease processes are characterized by high levels of oxidative stress and free radical formation, as well as abnormalities in glutathione metabolism and antioxidant defenses. Glutathione is the brains main antioxidant and also best kept secret to maintaining health. Glutathione is a small molecule made up of three amino acids, which exists in almost every cell of the body. However, Glutathione must be generated within the cell from its precursors before it can work effectively in the body [11]. Without glutathione, other important antioxidants such as vitamins C and E cannot do their job adequately to protect human body against disease.

Generation of reactive oxygen species (free radicals) and oxidative damage are an important cause of neuron (brain cell) death from brain injury. Chemicals that cause toxicity to certain brain cells are known to decrease cerebral glutathione (GSH), making the cells more vulnerable to reactive oxygen species (ROS) [12]. The free radical mediated oxidative injury in various sub types of schizophrenia has so far been reported in few literatures. To the best of our knowledge none of the reports in the literature have stated about the role of the non-enzymic antioxidants in patients with different schizophrenia symptoms such as positive, negative and cognitive. Therefore, the current study has been undertaken with the objective to explore the Status of Antioxidant Vitamins and Reduced Glutathione in schizophrenia patients with positive, negative and cognitive symptoms.

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, schizophrenics with positive symptoms, negative symptoms and cognitive symptoms with 20 patients in each group. They all met DSM-IV (Diagnostic and Statistical Manual of Mental Disorders-IV) criteria (American Psychiatric Association, 2000) [13] 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. All subjects did not take any antioxidant supplements such as vitamin C, vitamin E, β-carotene, any plant based Ayurvedha or siddha medicines or other similar substances within one month prior to blood draw. A standard diet was given to all inpatients. 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.

Blood Sampling: Blood collection was carried out in dust free environment and serum and plasma has been separated. Plasma was used for the estimation of ascorbic acid, α-tocopherol, and reduced glutathione. Serum was used for the estimation of vitamin B_{12} and folic acid.

Determination of non-enzymic antioxidants levels: Vitamin B_{12} activity in the serum was measured by the method of NCCLS method (1980) [14]. Activity of serum folic acid was measured by the method of Henderson *et al* (1986) [15]. Plasma Ascorbic acid levels were measured by the method of Omaye *et al* (1979) [16]. Vitamin E levels were measured by the method of Baker *et al* (1980) [17]. Erythrocyte GSH was measured by the standard method of Beutler *et al* (1963) [18]. All the operations accord with the guidelines of the apparatus and samples were done in triplets. Statistical analysis between control and patient groups were performed by students't' test using SPSS package (version 15.5). 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 summarize all analyzed biochemical parameters which showed a significant decrease in the levels of folic acid, vitamin B_{12}, ascorbic acid, plasma vitamin E and erythrocyte GSH (non-enzymatic antioxidant defense system) in patients with various symptoms of schizophrenia when compared to normal healthy controls. 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 defense against oxidant damage in schizophrenia. Plasma ascorbic acid and α-tocopherol levels were decreased statistically more significantly in schizophrenics with positive type (0.54 ±0.18 mg/dl, $p<0.001$ and 0.47±0.24 mg/dl, $p<0.001$) compared to control values (1.78±0.13mg/dl and 1.38±0.15 mg/dl). There is also statistically significant difference among schizophrenics with various symptoms ($p<0.01$). However, there was no significant difference in ascorbic acid and α-tocopherol activities between the negative and cognitive groups. The estimation of vitamin B_{12} and folic acid levels suggest a statistically significant decrease in all groups of schizophrenic patients, which was more expressed in schizophrenics with positive symptoms (468.69 ±96.53pg/ml, $p<0.001$; 7.42±2.07, $p< 0.001$) in comparison to control values (537.96 ±78.47 pg/ml and 14.47±1.43 ng/ml). The levels of erythrocyte GSH was significantly decreased in schizophrenics, compared to controls ($p<0.001$), but the decrease is statistically more significant in schizophrenics with negative symptoms ($p<0.001$).

DISCUSSION

The brain contains both enzymatic and non-enzymatic antioxidants against free radical damage. The intensity of lipid peroxidation and antioxidative defense in erythrocytes, to a certain extent reflects the state of the cell membranes of different tissues, including brain tissue [19]. So the analysis was made in the antioxidant vitamin status in erythrocytes of schizophrenic patients with positive, negative and cognitive symptoms.

Results showed that there is a significant decrease in the levels of plasma folic acid in all schizophrenic patients, when compared to controls. Also, no significant differences in folate activity between negative and cognitive symptomic groups have observed, but schizophrenics with positive psychosis have statistically highly significant depletion in folate activity. Folic acid deficiency occurs in 10 to 30% of hospitalized psychiatric patients. In addition to psychosis, the deficiency is associated with depression, confusion, disorientation and dementia as well as with neurological symptoms such as numbness, stiffness, plasticity and weakness, both with and without muscular atrophy [20].

In a randomized double-blind study reported in the Lancet, schizophrenics with low red cell folate levels, but without a deficiency of vitamin B_{12} or significant cognitive impairment, received either methylfolate 15mg daily or placebo in addition to standard psychotropic drugs. After 6 months, there were significant differences in mean clinical

outcome scores between groups in favor of the folate group [21]. Also, hyperhomocysteinemia due to an inborn error of folate metabolism may present as a schizophrenic syndrome sometimes accompanied by neurological signs. Case reports suggest that folic acid supplementation, usually along with either vitamin B_6 or vitamin B_{12} appears to be effective. Many people with schizophrenia, especially young males, tend to have a high level of the toxic protein called homocysteine, despite no obvious dietary lack of these vitamins. High levels of homocysteine and low blood levels of folic acid have been reported in schizophrenia by many research groups [22].

Specific mechanisms by which folate deficiency may be implicated are starting to be identified. Folic acid may be an essential cofactor in the conversion of the omega-6 fatty acids to prostaglandins, [23] and early evidence suggests that impaired prostaglandin metabolism may provoke a schizophrenic picture.

In the current study, vitamin B_{12} activities have been decreased significantly in schizophrenia patients with different symptoms [positive (167 %), negative (123%) and cognitive (143%)]. Vitamin B_{12}, which like folic acid is involved in methylation, has also been shown to help schizophrenic patients. Vitamin B_{12} is difficult to absorb, especially in large amounts, and some doctors have reported good results giving weekly, or twice-weekly, injections of 1mg of vitamin B_{12} A form of B_{12}, methyl B_{12} is more easily absorbed. It was proven that a combination of folic acid, B_{12} and vitamin B_6 has been shown to be most effective in improving the mental health, and lowering the homocysteine levels of schizophrenia patients with high homocysteine levels [24].

There is a significant decrease in the levels of erythrocyte-reduced GSH, ascorbic acid and plasma vitamin E (non-enzymatic antioxidant defense system) in schizophrenic patients has been noticed, when compared to controls. The decrease of antioxidants ascorbic acid and α- tocopherol is more significant in schizophrenia patients with positive symptoms ($p<0.001$) and at the same time, the decrease of glutathione is pronounced significantly in patients with negative symptoms. 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. 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 [25]. Similar reports of decreased GSH, ascorbic acid and vitamin E levels in schizophrenic patients were reported by various studies. There appears to be an inverse correlation between ascorbic acid intake and the risk of schizophrenia. Even when the dietary vitamin C intake is adequate for normals, schizophrenics may have depressed plasma levels and may demonstrate a greatly reduced urinary excretion of ascorbic acid after an ascorbic acid load and a number of studies have shown that people diagnosed with mental illness may have much greater requirements for this vitamin – often ten times higher – and are frequently deficient [26].

Vitamin E is a major lipid-soluble antioxidant, and is the most effective chain-breaking antioxidant within the cell membrane, where it protects membrane fatty acids from lipid peroxidation. Most *in vivo* and clinical studies of the effects of lipid soluble antioxidant supplementation on neurological diseases have focused on vitamin E. Recently, it was shown that the protein responsible for the uptake of vitamin E is in fact present in brain cells of patients suffering from vitamin E deficiency or diseases associated with oxidative stress [27]. In an Austrian study, serum concentration of vitamin E was found to be significantly associated with cognitive function in adults aged 50 - 75 years measured by a standardized test [28]. Glutathione is the most significant component which directly quenches ROS such as lipid peroxides and also it maintains ascorbate (vitamin C) and α-tocopherol (vitamin E), in their reduced forms, which also exert an antioxidant effect by quenching free radicals. Studies show a direct correlation with schizophrenia and decreased glutathione levels. Research shows that glutathione may slow down the progression and even lessen the symptom severity of schizophrenia [29].

It is confirmed that oxidative stress may be involved in schizophrenic patients. 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 the pathophysiology of schizophrenia.

CONCLUSION

A controlled trial involving 60 schizophrenia patients and 60 control subjects were described.

Standardized biochemical techniques were used to assess changes in the antioxidant vitamins. Results showed that schizophrenia patients are shown to have an unusually high demand for ascorbic acid, α-tocopherol, folate, vitamin B_{12} and glutathione. The demand is far higher for patients with positive symptoms. 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 as rightly pointed out by Surapaneni [30].

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Table 1: Levels of plasma antioxidant vitamins and glutathione in schizophrenia patients (with positive, negative and cognitive symptoms) and healthy controls (Values are mean ± Standard Deviation)

Vitamins	Control	Schizophrenia Patients with		
		Positive Symptoms	Negative symptoms	Cognitive symptoms
Vitamin C (mg/dl)	1.78±0.13	0.54 ±0.18a*	0.95±0.12ab	1.04 ±0.17ac
Vitamin E (mg/dl)	1.38±0.15	0.47±0.24a	0.63±0.18a	0.68±0.12a
Vitamin B12 (pg/ml)	537.96 ±78.47	468.69 ±96.53a	493.66 ±89.97ab	489.33 ±87.75ac
Folic acid (ng/ml)	14.47±1.43	7.42±2.07a*b	9.96±2.87a	10.35±2.20ac*
Glutathione (mg/dl)	49.79 ±3.12	27.09 ±3.17a	21.22 ±3.45ab	28.76 ±3.89a*c*d*

a b c d $p<0.01$, a* b*c* d*$p<0.001$

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)

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