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Original research article

The possible role of oxidants and antioxidant imbalance in pathophysiology of Schizophrenia

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Abstract

There are large growing data demonstrating that reactive oxygen species are involved in initiation and development of many different neuropsychiatric disorders including schizophrenia. Oxidative stress is a state of disequilibrium between oxidant process and the antioxidant defense system as a consequence of increased production of free radicals or when the antioxidant system is inefficient or a combination of both events. In order to examine lipid peroxidation and antioxidant status in study subjects (40 schizophrenic patients and 40 healthy controls), the levels of malondialdehyde (MDA) as an index of lipid peroxidation and free radical scavenging antioxidants like erythrocyte superoxide dismutase, glutathione, vitamin E, vitamin C and TAC have been evaluated. The objective of this study is to investigate the oxidant/ antioxidant imbalance leading to oxidative stress. Significantly lower levels of the antioxidant were found in patients as compared to normal controls with an increased oxidative stress as indicated by high plasma malondialdehyde levels and nitric oxide metabolites. This study shows that the dysregulation of oxidant and antioxidant defense system might be important mediator for development and progression of clinical conditions in schizophrenia. So, the findings also provide the theoretical basis to develop the new therapeutic approach towards antioxidant supplementation.

Key words: Reactive Oxygen Species, Lipid peroxidation, Nitric oxide. Superoxide dismutase, Schizophrenia.

Introduction

Schizophrenia is a devastating psychiatric disorder with a broad range of behavioural and biologic manifestations. It has a life-time prevalence of 1% of world's population.[1] There are several clinical characteristics of the illness that have been consistently associated with poor premorbid adjustment, long duration of psychosis prior to treatment and prominent negative symptoms. The etiopathogenic mechanisms of lack of insight in patients with schizophrenia are to date unknown, although several hypotheses have been suggested. Despite formulation of several hypotheses, the pathophysiology of schizophrenia remains also on large part unknown. In the last few decades, dopamine hyperactivity hypothesis predominates in the research field all the time. Recently more and more converging evidences indicate that oxidative

mechanism may play role in schizophrenia.[2, 3] Oxidative stress may be the central cause or the consequence of the disease process.[4]

The generation of ROS in normal cells, including neurons, is under tight homeostatic control. To help detoxify ROS, biological antioxidants, including glutathione, α-tocopherol (vitamin E), carotenoides and ascorbic acid (vitamin C) will react with most oxidants. In addition, the antioxidant enzymes catalase and glutathione peroxidase detoxify hydrogen peroxide by converting it to oxygen and water. However, when ROS levels exceed the antioxidant capacity of a cell, a deleterious condition known as oxidative stress occurs.[5] Unchecked, excessive ROS can lead to the destruction of cellular components including lipids, proteins, and DNA, and ultimately cell death via apoptosis or necrosis.[4]

While numerous studies have examined the existence of increased ROS in later-onset neurodegenerative diseases, the mechanism by which neurons die under condition of oxidative stress remains largely unknown. Some studies have suggested the interrelationship of antioxidants and their overall effects on regulation of oxidative stress.[6]

The disorder has bad outcome, regardless of different treatments. Schizophrenia is related to different neurodevelopmental, structural and behavioral abnormalities. It has been proposed that such abnormalities could originate from malfunctioning genes and /or non- genetic factors such as ethnicity, drug and alcohol abuse, life style, medication, pre-natal and neonatal infections, maternal malnutrition, complications during birth and many other factors, by inducing the cellular metabolic stress these factors appear to increase the possibility of oxidative stress and damage.[7] In schizophrenia patient's dysregulation of ROS and reactive nitrogen species (RNS) metabolism, as detected by abnormal activities of critical antioxidant enzymes and other indicators – lipid peroxidation in plasma, red blood cells, blood platelets, and cerebrospinal fluid is observed.[8] Many studies suggest that excess of ROS formation may play a critical role in the etiology of schizophrenia.[8, 9] A cell membrane dysfunction caused by lipid peroxidation can be secondary to a free radical- mediated pathology and may contribute to specific aspects of schizophrenic symptomatology, and complications of its treatment. Yao et al have previously demonstrated that individual antioxidants, albumin, bilirubin uric acid, were significantly reduced in plasma of patients with chronic schizophrenia, during on and off haloperidol treatment conditions.[2] Excess NO production leads to changes of neuron structure and function involving neuronal membrane damage,[10] and increased indices of lipid peroxidation.[11] Another study reported decreased SOD activity and unchanged Glutathione peroxidase activity compared to the control group in schizophrenia.[12,13]

Proceeding on existing knowledge and former evidences, we hypothesized that the levels of MDA, NO in patients are higher than that of controls; inversely the antioxidant level such as activities of SOD, GSH, vitamin E and ascorbic acid. The total antioxidant capacity is decreased in patients. These findings may lead to attempt new therapeutic approaches using appropriate antioxidants which might partially alleviate or prevent the symptoms of schizophrenia.

Materials and methods:

40 schizophrenia patients were consecutively recruited from outpatients or inpatients of the department of psychiatry of PDVVPF's medical college and hospital. The diagnosis of schizophrenia was made by using the ICD-10 criteria.[3] It includes 22 males and 18 females, the average age was 31.2 ± 11.0 years (range; 15- 56 years) and the duration of illness was ranges from 1 month to 3 years. None of the patient had significant other psychiatric or somatic co-morbidity. At the start of this study all patients had to be medication free for at least 2 weeks and not must have received any other kind of therapy. 40 normal healthy control volunteers of matched age and sex were recruited to participate.

Exclusion criteria:

The following exclusion criteria for patients and the control group were applied: any somatic disorders, especially circulatory diseases, disorders of lipid metabolism and diabetes mellitus, malnutrition, obesity and neurological disorders, and serious head injuries. The subject had normal body mass index (BMI) did not use any addictive substances or antioxidant supplementation. Their diet was balanced. Heavy smokers were excluded from both the group.

The project was carried out with the approval of the Institutional ethical committee and was in accordance with the declaration of Helsinki and written informed consent from all participants prior to examination.

Biochemical assays:

The heparinised venous blood samples obtained from these subjects were used for the analysis. Plasma was separated by centrifugation at 3000 g for 15 minutes. Separated plasma was used for the estimation of MDA, NO, vitamin E, vitamin C, TAC. The buffy coat was removed and the packed cells were washed three times with physiological saline. The erythrocytes suspension was prepared by the method of Dodge et. al.[14], modified by Quist.[15] The packed cells were used for the analysis of SOD and GSH. SOD activity was measured in hemolysate, according to the method of Kajari Das.[16] Erythrocyte GSH was estimated by the method of Beutler et. al. using dithio-bis-nitro benzoic acid. [17] Plasma vitamin E was determined by the method of Baker and Frank.[18] Ascorbic acid levels were estimated in plasma by the method of Tietz.[19] Plasma MDA and NO concentrations were determined as the measure of TBARS,[20] and Najwa and Cortas [21] respectively. TAC was measured by the assay of FRAP.[22] All the reagents used were of analytical reagent grade.

Statistical analysis:

Statistical analysis between controls and patients was performed by student's't' test using Grapad software. The data were expressed as mean± SD, $p< 0.05$ was considered as significant.

Results

Clinical data of patients and age-matched controls are summarized in Table 1. The means of BMI of the groups did not differ significantly. There were no differences between cases and controls with regard to body mass index and age.

The mean ± SD of plasma MDA, NO, erythrocyte SOD, GSH, plasma vitamin E, vitamin C, and TAC was illustrated in Table 2. Our studies have shown that plasma MDA was an indicator of oxidative stress and an end product of lipid peroxidation measured as thiobarbituric acid reactive substance (TBARS), which was significantly higher ($p < 0.001$) in schizophrenics as compared to normal controls. The levels of NO metabolites were significantly ($p < 0.001$) reduced in schizophrenia. The activity of erythrocyte SOD which acts as sinks for superoxides produced in plasma decreased significantly, along with decreased erythrocytes GSH, plasma vitamin E, vitamin C and TAC in patients with schizophrenia compared with controls.

Table.1: Sample Demographics

Parameter	Patients (n = 30)	Controls (n = 30)	P
Age	35.50 ± 9.4	37.2 ± 13.8	0.579
Weight	71.75 ± 9.7	73.50 ± 10.5	0.505
Gender	All males	All males	
BMI (kg/m^2)	25.67 ± 2.7	24.80 ± 5.5	0.439
PANSS score	84.45 ±9.3	-	-

Data presented as Mean ± SD

Table.2: Plasma MDA, NO metabolites Erythrocyte-SOD, GSH, Plasma Vitamin E, Vitamin C & TAC in controls and patients with schizophrenia.

Biochemical parameters	Healthy controls (n=40)	Schizophrenic patients (n=40)
MDA (nmol/L)	253.22±39.20	367.33 ± 58.49*
NO (mmol/L)	25.14 ± 5.50	36.76 ± 4.68*
SOD (U/gm Hb)	24.81 ± 6.38	20.05 ± 3.97*
GSH (mg/gm Hb)	34.68 ± 4.67	26.81 ± 3.53*
Vitamin E (mg/L)	10.13 ± 1.11	9.20 ± 0.82*
Vitamin C (mg/dL)	0.90 ± 0.21	0.63 ± 0.14*
TAC (nmol/L)	904.61 ± 11.40	761.93 ± 105.00*

Data presented as Mean ± SD *P < 0.001 Highly significant (comparison with control group)

Discussion

This study instigated both oxidant and antioxidant system in the same blood samples from schizophrenic patients. Our results suggest that there was serious dysregulation of oxidant/ antioxidant system during schizophrenia and increased oxidative stress seems to be result of the toxic effect of increased free radicals. They can cause membrane defect, oxidation of of the lipid membranes and also increase in catecholamine oxidative metabolism, [23] all of which may play an important role in the pathophysiology of schizophrenia.

The brain is particularly more vulnerable to the damaging effects of free radicals, when compared to the other organs of the body, because they have a high rate of oxidative metabolic activity (e.g. catecholamine degradation etc., a low level of protective antioxidants, a high ratio of membrane surface area to cytoplasmic volume, a neuronal anatomical network vulnerable to disruption, and high concentration of readily oxidisable membrane of polyunsaturated fatty acids.[5] Probably, catecholamines including dopamine and nor- epinephrine are related to free radical production.

In the present study, lipid peroxidation product i.e. MDA, levels have been increased significantly in plasma of the schizophrenics patients than that of control group. Similar results were obtained by many researchers.[3,24,25,26] The raised levels of MDA could be due to increased generation of ROS due to the excessive oxidative damage generated in these patients. This also reflects the oxidative injury due to schizophrenia, which is attributed to free radicals formation that abstract hydrogen atoms from lipoproteins, causing lipid peroxidation.[5] Elevated levels of thiobarbituric acid reaction products have been found in the cerebrospinal fluid of neuroleptic treated patients[27] and also in plasma of schizophrenics, with or without tardive dyskinesis.[28]

We observed a significant increase in the levels of NO metabolites in patients compared to controls, which is consistent with the finding of the previous studies.[29, 30,31] Reduced levels of NO metabolites found to be in other neurological disorders. However many other reports, decreased plasma NO metabolites and nitric oxide synthase activity were found in schizophrenics (32). It seemed that NO may play a critical role in schizophrenia. Previous studies suggested that NO is functionally linked to both dopaminergic and glutaminergic systems. NO, as a neurotransmitter, diffuses intracellularly and combines with guanylate cyclase which precipitates during transferring GTP into cGMP. Prolonged duration of ion channel results in increased calcium mobilization, and neuron necrosis occurs due to calcium overload. Moreover NO, as a free radical, also damage mitochondria, lipids, proteins and DNA.[8] Several NO metabolites are potent

oxidants which can depletes the GSH, uric acid and ascorbate.

In our study, the erythrocyte antioxidant enzyme i.e. SOD activity have been reduced significantly in patients with schizophrenia, compared to controls, which is consistent with many studies.[33] disturbances in the overall mechanism of generation of free radicals and their consequent neutralization due to oxidative stress in schizophrenia, where SOD is utilized for neutralizing free radical superoxide ion to H_2O_2 and oxygen. Further glutathione peroxidase reduces this H_2O_2 to water. Interestingly, the levels of SOD have been found to be high in chronic schizophrenic patients,[34, 35] and is due to over-expression of SOD might be an adaptive response, and it results in increased dismutation of superoxide radicals to hydrogen peroxide.

In present study, we have observed a significant decrease in concentrations of erythrocyte- reduced GSH, plasma vitamin E, vitamin C, and TAC in patients with schizophrenia, compared to control subjects. GSH, vitamin E and vitamin C are important chain breaking antioxidants, responsible for scavenging the free radicals and prevent the peroxidation in aqueous and lipid region of cell.[36]

The GSH deficit found in this study and previous reports indicates the role of GSH in pathophysiology of variety of neuropsychiatric disorders including schizophrenia. This may be involved in membrane peroxidation and micro lesions related to dopamine, which seem to be increased in schizophrenia, suggests that GSH may be a possible indicator of damage in neuronal membrane.[37]

Our finding regarding vitamin E and vitamin C suggested that, there was increased oxidatiuve stress induced lipid peroxidation in the patients that resulted in increased consumption of the antioxidants vitamins leading to significant reduction. Moreover due to a prevailing decreased ascorbate level in them, oxidatively modified, inactive α-tocopherol could not be regulated into its active form. This further exaggerated the oxidative stress induced lipid peroxidation in the brain cell membrane. The free radical scavenging function of ascorbate most probably protects the SOD enzyme integrity and activity against the free radical induced damage. Thus data analyses suggested that SOD activity became dependent primarily on the plasma ascorbate level in the schizophrenic patients in the present study. Also ascorbate was found as an important antioxidant that prevented dopamine against the oxidation by RNS derived from NO.[38, 39]

The levels of plasma TAC was measured to obtain the more information about antioxidant protection in schizophrenia. The TAC of plasma was found significantly lower in patients as compared to controls. Relative contribution of the TAC is not well defined. While some studies demonstrated that the decreased plasma TAC,[40] and others showed that it is unchanged.[11] Our data supports the view that schizophrenia is associated with a loss of antioxidant capacity in response to increased oxidative stress. Reduction in vitamin E and ascobate concentration may be responsible for reduction in TAC. These components of TAC prevent the reaction that causes lipid peroxidation.

Conclusion

In conclusion, oxidant/antioxidant imbalance may be involved in the pathogenesis of schizophrenia. The present study has clearly shown higher free radical production and decreased antioxidant defense activity supports the oxidative damage hypothesis in schizophrenia. There are some methodological limitation of the present study must be acknowledged. First, sample size is relatively small. Apart from this, it is essential to mention that some confounding factors related to the patients habits, i.e. life style, dietary changes and exercise which may affect the levels of antioxidant system. Of course, we got all the results from peripheral blood in this study; further advanced technique should be adopted to make sure whether it mirrored the exact status in the brain. Consequently, the growing consideration regarding free radical damage may have an important role in the development of preferable therapeutic approach towards antioxidants supplementation and may be to improve outcome and prognosis of schizophrenia.

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