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STUDY OF NITRIC OXIDE END PRODUCTS AS OXIDATIVE INJURY AND ANTIOXIDANT DEFENSE SYSTEM AND THEIR ASSOCIATION IN SCHIZOPHRENIA

C. B. Shinde[1*], A. N. Suryakar[2], A. S. Garad[3], A. A. Momin[4]

[1]Assistant Lecturer, Department of Biochemistry, Institute of Medical Sciences & Research, Mayani, Satara, Maharashtra, India.

[2]Registrar, Maharashtra University of Health Sciences, Nashik, Maharashtra, India.

[3]Assistant Lecturer, Department of Biochemistry, Institute of Medical Sciences & Research, Mayani, Satara, Maharashtra, India.

[4]Assistant Professor, Department of Biochemistry, Institute of Medical Sciences & Research, Mayani, Satara, Maharashtra, India.

*Corresponding Author Email: shindechaya_13@rediffmail.com

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 present study, 30 schizophrenic patients and 30 age and sex matched `healthy controls were included. The blood samples were collected, and investigated for nitric oxide end products and activity of antioxidant enzyme RBC-Superoxide dismutase and level of Vitamin C in schizophrenic patients and control subjects. The level of nitric oxide end product was found to be significantly increased in patients than healthy controls; also a significant decrease in vitamin C in schizophrenic patients was seen as compared to controls. The negative correlation of nitric oxide end products was found with RBC-Superoxide dismutase and vitamin C level among patients. The difference in the activity of the erythrocyte SOD was not significant when compared between patients and controls. The increase in nitric oxide end products may reflect increased oxidative stress in brain tissue of schizophrenics, while decreased level of vitamin C is suggestive for worsening of the disease. Intensive oxidative stress and decreased antioxidants may contribute to neuronal death and alter the information processing in schizophrenia. These findings also provide theoretical basis for the development of novel therapeutic strategies such as antioxidant supplementation.

KEYWORDS:

Superoxide Dismutase (SOD), Reactive Oxygen Species (ROS), Nitric oxide end products ($NO^{\bullet}$) Vitamin C, Schizophrenia.

INTRODUCTION

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[1] The chemical nature of the schizophrenic brain is still not completely understood and has a lifetime risk of 1% and affects at all age groups in many cultures around the world. [2]

Free radicals and Reactive Oxygen Species (ROS) generated during aerobic metabolism can affect certain processes leading to clinical manifestations. There is abundant evidence that free radicals involved in membrane pathophysiology in the central nervous system and may play a role in schizophrenia.[1] Moreover, the body's defense mechanisms would play an important role in the form of antioxidants and try to minimize the damage, adopting itself to the stressful situation.[1,2] Alterations in the oxidant and antioxidant profile is known to occur in pathophysiology of many disorders including schizophrenia.

If, the homeostasis between rate of formation of free radicals and the rate of their neutralization is not occurred or maintained, an oxidative damage can occur which is known as oxidative stress. Oxidative stress is a result of increased formation of free radicals or reduced anti oxidative capacity.[3] The brain is most susceptible to attack by the free radicals as it is rich in polyunsaturated fatty acids and highly oxygenated and the damage caused to the neurons by these free radicals cannot be repaired.[4]

Nitric oxide is a free radical also acts as neurotransmitter, can react with superoxide radical to form highly toxic peroxynitrite ($ONOO^{-}$). When peroxynitrite reacts with human body fluids nitro tyrosine is generated, which, have been detected in human brain and may be increased in schizophrenia.[3,5]

Antioxidant enzyme Superoxide Dismutase (SOD), is the primary enzyme involved in direct elimination of ROS which block the initiation of free radical chain reactions and the non enzymatic antioxidant components

consist of molecules such as vitamin E, ascorbic acid that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions.[5] Vitamin C is considered the most important water soluble antioxidant enzyme in extracellular fluid, as it is capable of neutralizing ROS in the aqueous phase before lipid peroxidation initiated and very effectively protects LDL cholesterol against oxidation and acting as co-substrate in pro-collagen, catecholamine and carnitine biosynthesis.

The present study was aimed to study the oxidative stress by means of nitric oxide end products, and the activity of RBC superoxide dismutase & level of vitamin C antioxidants was measured in schizophrenics.

MATERIAL AND METHODS

The present study was carried out in Department of Biochemistry Dr. V. M. Government Medical College, Solapur, in collaboration with Shree Chhatrapati Shivaji Maharaj General Hospital, Solapur, (Maharashtra). The protocol was approved by Ethical committee of the institute. The consent form was obtained from the relatives of patients. A total of 60 individuals were included in this study. Out of these, 30 were clinically diagnosed as schizophrenic patients.

The study subjects having disorders associated with heart, lung, liver, kidney and other pivotal organs were excluded from the study. All the patients were comprised of clinically diagnosed schizophrenic patients of age group between 20 to 55 years. Diagnosis of schizophrenia was made by Psychiatrists by using Diagnostic and statistical Manual of Mental Disorders (DSM-IV) classification (American Psychiatric Association, 1994). [6]

The venous blood samples were collected from the subjects under aseptic condition by venipuncture using 10 ml sterile syringe and needle. About 8ml of random blood was collected of which 3ml was poured into sterile vacutainer containing heparin for the estimation of RBC-SOD. Remaining blood was taken into sterile vacutainer without anticoagulant for estimation of other parameters. Then the plasma and serum were separated by centrifugation at 3000 rpm for 5-10 minutes and un-hemolyzed samples were taken for the assays. Serum nitric oxide end products were determined by a kinetic cadmium reduction method.[7] The activity of RBC-Superoxide dismutase was determined using Method given by Kajari Das.[8] Plasma vitamin C was estimated by method of Caraway et al. [9]

All the values of biochemical parameters of patients and controls were expressed as mean ± SD. All the biochemical parameters measured in study group were statistically compared with those estimated in controls by using independent samples student "t" test. Correlations between the variables were estimated by Pearson's correlation coefficient. The difference was considered significant, when the $p<0.05$.

RESULTS

The present study was aimed to study the biochemical parameters viz. nitric oxide end products, RBC SOD and vitamin C in the patients of schizophrenia and healthy controls, as markers of oxidative stress and antioxidant defense system status. The levels of nitric oxide end products was increased significantly ($p<0.05$) in patients of schizophrenia when compared with healthy controls, while there was no significant ($p>0.05$) difference found in RBC- SOD in patients and controls. The level of vitamin C was found to be significantly ($p<0.05$) decreased in patients of schizophrenia than the healthy controls. The results are depicted in **Table 1**.

The correlation between Erythrocyte SOD activity and vitamin C was studied with nitric oxide end products. We found negative association between erythrocyte SOD activity and nitric oxide end products with r value of -0.718 and associated p value <0.01. Vitamin C activity was also negatively correlated with Nitric oxide end products in patients with r value of -0.793 and associated p value <0.01. The values are depicted in **Table No.2** and scatter diagrams are shown in **Figure 2 & 3**.

Table No 1: Nitric oxide end products, activity of erythrocyte Superoxide Dismutase (SOD) and Plasma Vitamin C levels in healthy controls and schizophrenic patients

Biochemical Parameters	Healthy controls (n=30)	Patients (n=30)
Serum $NO^{\bullet}$ end Products (µmol/l)	66.70 ± 36.82	126.48 ± 56.59*
Erythrocyte-SOD (U/gm of Hb)	1187.33 ± 726.13	863.33 ± 629.39#
Plasma vitamin C (mg/dl)	4.19 ± 1.67	0.49 ± 0.27*

*** Significant**

 # not significant

Table No.2: Association of Nitric oxide end products with erythrocyte SOD activity and Vitamin C.

Parameters	Nitric oxide end products	
	'r'	P
Erythrocyte SOD activity	-0.718	<0.01*
Vitamin C	-0.793	<0.01*

*** Significant**

Figure 1: Scatter diagram showing association between serum Nitric Oxide end products and Erythrocyte superoxide Dismutase (SOD) activity in schizophrenic patients.

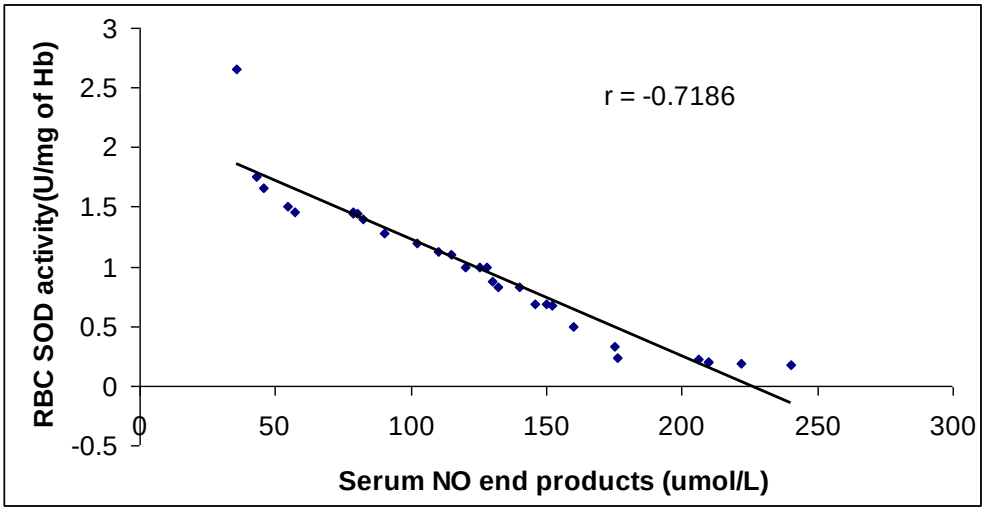
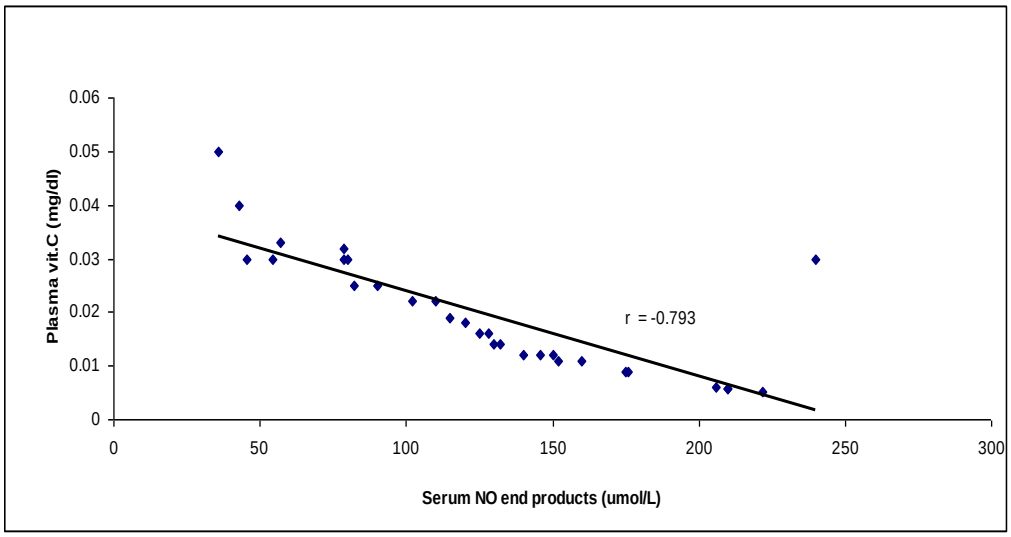


Figure 2: Scatter diagram showing association between serum Nitric Oxide end products and vitamin C levels in schizophrenic patients.



DISCUSSION

Among the different brain regions, the basal ganglia may be particularly at risk of radical induced damage because they contain large amount of iron, which can be associated with increased free radical production through the Fenton reaction. There are several ways to excess free radicals may be generated in the brain. The metabolism of catecholamines, such as norepinephrine and dopamine, is probably associated with increased catecholamine metabolism may increase the free radical production. Antipsychotic drugs also cause an increase in metabolic turnover of catecholamines. [10]

There was remarkable increase in nitric oxide levels ($NO^{\bullet}$) in patients with schizophrenia, which indicates a possible role of $NO^{\bullet}$ in pathogenesis of it. Increased oxidant end products by the reactions of $NO^{\bullet}$ with other free radicals may probably contribute to the neuro-pathophysiology, thereby psychopathology of schizophrenia because of the preferential vulnerability of the brain to oxidative injury.[11]

In previous studies by showed the activity of SOD and vitamin C level along with some free radicals were evaluated in different psychiatric disorders.[11,12,13] They have found significant differences among free radical & antioxidant levels in patients than controls. So we planned the study of these antioxidants along with nitric oxide end products in schizophrenic patients and healthy controls.

The results of present study indicate, there is a significant increase in free radical generation & antioxidant defense system is impaired in schizophrenic patients. The level of nitric oxide end products was increased significantly, and Vitamin C level was significantly decreased in patients of schizophrenia when compared with healthy controls. Erythrocyte SOD activity was not differed statistically among study group and controls.

Our findings were consistent with those reported by **Sadik S. [11] et al, Herken H. [14] et al and Yao [15] et al**, no significant difference in SOD activity was found in schizophrenics and controls. **Reddy R. [16] et al Vaiva G. [17] et al** reported increased activity of SOD in chronic schizophrenic patients, while low erythrocyte SOD activities had been reported by **Mukherjee S. [18] et al** at onset of psychosis. This discrepancy may be due to the different duration of the disease in different studies.

Nikam S. [13] **et al**, **Sadik S. [11] et al** found increased level of nitric oxide in patients with parkinson's disease and autism respectively. Our results support the findings of **Li H.C. [19] et al** they had shown that, the levels of nitric oxide was increased in Schizophrenic patients than the healthy controls. Our results are also supported for Vitamin C levels by **Gerard D. [1] et al**, **Surapaneni K.M. [12] et al** who had shown decreased levels of Vitamin C in patients of schizophrenia than healthy controls.

Nigral dopaminergic neurons are particularly vulnerable to oxidative stress because the metabolites of dopamine can act as endogenous toxins. Dopamine auto oxidizes at normal pH into toxic dopamine quinine species, O_2^{-} radicals and H_2O_2. Superoxide can be converted into H_2O_2 by SOD or into labile $ONOO^{-}$ radicals in the presence of NO•. NO• overproduction also plays a key role in the pathogenesis of schizophrenia. NO• and $ONOO^{-}$ impair the mitochondrial respiratory chain by affecting the complex I, II and IV leading to energy failure and ultimately cell death. Moreover, $NO^{\cdot}$ as a free radical, damages mitochondria, lipids, proteins and DNA.[20,21,23]

Decrease in the levels of plasma vitamin C in schizophrenia may be due to increased oxidative injury. For prevention of oxidative injury suggest an increased defense against oxidative damage in schizophrenia. Increased oxidative stress in schizophrenics leads to decrease in the levels of antioxidant like vitamin C and disturb their metabolism which weaken their ability to fight against growing stress. [19,23, 24]

Also found negative correlation between nitric oxide end products & SOD may suggest that SOD is involved in the antioxidant defense system against free radicals, as well as between nitric oxide end products & vitamin C. The negative correlation of nitric oxide end products with vitamin C and SOD in our findings show the effect of free radicals on antioxidants. Because, increased lipid peroxidation causes free radical generation, these free radicals are scavenged by antioxidants like RBC-SOD &Vitamin C in the process where it gets utilized and another reason is the loss of vital antioxidants of the body due to stress, changes in the concentration of different fractions of ascorbic acid sensitively reflects oxidative stress in tissues.

Thus, in Schizophrenia gives an opportunity for using these parameters as markers for evaluation of this disease and findings provide to develop novel strategies for diagnosis, prognosis, and also provide theoretical basis for the development of novel therapeutic strategies such as antioxidant supplementation of schizophrenic patients are warranted.

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***Corresponding author address**:
Ms. Chhaya B. Shinde,
Assistant Lecturer, Department of Biochemistry,
Institute of Medical Sciences & Research,
Mayani, Dist- Satara,
Maharashtra, India.
Phone Number: +919975378101
E_mail ID: shindechaya_13@rediffmail.com