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RESEARCH ARTICLE

THE STUDY OF POSSIBLE ASSOCIATION OF HOMOCYSTEINE-INDUCED OXIDATIVE STRESS AND NEUROPSYCHIATRIC DISORDERS

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ABSTRACT

Context- The mechanisms of oxidative stress in neuropsychiatric disorders patients are not fully understood. Increased levels of Homocysteine (Hcy) are associated with risk of some neuropsychiatric disorders. Hcy may cause this risk by depleting the antioxidant system or generation of ROS.
Objectives – The present study planned to investigate the association of elevated level of with Hcy oxidative stress, by measuring biochemical markers namely Malondialdehyde (MDA), an index of lipid peroxidation in plasma, the level of Vitamin E, Reduced Glutathione (GSH) and plasma Total Antioxidant Capacity (TAC) in schizophrenic and major depression patients.
Results- Significantly increased plasma Hcy, MDA levels and significantly lower levels of plasma vitamin E, TAC and blood GSH in schizophrenia and depressed patients were observed. The obtained results indicates that the pro-oxidant disturbance occur in neuropsychiatric disorders.
Conclusion- The data indicates a link between Hcy and neuropsychiatric disorders. Our results showed that in schizophrenic patients the amount of homocysteine in plasma was higher in comparison with the control group. We also observed a statistically increased level of biomarkers of oxidative stress such as MDA, vitamin E, GSH and TAC in patient groups. Considering the data presented in this study, we suggest that the elevated Hcy may acts as stimulant for the oxidative stress in neuropsychiatric patients. Finally our results support the further studies of the possible neuro-protective role of antioxidants as therapeutic strategies for neuropsychiatric disorders.

INTRODUCTION

The multiple disease etiologies that lead to neuropsychiatric disorders, such as Parkinson's and Alzheimer's disease, Huntington disease, schizophrenia, depressive illness and stroke, offer significant challenges to drug discovery efforts aimed at preventing or even reversing the progression of these disorders. Further, they are thought to be initiated by a cascade of molecular events that involve several neurotransmitter systems (Cornelis *et al.,* 2006). Hcy is directly toxic to neuron and blood vessels and can induce DNA breakage, oxidative stress and apoptosis. The methionine-Hcy metabolic pathway produces methyl group required for the synthesis of catecholamine's and DNA. This is accomplished by remethylation of Hcy using B_{12} and folate as cofactors-back to methionine. Hcy is cleared by transulfuration reaction to give cysteine and GSH, an important antioxidant. Transulfuration requires vitamin B_6 and B_{12}. Since Hcy is a sensitive indicator of B vitamin deficiency, an elevated Hcy level is marker for a pathogenic process as well as a cause of pathology (Rogers and Chen 2007) (Fig.1). Recent findings show a relationship between elevated tHcy and neurodegenerative diseases, in particular with Alzheimer's disease. Relatively small changes in tHcy may dispose to pathological outcomes (Brosnan *et al.,* 2004). Muntjewerff *et al.* (2006) and Almeid *et al.* (2008) provides the evidence for an association of Hcy with schizophrenia with the help of homozygous genotype of MTHFR 677C>T polymorphism and risk of schizophrenia (Muntjewerff *et al.,* 2006; Almeid *et al.,* 2008). Oxidative stress has been implicated in the pathophysiology of several neuropsychiatric diseases, as the brain has comparatively greater vulnerability to oxidative damage. Numerous experimental studies have revealed the significant role of oxidative stress in the development, progression and severity of psychiatric disorders, among which are depression and schizophrenia. Some of them proved this link directly, by accessing the concentrations of biomarkers of redox imbalance, while others used an indirect approach by administering antioxidant substances and revealing the clinical improvement (Sayre *et al.,* 2008; Lačković *et al.,* 2013). The idea that HHcy can promote formation of free radicals (FR) is supported in the majority of cases with only indirect evidence (Racek *et al.,* 2005). Dietrich-Muszalska *et al.* (2010) and Brown *et al.* (2007) Gariballa *et al.* (2011), Almeida OP *et al.* Naismith *et al.* (2002) and Bottiglieri *et al.* (2000) suggested that

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increased Hcy may associated with oxidative stress and have a significant influence on the development and clinical symptoms of schizophrenia. The antioxidant defense system, which is known to be dysregulated in schizophrenia, is closely linked to the dynamics of Hcy pathway. Thus, alterations in the homeostatic balance in the Hcy pathway may be involved in the pathophysiology of neuropsychiatric disorders including schizophrenia (Reif *et al.,* 2003). GSH, vitamin E and vitamin C are important chain breaking antioxidants, postulated to protect against damage to biological membranes by their ability to scavenge free radicals and prevent the peroxidation in aqueous and lipid region of cell (Ghodake *et al.,* 2012). As a main cellular non-protein antioxidant and redox regulator, glutathione (GSH) plays a major role in protecting nervous tissue against reactive oxygen species and in modulating redox-sensitive sites, including NMDA receptors (NMDA-R). Although Hcy has been shown to act as an NMDA receptor agonist when glycine levels are pathologically elevated, this amino acid acts as a partial antagonist at the glycine site of the NMDA receptor when glycine levels are in physiologic range (Kumari *et al.,* 2011). GSH is an important protector of energy metabolism (mitochondrial function) during periods of oxidative stress. Dopaminergic neurons are very sensitive to changes in the internal oxidant buffering capacity of the cell caused by reductions in GSH levels that can lead to disruption of calcium homeostasis and cell death (Dringen 2000). The purpose of the present study were 1) to assess whether plasma Hcy, MDA, vitamin E, GSH and TAC levels were altered in the patients compared to control subjects, 2) if so, to further test whether altered balance of oxidative stress and antioxidant defenses were associated with Hcy and neuropsychiatric disorders. These findings may lead to attempt new therapeutic approaches using appropriate antioxidants which might partially alleviate or prevent the symptoms of diseases.

MATERIALS AND METHODS

The study was approved by UDIRT, MUHS (University ethical and review board) for the patients and healthy control subjects. Patients were consecutively recruited from outpatients or inpatients of the department of Psychiatry of PDVVPF's Medical College and hospital and Dr. Jhalani's Psychiatric Hospital, Ahmednagar. All subjects provided written informed consent (from patient's relatives). The patients group was composed of 30 patients with acute schizophrenia and 30 patients with major depression (age range 19-55 years) diagnosed according to DSM-IV (American Psychiatric Association Diagnostic and Statistical Manual of Mental disorders) (American Psychiatric Association 1994). 30 normal healthy control volunteers of matched age and sex were recruited to participate (included in the study for comparison). The oxidative stress markers determinations were performed in the laboratory during the same time period as samples from patients.

Exclusion criteria

The following exclusion criteria for patients and the control group were applied: The patients and controls none had a history of any cardiovascular or neurological or drug or alcohol abuse. No patient was being treated with antipsychotic or antidepressant medications. Not having any somatic disorders, especially, 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. All subjects were not showing any abnormalities of immunoresponse.

Blood sampling / collection and measurements –

The heparinized 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 Hcy, MDA, vitamin E, and TAC. Plasma Hcy was measured by kit method, using direct chemiluminescent immunoassay. For estimating plasma MDA, an indicator of oxidative stress, measuring and a secondary fragmentation product of PUFA peroxide, a thiobarbituric acid-reactive substance (TBARS) that gives a pink color complex with TBA. It was read on a spectrophotometer at 535 nm wavelength (Bird and Draper 1984). Erythrocyte GSH was assayed by reading the reduction of dithio-bis- nitro benzoic acid (DTNB), forming yellow anion at 412 nm (Burtis and Ashwood 1999). Plasma vitamin E was determined by the method of Baker and Frank (1988) and TAC was measured by the assay of FRAP (Benzie and Strain 1996). All the reagents used were of analytical reagent grade.

Statistical analyses-

Statistical analysis between controls and patients was performed by student's't' test using Graphpad software. The data were expressed as mean ± SEM. A p value of < 0.05 was considered statistically significant.

RESULTS

Table no. 1 showing comparison of Hcy and oxidative stress parameters between three groups. Table No. 2 and 3 showing the mean differences in the levels of plasma Hcy and MDA in schizophrenic and depressed patients, which were significantly higher ($p<0.0001$) than that in the healthy control subjects. However, the mean levels of plasma Hcy ($p<0.05$) and serum MDA ($p<0.0001$) in schizophrenic patients were significantly higher than that of depressed patients. It indicates that in schizophrenia Hcy abnormalities and oxidative stress are to greater extent as that of patients with major depression. The levels of plasma vitamin E, erythrocyte-GSH and TAC were significantly lowered ($p<0.01$) in both patients group than that of control subject. The mean depleted levels of vitamin E, GSH and TAC indicated its utilization during stress condition.

DISCUSSION

In order to investigate for a possible relationship of Hcy with oxidative stress in neuropsychiatric disorders, we screened outpatients and inpatients with schizophrenia and depression from a small catchment area, for Hcy, MDA, vitamin E, GSH and TAC levels in an observational trial. Oxidative stress is

Table 1. Patients and controls data

	Controls (n=30)	Patients	
		Schizophrenia (n=30)	Depression (n=30)
Hcy(µmol/L)	7.09±4.28	16.85±6.74**	14.15±5.12##
MDA(µmol/L)	255.43±19.61	342.78±45.42**	289.81±60.75#
Vitamin E(mg/L)	10.92±1.48	9.45±1.59*	9.15±1.48##
GSH(µmol/L)	34.09±3.76	24.84±4.98**	26.31±5.01##
TAC(µmol/L)	888.40±131.95	770.07±124.64**	761.00±111.28##

All values are expressed as Mean ± SD

The levels of statistical significance is P<0.05
* controls v/s Schizophrenia, # controls v/s Major depression.
**, ##- Highly significant, *, #- Significant

Table 2. Showing paired't' test for Hcy levels

Paired Differences	Mean difference	Std. Error Mean	95% CI of the Difference		t test value	df	p value
			Lower	Upper			
Controls v/s Schizophrenic patients	-9.753	1.556	-12.936	-6.571	6.268	29	<0.0001**
Controls v/s major depression patients	-7.053	1.365	-9.845	-4.262	5.167	29	<0.0001**
Schizophrenia v/s Major depression	2.700	1.208	0.229	5.171	2.2344	29	0.03*

*Significant, ** Highly significant*

Table 3. Showing paired 't'test for MDA levels

Paired Differences	Mean difference	Std. Error Mean	95% C I of the Difference		t test value	df	p value
			Lower	Upper			
Controls v/s Schizophrenic patients	-87.3450	-87.34	-107.73	-66.95	8.761	29	<0.0001**
Controls v/s major depression patients	34.3750	12.413	-59.76	-8.98	2.769	29	0.009*
Schizophrenia v/s Major depression	52.970	10.062	32.39	73.54	5.264	29	<0.0001**

*Significant, ** Highly significant,*

systemic and some of the oxidative products of the brain tissue do end up in the blood, so peripheral indices have been accepted to reflect the brain oxidative injury (Raffa *et al.,* 2011).

The concentration of Hcy in patients with schizophrenia and depression was significantly higher than that of control subjects. Hcy may also enhance oxidative stress. Our present and the results of other researchers suggest that schizophrenia and depressive disorders are characterized by abnormal oxidative stress (Tyagi *et al.,* 2005; Akanji *et al.,* 2007; Dietrich-Muszalska *et al.,* 2009). Hcy has been suggested as a potentially useful biomarker of oxidative stress in schizophrenia. The harmful effect of Hcy arises from generation of ROS during its catabolism, which could oxidize membrane lipids and proteins including enzymes. In fact, it has been suggested that Hcy may have a significant influence on the development of clinical symptoms of neuropsychiatric disorders (Dietrich-Muszalska *et al.,* 2012). Some studies observed that a significant relationship of hyperhomocysteinemia and depression that were due to physical co-morbidity and cardio-vascular risk factors in subjects with depression (Tiemeier *et al.,* 2002). Brown *et al.* (2007) suggest that elevated third-trimester homocysteine levels may increase schizophrenia risk through developmental effects on brain structure and function and/or through subtle damage to the placental vasculature that compromises oxygen delivery to the fetus. Hcy (with –SH group) continuously gets oxidized resulting in Hcy (S-S group) and homocysteinylated proteins. This leads to the formation of ROS such as superoxide, hydrogen peroxide and hydroxyl radicals. The damaging effects of ROS on biomolecules (proteins, lipids) are well known. Further, superoxide converts nitric oxide to peroxinitrite thereby depleting tissue NO levels. Again homocysteine interacts with the N-methyl-D-aspartate receptor, causing excessive calcium influx and free radical production, resulting in neurotoxicity (Uppala and Badikillaya 2012). Our earlier (Ghodake *et al.,* 2012) and present results (Table No.1) suggest that schizophrenia is characterized by abnormal oxidative stress. In our study, the administration of antioxidants caused an increase in plasma antioxidant capacity and GSH concentration. In accordance with the improvement of antioxidative defense, we found a lower MDA level following supplementation with antioxidants as a result of a reduced lipid peroxidation degree. In the present study, serum MDA as an index of lipid per oxidation increased significantly in psychiatric patients compared to controls. Increased peripheral lipid peroxidation in patients may reflect such activities in the CNS. This study supports the results obtained by Dietrich-Muszalska *et al.* (2009, 2010), Ben Othmen *et al.* (2008) (Ben

Othmen *et al.,* 2008), Zhang *et al.* (2007) and Dadheech *et al.* (2006). Vitamin E and GSH are the most well known radical scavenging antioxidants in animals. Our results support the studies by Bal *et al.* 2012), and Yanik *et al.* (2004) who found that low vitamin E levels at MDD compared to controls. However, Teimer *et al.* (2002) found normal vitamin E levels in major depressive patients. Singh *et al.* (2008), Dadheech *et al.* (2006) and Surapaneni *et al.* (2007) reported that reduced concentration of vitamin E in schizophrenics compared to controls. GSH is involved in the disposal of peroxides by brain cells and in the protection against reactive oxygen species. The effects of GSH in the synaptic transmission of the mammalian brain have been reviewed recently). Available literature on oxidative stress and neurological disorders, as well as the involvement of the glutathione system in such processes, has recently been reviewed (Kumari *et al.,* 2011; Janaky *et al.,* 1999). The GSH deficit found in this study and in previous reports (Dietrich-Muszalska *et al.,* 2009; Dadheech *et al.,* 2006; Janaky *et al.,* 1999; Yao *et al.,* 2006) shown that GSH may be a possible indirect indicator of damage in neuronal membranes. The study on the drug-naïve first episode schizophrenic patients also suggests that the deficit in GSH may underlie the pathophysiology of the disease and is not a consequence of treatment. The converging data in literature, in agreement with our results in FESP, indicate that psychosis is associated with an important brain glutathione deficit (Tyagi *et al.,* 2005). In fact, it could be considered that different etiological mechanisms converge into precipitating a first psychotic episode in individuals with a limited GSH synthesis capacity, after which the psychotic episode develops into a degenerating condition that we call schizophrenia.

Conclusion

In summary, the present study have shown that higher levels of oxidative stress in schizophrenic and depressive patients than the controls. An association between oxidative stress and elevated Hcy in neuropsychiatric disorders might be informative about the hypothesis that higher levels of Hcy play a casual role in the etiology of disorders. On the basis of various observed facts, it has been proposed that antioxidants can inhibits the oxidative stress induced by Hcy; therefore, the next step of future studies is to evaluate the therapeutic effect of different antioxidants on oxidative stress in patients with neuropsychiatric disorders, which have the hyperhomocysteinemia.

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