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Plasma levels of trace elements in patients with different symptoms of Schizophrenia

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ABSTRACT

To determine changes of plasma trace elements levels (zinc, copper, magnesium, and iron) in schizophrenic patients with positive, negative and cognitive symptoms. The observations in the present study suggest that alterations in essential trace elements Mn, Cu, Zn, Se and Fe may play a role in the pathogenesis of schizophrenia. Supplementation of trace minerals -rich diet, which are thought to increase the antioxidant system activity, can be useful and effective in the treatment of the schizophrenia.

Key words: Schizophrenia, Trace elements, Flame atomic absorption spectroscopy, clinical significance.

INTRODUCTION

Schizophrenia is a disabling group of brain disorders characterized by symptoms such as hallucinations, delusions, disorganized communication, poor planning, reduced motivation, and blunted affect. While the incidence of the disorder is relatively low (median value 15.2 per 100,000 persons per year)[1], the condition is one of the major contributors to the global burden of disease[2]. The substantial burden of disease is a reflection of two features of schizophrenia: (a) the disorder usually has its onset in early adulthood, and (b) despite optimal treatment, approximately two-thirds of affected individuals have persisting or fluctuating symptoms[3].The symptoms of schizophrenia fall into three broad categories: Positive, negative and cognitive symptoms[4].

Recently more and more converging evidence indicates that oxidative mechanisms may play a role in schizophrenia[5]. The essential trace elements, play a major role in many metabolic pathways, play a vital role in immune system and are essential for the optimal function of a variety of antioxidant enzymes[6].

Copper and zinc, two essential trace elements have been studied in many diseases, including autoimmune, neurological and psychiatric disorders. Copper is a component of several metallo enzymes, including tyrosinase and dopamine b hydroxylase. On the other hand, the copper plays a well-known role in neurological disorders. Schizophrenia patients have been found to have raised levels of serum copper. Copper has been shown to be reduced after antidepressant treatment in depressive patients[7]. Excessive copper, however, is a cause of emotional instability as found in hyperactive children, and can lead to depression of so-called “unknown Causes[8].

Zinc is implicated in the functioning of more than 200 enzymes, some of them related with DNA and RNA synthesis. Schizophrenia patients have been described to possess lower levels of Zn than normal controls. Some studies have shown lower levels in acute psychiatric admissions of

schizophrenic patients but not in long-stay hospitalized schizophrenic patients[9].

Magnesium (Mg) is a trace mineral that is known be part of many different enzyme systems and is involved in controlling various metabolic functions. The role of magnesium in the central nervous system could be mediated via the N-methyl-d-aspartate-antagonistic, -amino butyric acid A-agonistic or a angiotensin II-antagonistic property of this ion. A direct impact of magnesium on the function of the transport protein p-glycoprotein at the level of the blood-brain barrier has also been demonstrated, possibly influencing the access of corticosteroids to the brain. Furthermore, magnesium dampens the calcium ion-protein kinase C related neurotransmission and stimulates the Na-K-ATPase. All these systems have been reported to be involved in the pathophysiology of schizophrenia[10].

Iron is necessary for life, but if too much or not enough iron is stored, health is seriously impaired. Low serum iron has been reported in a variety of neuropsychiatric motor disorders[11]. Low level of iron affects dopaminergic system. Many researchers found abnormal iron deposits in the brains of schizophrenics[12]. It is suggested that Ca is capable of inducing structural and cognitive deficits seen in schizophrenia and it has been proposed that altered Ca signaling may constitute the central unifying molecular pathology in schizophrenia[13].

Selenium is an essential, natural antioxidant, is most notable for its antioxidant properties. In 1973, Rotruck and colleagues provided rationale for identifying selenium as an antioxidant. The selenium-dependent enzyme glutathione peroxidase (GPX) recycles glutathione, reducing lipid peroxidation by catalyzing the reduction of peroxides, including hydrogen peroxide. Selenium is a cofactor within several metabolic pathways, including the GPX pathway, where it is present as selenocysteine[14,15].

Schizophrenia has a diverse nature of clinical symptoms and a number of hypotheses have been suggested to explain its aetiological basis. There is a substantial published literature on concentrations of trace metals in the blood of patients with various diseases, but to our knowledge, there has been no published paper on plasma trace elements in schizophrenia patients with different symptoms. There is some evidence that the concentrations of these metals may deviate from "normal" in plasma in some neurological diseases.

In an attempt to determine if blood plasma trace-metal concentrations, or their ratios, are of value in the diagnosis, understanding, or management of schizophrenia patients, in the present study, we aim to measure the concentrations of copper, zinc, iron, magnesium, calcium and selenium in the plasma of schizophrenia patients with positive, negative and cognitive symptoms. The present study was carried out during the month of September 2004 to June 2007, in the Postgraduate and Research Department of Biochemistry, Dr. N.G.P Arts and Science College, with the collaboration of Kovai Medical Centre and Hospital (KMCH), a multispeciality hospital with a separate division for psychiatry.

MATERIAL 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 with 20 patients in each group. (1) schizophrenics with positive symptoms (2) schizophrenics with negative symptoms and (3) schizophrenics with cognitive symptoms. They all met DSM-IV (Diagnostic and Statistical Manual of Mental Disorders-IV) criteria (American Psychiatric Association, 2000)[16] 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. All patients were treated with daily stable doses of various neuroleptics.

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

A total of 60 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.

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. Informed and written consent was obtained from all subjects prior to examination.

Blood sampling

Blood from forearm vein was collected into 5-ml Vacutainer tubes containing potassium EDTA. Fasting blood samples obtained by venupuncture from patients and controls were drawn into heparinised tubes, which were then centrifuged at 2000 g for 15 min, plasma was carefully removed and the separated plasma was stored at 4°C immediately. Plasma was used for the estimation of trace elements. Blood collection and plasma separation was carried out in dust free environment.

Determination of trace elements levels

Analysis of the trace element (Cu, Mg, Zn and Fe) was carried out by using flame atomic absorption spectrometry (Varian Spectra AA 220, Australia) according to the method of kaneko[17]. Plasma selenium was estimated by graphite furnace atomic absorption analysis as proposed by Jacobson and Lockitch, 1988 [18]. The level of calcium in plasma was estimated by kit method of Endres and Rude, 1999 [19].

Statistical analysis

The results were expressed as mean ± SD by using SPSS (Windows Version 15.5) software. One way analysis of variance (ANOVA) was performed to determine the variations in the levels of trace elements among groups. The significance level was set at $P<0.05$ and $p<0.001$.

RESULTS AND DISCUSSION

Table 1 shows the mean level of Cu was significantly raised in schizophrenia groups compared with controls ($p<0.01$). The raise in copper level is statistically more significant in patients with positive symptoms ($p<0.01$). The mean levels of zinc and selenium were significantly lower in schizophrenia groups compared with controls ($p<0.01$) and there was no significant difference was found among schizophrenia patients with different symptoms. There was also significantly decreased mean levels of iron in study groups when compared with the controls ($p<0.01$).

The mean level of calcium was raised very little, which is statistically not significant in schizophrenia groups compared with controls. But schizophrenia patients with cognitive symptoms were showing profound decrease in the calcium status ($p<0.01$). Also there was no significant increase in the levels of magnesium in schizophrenia patients with positive symptoms and control groups. But, there was statistically comparable increase in the levels of Mg was found in patients with negative and cognitive symptoms when compared to that of control and patients with positive symptoms ($p<0.001$).

Imbalances in the optimum levels of trace elements may adversely affect biological processes, and are associated with many diseases. In this study, levels of plasma zinc was significantly low in schizophrenia patients with different symptoms. Two main changes might be considered in the tissues with decreased plasma zinc level (20,21). (1) Cell membrane damage. (Shortage of Zn lowers the stability and integrity of the cell membrane,

Table 1: Levels of plasma Trace elements in Schizophrenia patients (with positive, negative and cognitive symptoms) and Healthy controls

Trace Elements	Control	Schizophrenia Patients with		
		Positive Symptoms	Negative Symptoms	Cognitive Symptoms
Copper (µg\dl)	79±22.08	87 ±23.74a*	83 ±19.05ab	84 ±213.92ac
Zinc (mg\L)	128±13.42	123±17.28a	122±16.94a	124±12.74a
Magnesium (mg\L)	6.9 ±2.32	7.1 ±2.06	7.8 ±1.97b	7.6 ±1.98c
Iron (µg\dl)	164.10±1.22	149.21±1.09a	143.44±1.02a*b	148.24±1.28ad
Calcium (mg\dl)	19.47 ±0.39	19.72 ±0.81	18.08 ±1.27ab	17.43 ±0.98a*c*d*
Selenium (µg\dl)	93.25±0.12	83.19±0.43a*	83.56±0.18a*	84.55±0.25a*

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).

leading to release of enzymes from lysosome and histamine from mastocytes. The ability of the membrane to resist free radicals is impaired). (2) Cu-Zn SOD structure changes. (Lower Zn damages the structure and affects the activity of Cu-Zn SOD, consequently reduces the ability of Cu-Zn SOD to scavenge free radicals.). When the content of Zn decrease Cu-Zn SOD activity also diminishes and free radicals cause increased tissue injury.

Another finding which confirms low zinc in schizophrenics were come from the study of Pfeiffer, 2007[22] who stated that pyrrole is a chemical substance that is involved in the formation of heme, which makes blood red. Pyrroles bind with B6 and then with zinc, thus depleting these nutrients. Abnormal production of pyrroles and their appearance in the urine of psychotics was first noticed in 1958 during LSD experimentation. Approximately 15-30% of "schizophrenics" have pyroluria. (At least 10% of these also have histamine problems.) Symptoms include (mental phenomena) include delusions, hallucinations, paranoia, and occasional loss of contact with reality, amnesia spells, and low stress tolerance.

The findings of the present study indicates that the schizophrenia subjects had significantly high plasma copper values than the controls; which is consistent with the hypothesis that there is increased central activity of copper-dependent enzymes in schizophrenia. These enzymes, tyrosine hydroxylase and dopamine-f3-hydroxylase, are involved in the synthesis and catabolism of dopamine[23].

One of the most favored theories on the etiology of schizophrenia is concerned with the role of dopamine. It is known that the majority of drugs effective in treating schizophrenia blocks the receptors at which dopamine acts. At least two enzymes involved in dopaminergic regulation are copper-dependent-dopamine- f3-hydroxylase (DBH), which converts dopamine to nor epinephrine, and tyrosine hydroxylase, which catalyzes the hydroxylation of tyrosine to produce dopa. Copper deficiency may be associated with reduced activity of these enzymes and cupper overload is concerned with the increased activity of these enzymes. The results of the present study support this theory because schizophrenia patients with positive symptomology can usually have increased dopamine production. A number of studies[24,25] have demonstrated raised serum copper in schizophrenia and Abul Hasnet et al (2007)[26] also demonstrated the raised cupper level in schizophrenia patients

with somatization disorders. To determine whether abnormality in copper metabolism is central in schizophrenia, Greiner and associates (1975)[27] looked at the copper content in the brains of individuals with this disease. In a postmortem study on 12 subjects, they showed that there was no difference in the amount of copper in the brains of schizophrenic, brain-damaged, and normal subjects.

Histapenia (hista-: histamine; -penia: deficiency of) is a shortage of histamine in the body. Histamine is an important brain chemical involved in many reactions. It has been found that 50% of patients classified as "schizophrenic" have low histamine levels in the blood and it rises to normal as they improve. These same patients are found to have high copper levels. Elevated copper decreases blood histamine. Excess copper is linked with psychosis. According to Pfeiffer (2007)[22], people with histapenia tend to have classic signs of positive symptoms, including, ideas of grandeur, undue suspicion of people, racing thoughts, the feeling that someone controls one's mind, seeing or hearing things abnormally, ringing in the ears, and others. Dopamine appears to be a factor in producing hallucinations, voices and other symptoms associated with schizophrenia. Those with histapenia may have elevated dopamine levels.

"Copper overload with zinc deficiency will explain the present dopamine theory of simplistic schizophrenia since this condition occurs only in one-half of patients labeled schizophrenic", that is, in low histamine schizophrenia. Copper oxidizes catecholamine such as dopamine and therefore propagates neurotoxin formation. Zinc imbalance is associated with central nervous system disorders such as schizophrenia and autism and several other pathologies[28,29].

The results of the present study suggest that there are no significant differences in the content of calcium in plasma between controls and schizophrenic patients with positive and negative patients. But there was a statistically significant decrease in the levels of calcium in patients with cognitive symptoms. It is suggested that Ca is capable of inducing structural and cognitive deficits seen in schizophrenia and it has been proposed that altered Ca signaling may constitute the central unifying molecular pathology in schizophrenia[13].

Ca (2+) is capable of inducing structural and cognitive deficits seen in schizophrenia. The evidence of the ability of antipsychotic drugs to affect Ca (2+) signaling is also presented. Based on these data, it is proposed that altered Ca (2+) signaling may constitute the central unifying molecular pathology in schizophrenia. According to this hypothesis schizophrenia can result from alterations in multiple proteins and other molecules as long as these alterations lead to abnormalities in certain key aspects of intracellular Ca (2+) signaling cascades[13].

It was also found in our study that plasma Fe concentration was significantly lower ($p < 0.01$) in schizophrenic patients than in controls. Iron (Fe), apart from its presence in all body cells, plays a role in the oxygenation of tissues as it is incorporated in the haem structure of hemoglobin. Low serum iron has been reported in a variety of neuropsychiatric motor disorders (11,30). Low level of iron affects dopaminergic system. Many researchers found abnormal iron deposits in the brains of schizophrenics[12]. When our results are stating that plasma iron concentrations decreased in schizophrenia patients, Abul Hasnet *et al.,* (2006)[31] comes in opposite directions. They stated that plasma concentrations of iron increased significantly when compared with control groups.

Magnesium is known to be part of many different enzyme systems and is involved in controlling various metabolic functions. Plasma magnesium (Mg) levels were estimated and results showed that there was a slight increase in patients with positive symptoms while patients with negative and cognitive patients showed significant elevation in the levels of magnesium when compared with control subjects($p<0.001$). Hypermagnesemia is present in adrenal insufficiency and rhabdomyolysis. High levels may reduce neuromuscular transmission and can act as a nervous system depressant. Some studies have been found lower levels in schizophrenics than in controls (32, 33), and other works have not found a correlation between magnesium levels and psychiatric symptoms in in-patients. A hypothesis is proposed according to which

the high level of stress found in severely ill psychiatric patients can lead to marginal Mg difference in susceptible individuals. This could exacerbate symptoms such as anxiety, fear, hallucinations, weakness and somatic complaints [32,34].

It is shown in the present study that plasma selenium levels decreased significantly in all the patients compared with control groups. When intragroup comparison was made, there is no noticeable difference among schizophrenia patients with different symptoms. Selenium is involved primarily in enzymes that are antioxidants. The effect of Selenium deficiency or imbalance plays a role in the symptoms of mood disorders. Observational and experimental studies have shown an association between selenium and schizophrenia[35,36].

A defective selenium transport protein and consequent low levels of selenium might adversely affect multiple enzyme systems. Selenium-enzyme interactions are discussed and the effect of selenium on arachidonic acid and its metabolites, especially 12-HPETE, are examined by Berry (1994). If the Berry model is essentially correct, selenoprotein P, a hypothesized selenium transport protein, is a likely candidate for a protein involved in the etiology of a form of schizophrenia[37].

There is direct evidence that the nutritionally essential trace element selenium has a pivotal role in neuronal susceptibility to excitotoxic lesions. Nicolai *et al.*,[38] observed in neuronal cell cultures that addition of selenium in the form of selenite within the physiological range protects against excitotoxic insults and even attenuates primary damage. The neuroprotective effect of selenium is not directly mediated via antioxidative effects of selenite but requires de novo protein synthesis. These findings indicate the importance of selenium for prevention and therapy of excitotoxic brain damage.

Deficiency in selenium may be associated with increased anxiety; depression and fatigue. The men taking more selenium reported feeling more confident and less anxious, depressed and confused[39].

A randomized, double-blind study involving 50 individuals also demonstrated the importance of selenium for maintaining emotional well-being. Some people in the trial received a daily supplement of 100 mcg selenium for five weeks, while others received placebo. Both groups continued to eat their usual diets. The individuals taking the placebo reported no change in mood, but those taking selenium reported a substantial improvement - including decreased feelings of anxiety - at both 2.5 and 5 weeks[40]. Interestingly, individuals receiving the least selenium from their regular diets benefited the most from taking extra selenium. After five weeks of supplementation, reports of anxiety, depression, and fatigue decreased most drastically for these individuals. This finding suggests that diets lacking in selenium can contribute to feelings of depression and anxiety[41].

Our results supports the findings of Vaddadi *et al.,* 2003,[42] who compared the plasma and red-cell selenium concentrations of schizophrenic patients treated with clozapine, with healthy controls and patients with mood disorders and interpreted that Selenium concentrations in plasma and red cells were found to be significantly lower in schizophrenic patients treated with clozapine as compared with all other control groups.

In conclusion, the results of the present study demonstrate that the serum selenium, zinc and iron were found in low levels in schizophrenia patients. The levels of copper and magnesium seems to be elevated. These observations support that alterations in essential trace elements either deficiency or overload may play a role in the pathogenesis of schizophrenia. Both under and malnutrition have been found to impair the brain and behavioral development as well as, affect the health of the body and mind in the adult. Although several prenatal under nutrition have been found to affect the brain growth and maturation of fetus, often resulting in cognitive , and psychomotor retardation, and under nutrition per se may not be related to schizophrenia, since schizophrenia patients who have adequate calorie intake in developed countries and upper socioeconomic classes can suffer from a more unfavourable course and outcome. Malnutrition refers to the lack of essential nutrients, such as essential amino acids, vitamins, lipids and trace elements and may be more relevant to schizophrenia than under nutrition.

It is suggested that schizophrenia is associated with the reduced availability or lack of some essential nutrients during the critical stage of brain development, and a continued lack of these essential nutrients after birth through adolescence may contribute to an unfavorable course and outcome of the disease.

Nutritional needs of people with schizophrenia ultimately involve neurotransmitter issues of production, release, inhibition, transmission, and receptor formation. In schizophrenia, nutrition must also address neuron cell degeneration. - an unfortunate common finding in chronic schizophrenia. Neurotransmitter metabolism is intricately involved with chemical reactions, which are in turn dependent on vitamins, minerals, and other substances. This study provides a prognostic tool for the diagnosis of schizophrenia and stresses the need of supplementation of trace minerals which could alleviate the schizophrenia.

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