Comparative Analysis of Serum Manganese, Zinc, Calcium, Copper and Magnesium Level in Panic Disorder Patients

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Abstract The purpose of the study was to determine the serum concentration of trace elements of panic disorder patients and to find out the relationship between trace element levels and nutritional status or socio-economic factors. The study was conducted among 54 panic disorder patients and 52 healthy volunteers. Patients were recruited from Bangabandhu Sheikh Mujib Medical University by random sampling. Serum trace element concentrations were determined by flame atomic absorption spectroscopy (for Mg, Zn, Ca, and Cu) as well as graphite furnace (for Mn). Data were analyzed by independent t test, Pearson's correlation analysis, regression analysis, and ANOVA. The serum concentration of Mn, Zn, Ca, Cu, and Mg in panic disorder patients were 0.37 ± 0.30, 0.67 ± 0.20, 99.91 ± 15.15, 0.83 ± 0.23, and 21.14 ± 3.72 mg/L, while those were 0.4163 ± 0.2527, 0.86 ± 0.3, 106.6073 ± 18.6531, 0.8514 ± 0.3646, and 21.37 ± 2.03 mg/L in control subjects, respectively. The serum concentration of Zn decreased significantly ($p=0.001$) in patient group. But the differences of the concentration of Mn, Ca, Cu, and Mg between patient and control group were not significant ($p=0.522$, $p=0.065$, $p=0.800$, and $p=0.712$, respectively). Socio-economic data reveal that most of the patients were very poor and middle aged. Mean BMIs of the control group (23.74 ± 2.71 kg/m^2) and the patient group (22.62 ± 3.74 kg/m^2) were within the normal range (18.5–25.0 kg/m^2). There was no significant

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relationship between serum zinc level and BMI of patients ($r=0.038$; $p=0.809$). So the decreased level of serum zinc in panic disorder patients was not because of other reasons, but rather it may provide a prognostic tool for the diagnosis and treatment of this disease.

Keywords Panic disorder · Trace elements · Socio-economic factors

Introduction

Panic disorder, as described by the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV), is a psychological and psychiatric condition characterized by recurring panic attacks in combination with significant behavioral change or at least a month of ongoing worry about the implications or concern about having other attacks. Panic disorder often begins in late adolescence or early adulthood and is twice as common in women as men [1]. Panic disorder is associated with several psychiatric conditions such as depression and other anxiety disorders [2]. Symptoms of panic disorder as described by DSM-IV include palpitations, sweating, shaking, shortness of breath, chest pain or discomfort, depersonalization, losing control, and numbness or tingling sensations [3]. Panic disorder is often accompanied by other serious problems such as depression, drug abuse, or alcoholism [4, 5]. Panic disorder may result from an abnormally sensitive fear network, which includes the prefrontal cortex, insula, thalamus, amygdala, and projections from the amygdala to the locus ceruleus, hypothalamus, periaqueductal gray substance, and parabrachial nucleus [6]. But the exact etiology of panic disorder (PD) remains a mystery. In panic disorder, the body's normal "alarm system" tends to be triggered unnecessarily and the reason is not well understood [7]. However, distinguishing panic attacks from medical disorders is difficult [8]. Alternatively, the pathophysiology of panic disorder remains unknown and the diagnosis of panic disorder also remains difficult [9, 10], and currently there is no medical or laboratory diagnostic test to confirm panic disorder.

Trace elements play a dual role in the biological system through their interaction with biomolecules. They regulate a number of cellular metabolic reactions while a few of them act as etiological agents in many environmentally induced neurological disorders [11]. In addition, they are involved in the function of sub-cellular systems such as mitochondria as well as in membrane transport, nerve conduction, and muscle contraction. Some of them (Zn, Cu, Mn, and Se) act as antioxidants. The very complex balance of trace elements is crucial for all areas of maintaining human health, preventing as well as overcoming health problems [12]. Therefore, altered homeostasis of trace elements may adversely affect biological processes, and are associated with many diseases [13]. Copper is an important component of several metalloenzymes, including tyrosinase and dopamine hydroxylase [14]. It also plays a vital role in neurological disorders [15]. Zinc plays an essential role in many aspects of metabolism including the activity of more than 300 enzymes, the structure of many proteins, and control of genetic expression [16, 17]. Zinc is an important cofactor in many biochemical reactions essential for normal neurological function and a cofactor for several neurotransmitters. Zinc status affects basic processes of cell division, growth, differentiation, development, performance, and aging through its requirement not only for synthesis and repair of DNA, RNA, and protein but also for many other aspects of metabolism [18, 19]. Zinc is unevenly distributed within the brain particularly at high concentrations in the hippocampal mossy fiber system where it acts as a neuromodulator [20]. Interactions with inhibitory [21] and excitatory amino acid neurotransmitter are well known with Zn [22] and high concentration of zinc is neurotoxic [23]. Paul et al. stated that

panic attacks are highly associated with copper toxicity. Zinc deficiency due to copper excess may contribute to this problem. Brain is a major target tissue for higher level of manganese. Manganese has been shown to accumulate in vivo in the mitochondria of brain areas associated with neurological symptoms [24]. Lower level of Mn plays a significant role in the development of schizophrenia possibly by interfering with the membrane stability [25]. Magnesium is a natural calcium channel blocker and significant magnesium deficiency has been reported in depression [26].

Calcium and magnesium are necessary for relaxation and proper nerve function. Calcium excites nerves; magnesium calms them down. Ca is also capable of inducing structural and cognitive deficits in schizophrenia and it has been proposed that altered Ca signaling may constitute the central unifying molecular pathology in schizophrenia [27]. A lack of calcium or magnesium may increase susceptibility to anxiety and panic. Low Mg/Ca ratio may trigger the stress response [28].

These observations suggest that alterations in essential trace elements like Cu, Zn, Mn, Ca, and Mg may play a role in the pathogenesis of panic disorder. As no work has been reported regarding serum trace element concentration in panic disorder patients, therefore attempts were made to investigate the correlation between trace element level and the degree of disorder, if any.

Materials and Methods

Fifty four panic disorder patients (44 males and 10 females of mean age 29 ± 7 years) were randomly recruited from Bangabandhu Sheikh Mujib Medical University (BSMMU) by a specialist psychiatrist who was trained in Diagnostic and Statistical Manual of Psychiatric Disorders (Fourth Edition) and 52 sex-, education-, and age-matched healthy volunteers (42 males and 10 females mean age 30 ± 6 years) were recruited purposively as control. The study subjects were briefed about the purpose of the study and written consent was obtained from each of them. All subjects had to go through clinical examinations to find out the existence of other diseases that might alter trace element level. Subjects also had to undergo a routine physical checkup including their organ activity, weight, nutritional condition, blood pressure, chest X-ray, and ECG. Hematocrit, blood urea, nitrogen, creatinine, glucose, and liver enzyme tests were also done for all subjects to find out their actual pathological condition. These subjects did not have diabetes, kidney failure, or other disease nor had they been treated with drugs, which can interfere with concentration of the elements (diuretics, antihypertensive drugs or mineral supplements, etc.). Patients who were mentally retarded and suffered from co-morbid psychiatric disorders were also excluded from the study. Socio-demographic data were also collected in a questionnaire form which contains important information including socio-economic data, history of illness, family history, etc. Ethical approval was obtained from the BSMMU authority.

Five milliliters of venous blood was drawn from each patient and controls after overnight fasting using a plastic syringe fitted with a stainless steel needle. The blood sample was collected into a metal-free plastic tube and allowed to clot at room temperature for 1 h, so that clotting factors could be removed from the serum. Then the blood sample was centrifuged at 3,000 rpm for 15 min at room temperature to separate the serum. The serum was aliquoted into an Eppendorf tube and was left to stand at $-80°C$ until analysis. Blood collection and serum separation were carried out in a dust-free environment.

Analysis of the trace element was carried out by using flame atomic absorption spectrometry (Varian SpectraAA 220) as well as graphite furnace [29]. Serum samples were

diluted by deionized water by a factor of 10. Different concentrations (0.5, 1.0, 2.0, 5.0, and 10.0 mg/L) of trace elements were used for calibration of standard graphs. Absorbances were read at 324.8, 213.9, 279.5, 285.2, and 422.7 nm for copper, zinc, manganese, magnesium, and calcium, respectively, in the atomic absorption spectrometer. To verify the assay accuracy and to maintain quality, the standard solutions were run for every 10-test sample. A software package (SpectrAA software) was used to calculate concentration of copper, zinc, manganese, magnesium, and calcium. The precaution for both collection and subsequent handling of serum were taken in order to avoid or minimize trace elements contamination. The results were expressed as mean±SD. Differences between groups' means were determined by independent sample t test. The significance level was set at $p < 0.05$. Pearson's correlation analysis was performed to find out the correlation of BMI and socio-economic factors with the trace element concentration of the patients. A multiple regression analysis and one-way analysis of variance (ANOVA) were also performed to

Table 1 Socio-economic and Nutritional Status of Panic Disorder Patients ($n=54$) and Controls ($n=52$)

Parameter	Patients n (%)	Mean±SD	Control n (%)	Mean±SD	p value
Education					
Illiterate	3 (5.56)		3 (5.77)		0.834
Read/write	13 (24.07)		12 (23.08)		
Secondary	21 (38.89)		18 (34.62)		
Higher secondary	6 (11.11)		9 (17.30)		
Graduate and above	11 (20.37)		10 (19.23)		
Occupation					
Employed	18 (33.33)		17 (32.69)		0.653
Business	19 (35.19)		17 (32.69)		
Unemployed	9 (16.67)		11 (21.15)		
Housewife	8 (14.81)		7 (13.46)		
Monthly income in US $					
0–66	26 (48.15)	92±81	29 (55.78)	101±69	0.714
67–200	24 (44.44)		18 (34.62)		
201–400	4 (7.41)		5 (9.60)		
Age in years					
18–30	34 (62.96)	29±7	31 (59.62)	30±6	0.496
31–40	17 (31.48)		17 (32.69)		
>40	3 (5.56)		4 (7.69)		
Smoking habit					
Non-smoker	31 (57.41)		31 (59.62)		0.619
Smoker	13 (24.07)		11 (21.15)		
Ex-smoker	10 (18.52)		10 (19.23)		
BMI (kg/m^2)*					
Below 18.5 (CED)	6 (11.11)	22.62±3.74	1 (1.92)	23.74±2.71	0.150
18.5–25 (normal)	36 (66.67)		36 (69.23)		
>25 (obese)	12 (22.22)		15 (28.85)		

*$p < 0.05$ (significant difference between panic disorder patients and controls at 5% level of significance)

determine the extent of involvement of socio-economic factors and BMI that affects the level of trace elements.

Results and Discussion

Socio-economic data of the schizophrenic patients and control subjects have been shown in Table 1. It was found that most of the patients were literate (94.43%), non-smokers (59%), and very poor (income 91.95±80.93 US $ or less per month). Average age of patient and control group was 29±7 and 30±6 years, respectively, which was very similar to previous findings with other psychiatric disorder like somatization disorder, schizophrenia, mania, depression, anxiety, etc. [30–32].

Serum trace element concentrations are presented in Table 2. The concentration of Zn decreased significantly ($p<0.05$) while the change of concentrations of Ca, Cu, Mn, and Mg were not statistically significant ($p>0.05$). Difference between the mean BMI of patients (22.62±3.74 kg/m^2) and controls (23.74±2.71 kg/m^2) was not significant ($p=0.100$). Correlative analysis was also performed using the data of serum concentration of trace elements and BMI of panic disorder patients. It was found that there is no significant correlation with the trace elements and the BMI of patient group. Correlative analysis was also performed to evaluate the effect of BMI, age, and income of patient group on serum trace element levels. No significant correlation was observed ($p>0.05$). Also using serum Zn concentration of patient as dependent variable and smoking as independent variable, regression analysis was also carried out to find out the effect of smoking on serum Zn levels. Regression analysis depicted that the concentration of Zn had not changed

Table 2 Serum Trace Element Concentration of Panic Disorder Patients ($n=54$) and Controls ($n=52$)

Trace elements of serum samples (mg/L)	Patients n (%)	Mean±SD	Controls n (%)	Mean±SD
Ca*				
0–85	15 (25)	99.91±15.15	3 (5.77)	106.60±18.65
85–102	19 (36.54)		15 (28.85)	
102–128	20 (38.46)		34 (65.38)	
Mg**				
0–18	14 (25.93)	21.14±3.72	1 (1.92)	21.37±2.03
18–30	40 (74.07)		51 (98.08)	
Cu***				
0–0.9	42 (77.78)	0.83±0.23	35 (67.31)	0.85±0.36
0.9–1.5	12 (22.22)		14 (26.92)	
1.5–2.65	0		3 (5.77)	
Zn****				
0.3–0.9	45 (83.33)	0.67±0.20	30 (57.69)	0.86±0.3
0.9–1.4	9 (16.67)		22 (42.31)	
Mn*****				
0–0.8	53 (98.15)	0.37±0.30	47 (90.38)	0.41±0.25
0.8–1.4	1 (1.85)		4 (7.69)	
1.4–1.9	0		1 (1.92)	

Significance $p<0.005$. *$p=0.065$, **$p=0.712$, ***$p=0.800$, ****$p=0.001$, *****$p=0.522$

significantly with smoking behavior ($R^2=0.00\%$, $t=0.077$; $p=0.939$). Finally, ANOVA test was done with the same parameters that did not show a significant relationship between smoking and serum Zn concentration of the patients ($F=0.006$; $p=0.939$).

Other studies showed that lower magnesium level can cause depression, behavior and personality changes, apathy, irritability, and anxiety [33]. Maes et al. conducted three consecutive studies and found that serum zinc levels were significantly lower in depressed patients as compared to healthy matched controls. Studies showed that serum Cu and Mn levels were significantly higher in somatization disorder patients than in controls [34]. Yanik et al. showed that plasma Cu concentrations were significantly higher ($p<0.01$) and Mn and Fe concentrations were lower ($p<0.05$ and $p<0.05$, respectively) in schizophrenic patients than in controls. The same study observed that alterations in essential trace elements Mn, Cu, and Fe may play a role in the pathogenesis of schizophrenia [35].

Psychiatric manifestations of zinc deficiency include behavioral disturbances, depression, and mental confusion. Within major depression populations, lower zinc levels correlate with higher depression severity [36]. Zinc is commonly found to be deficient in people with depression. Mental confusion and mental illness are strongly associated with zinc deficiency. Present finding of significant correlation of Zn level with the panic disorder patients and no significant correlation of Zn with the BMI as well as smoking and gender differences justifies that zinc may be directly linked to this disease and zinc supplement may be beneficial to treat panic disorder patients.

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