

Toxicity, Deficiency and Dysmetabolism of Trace Elements in Ghanaian Clinically Stable Schizophrenics

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Abstract

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AIM: The purpose of the study was to determine the levels of Copper (Cu), selenium (Se), Zinc (Zn), Lead (Pb) and Lithium (Li) in patients in Accra and Pantang Psychiatric Hospitals in Ghana since no data exist.

SUBJECTS AND METHODS: Simple random sampling of age-matched subjects was used to recruit 81 schizophrenics and 25 mentally healthy controls in 2012. Serum levels of Cu, Se, Zn, Pb and Li were determined by flame atomic absorption spectroscopy (FAAS).

RESULTS: Mean levels were as follows: Cu_t 766 ± 250 µg/L and Cu_c 855 ± 270 µg/L (p = 0.168). Se_t 149 ± 72 µg/L and Se_c 108 ± 61 µg/L (p=0.009). Zn_t 702 ± 438 µg/L and Zn_c 1007 ± 593 µg/L (p = 0.028). Pb_t 1.38 ± 0.05 µg/L and Pb_c 0.10 ± 0.05 µg/L (p = 0.000). Li levels for the test group (Li_t) was 4077 ± 2567 µg/L, while that of the controls was undetectable < 0.02 µg/L. Se, Pb and Li levels were significantly higher in schizophrenic patients compared to controls. While Zn and Cu levels were lower in the same group.

CONCLUSION: Trace elements dysmetabolism exist among Ghanaian schizophrenics and monitoring is essential to avoid the adverse effects of metal overload or deficiency.

Introduction

In 2001, 450 million people worldwide suffered from some form of mental disorder or brain condition [1]. Of the 21.6 million people living in Ghana, 650,000 were reported suffering from severe mental disorders and a further 2,166,000 from moderate to mild mental disorders in 2007 (WHO, 2007) [2]. Globally, the most frequent disorders are schizophrenia, anxiety disorders, depression, somatoform and substance abuse disorders [3].

Traditional prevalence or "core," is studies-generated estimates based on the population residing within a defined catchment [4]. The prevalence estimates would therefore differ within lifetime, period, and point prevalence [4, 5]. Furthermore, in developing countries the proportion of cases with acute onset schizophrenia was twice as high as in developed countries [5]. This could be an underestimation [6].

Schizophrenia is characterized by symptoms that may include delusions, hallucinations, catatonia, negative symptoms, and disorganized speech or behavior. Genetic pre-disposition, pregnancy and its complications are said to be some of the risk factors, while viral infections and subsequent autoimmune reactions are causative factors [7].

Trace elements (TE) are suspected to be causative agents in the development of schizophrenia although the mechanism is poorly understood [8]. Cu, a component of several enzymes linked to dopamine synthesis, may play a role in schizophrenia by exacerbating or perpetuating dopaminergic dysregulation. Furthermore, it is suggested that ceruloplasmin elevation in schizophrenia is specific, and not simply an elevation of plasma copper-containing oxidative enzymes [9]. Abnormalities in Cu metabolism have been demonstrated in schizophrenics [10, 11]. This link is attributed to several metalloenzymes associated with dopamine

synthesis and regulation [12]. Dopamine and other catecholamines act as important hormones and neurotransmitters of the central and peripheral nervous system and have functions in the brain that control emotions [13, 14] and cognition [15].

Se is an integral component of glutathione peroxidase (GPx) which protects against reactive oxygen species (ROS). Selenoprotein R and thioredoxin, another selenoprotein, play similar protective roles [16]. Because oxidative stress increases DNA damage, Se deficiency could result in a higher mutation rate, increasing the likelihood that the sequence of a schizophrenia-related genetic region will be altered [17-18].

Zinc deficiency in the latter part of gestation impair fetal growth, brain size, total brain cell count, and the period of macroneuronal proliferation [19-20] and neurotransmitter functions. One explanation is attributed to the failure of thymidine incorporation into brain DNA, a decreased rate of protein synthesis and a smaller hippocampus [21]. Low and normal serum zinc levels have been demonstrated in studies with Bangladeshi and Turkish schizophrenics, respectively [11, 22].

Lead (Pb^{2+}) is a neurotoxicant present in the environment and capable of producing biochemical, anatomical, and neurological changes that altogether produce behavioural changes in human development and the manifestation of schizophrenia later in life. Pb exposure in the course of development manifests in expressions of increased spontaneous activity, decreased social interaction, and learning deficits presents also in schizophrenics [23].

Lead is a potent antagonist of the *N*-methyl-D-aspartate (NMDA) receptor (NMDAR). It is the reduced expression of NMDAR that is ideated to be associated with the pathophysiology of the disease [24]. Furthermore, reduced dentate gyrus (DG) neurogenesis has been demonstrated in an animal model using phencyclidine which is an NMDAR antagonist [25].

The biochemical mechanisms of action of Li appear to be extraordinarily complex, multifactorial and strongly intercorrelated with the functions of other elements, drugs, enzymes, hormones, vitamins, growth and transforming factors [26]. Rats on a Li-deficient diet demonstrated suppressed liver-press avoidance behavior [27]. Furthermore, rats on a Li-deficient diet exhibited diminished wheel-running activity, decreased response to handling and lower aggression in social interactions [28]. Evidence linking low Li intake with altered behavior and aggressiveness in humans has been reported [29]. Genetic factors have also been implicated in the metabolism of Li. The precise therapeutic mechanism of Li remains unknown, but may involve the circadian clock. Molecular components of the circadian clock are known to be Li sensitive [30-34]. Li also inhibits

glycogen synthase kinase 3β (GSK3 β) [33], which in turn regulates protein turnover and stability in the clock network by phosphorylating target proteins, thereby modulating circadian rhythms [34].

The aim of the study therefore was to determine deficiency, toxicity or dysmetabolism of Cu, Se, Zn, Pb and Li in Ghanaian schizophrenic patients in Accra since no such data exist.

Subjects and Method

This was a case-control study. Ethics approval was obtained from the Ghana Health Service (GHS-ERC: 16/05/12) and the University of Ghana School of Allied Health Sciences (SAHS-ET./10285645/AA/26A/2012-2013). A total of 81 clinically diagnosed psychiatric patients made up of 42 males and 39 females, from the Pantang Hospital (Accra) and 25 apparently mentally healthy age-matched and sex-matched controls (13 males and 12 females) were recruited for the study in July 2013. Simple random sampling was used to recruit subjects. Psychiatric patients should have been on admission for not less than five (5) years.

Treatment of Glassware

All glassware and tubes were soaked in 10% (w/v) HNO_3 (Fluka, Germany) overnight and then rinsed with deionized water.

Digestion Protocol for Sample

Digestion of samples was done according to standard trace element analysis protocols using milestone microwave labstation ETHOS 900 (INSTR: MLS-1200 MEGA) programme (Table 1).

Table 1: Microwave digestion programme.

Step	Time	Power	Pressure	Temp °C [1]	Temp °C [2]
1	00:02:00	250	100	400	500
2	00:02:00	0	100	400	500
3	00:06:00	250	100	400	500
4	00:05:00	400	100	400	500
5	00:05:00	650	100	400	500
Vent:00:05:00		Rotorctrl on		Twist on	

(Milestone Acid Digestion Cookbook update, 1996)

After cooling, the digestate was then transferred into a volumetric flask and diluted to 20 mL with deionized water. The diluted digestate was assayed for the level of Cu, Se, Zn, Pb and Li using VARIAN AA 240FS-Atomic Absorption Spectrometer (Kungsbacka, Sweden) along with standards of high purity metals from Teknolab AS (Kungsbacka, Sweden) and reference material SeronomTM Trace Elements Serum L-1, L-2 (Billingstad, Norway) under conditions in Table 2. Blank samples were also digested and analyzed along with the samples.

Table 2: Analytical conditions of elements.

Element	Wavelength nm	Lamp Current mA	Slit Width nm	Fuel	Support
Se	196.0	10	1.0	ACETYLENE	AIR
Pb	217.0	5	1.0	ACETYLENE	AIR
Cu	324.7	4	0.5	ACETYLENE	AIR
Li	670.8	5	1.0	ACETYLENE	AIR

(VARIAN, 1989)

Quality Assurance and Control Measures

The method detection limit was calculated as the mean of the blank concentration and three times the standard deviation. Values below detection limit of the instrument were replaced by the method detection limit divided by the square root of two. Control samples and standards were measured. Linearity was assessed by the calibration curve of all the elements measured. Replicate measurement for each sample was taken to ensure precision.

Statistical Analysis

The statistical analyses of the data was done using SPSS (Statistical Package for Social Sciences) version 16.0. Means \pm standard deviation were determined for quantitative variables. Chi-square was used for categorical data and independent t-test for demographic data. Comparison of Cu, Se and Zn levels between the psychiatric patients and the control group were performed with unpaired t-test. Abnormally distributed data (Pb) was analyzed with Wilcoxon-Mann/Whitney test. Pearson's correlation was used to test the relationship between the trace elements, gender and age. P-values \leq 0.05 were considered statistically significant.

Results

Demographic Data

A total of 81 clinically diagnosed psychiatric patients (20-57 years), made up of 42 males (51.2%) and 39 females (48.1), and 25 apparently mentally healthy controls (13 males and 12 females) (23-51 yrs) were recruited for the study. The mean age was 32.32 ± 0.84 yrs for cases and 33.64 ± 1.64 years for controls. Cases were found to be on haloperidol (0.5 mg – 3 mg).

Trace Elements Concentrations

The mean Cu, Se, Zn and Pb levels for the test and control groups were 766 ± 250 $\mu\text{g/L}$, 149 ± 72 $\mu\text{g/L}$, 702 ± 438 $\mu\text{g/L}$, 1.38 ± 0.05 $\mu\text{g/L}$ and 855 ± 270 $\mu\text{g/L}$, 108 ± 61 $\mu\text{g/L}$, 1007 ± 593 and 0.10 ± 0.05 $\mu\text{g/L}$, respectively. Graphical representations of Cu, Se, Zn, Pb and Li concentrations of the subjects are shown in Figure 1, 2, 3, 4 and 5, respectively. Statistical differences found between the psychiatric patients and the controls for the elements Cu, Se, Zn and Pb

were $p = 0.168$, $p = 0.009$, $p = 0.028$ and $p = 0.001$, respectively (Table 3).

Table 3: The levels of trace elements of Schizophrenic patients (cases) and controls.

	Controls		Cases		p values
	N	Mean	N	Mean	
Copper ($\mu\text{g/L}$) ^a	25	855 ± 270	81	766 ± 250	0.168
Selenium ($\mu\text{g/L}$) ^b	25	108 ± 61	81	149 ± 72	0.009
Zinc ($\mu\text{g/L}$) ^c	25	1007 ± 593	81	702 ± 438	0.028
Lead ($\mu\text{g/L}$) ^d	25	0.10 ± 0.05	81	1.38 ± 0.05	0.001
Lithium ($\mu\text{g/L}$)	25	BDL	81	4077 ± 2567	-

^{a, b, c} Independent t-test; ^d Wilcoxon - Mann/Whitney Test; BDL = Below Detection Limit

Li levels could be detected in only 16 samples of the test group, giving a mean value of 4077 ± 2567 $\mu\text{g/L}$. The controls were also below the detection limit of the equipment (< 0.02 $\mu\text{g/L}$). Therefore, only descriptive statistics was done for Li (Table 3). Values that could not be detected by the FAAS were replaced with the method detection limit (MDL) which is:

$$\text{MDL} = \text{Mean of Blank Concentration} * 3 * \text{SD},$$

$$\text{Non Detected Values} = \text{MDL} / \sqrt{2}$$

Table 4: The levels of trace elements in Psychiatric patients and controls according to gender.

		Cu	Se	Zn	Pb	Li
		($\mu\text{g/L}$)	($\mu\text{g/L}$)	($\mu\text{g/L}$)	($\mu\text{g/L}$)	($\mu\text{g/L}$)
Cases	Males	775 ± 270	124 ± 52	639 ± 439	0.48 ± 0.28	3233 ± 1038
	Females	757 ± 230	181 ± 82	774 ± 433	1.63 ± 0.75	3549 ± 1341
Controls	Males	799 ± 320	113 ± 77	945 ± 539	0.12 ± 0.05	BDL
	Females	916 ± 200	102 ± 39	1064 ± 609	0.08 ± 0.04	BDL

* = 0.05; BDL = Below Detection Limit.

Female values were higher in all trace elements except for Cu. However, for the control group, copper was higher in males (Table 4). Furthermore, females had very high Se (181 ± 82 $\mu\text{g/L}$) compared to males (124 ± 52 $\mu\text{g/L}$) ($p=0.05$) and low Zn (639 ± 439 $\mu\text{g/L}$) compared to females (774 ± 433 $\mu\text{g/L}$) ($p>0.05$) (Table 4).

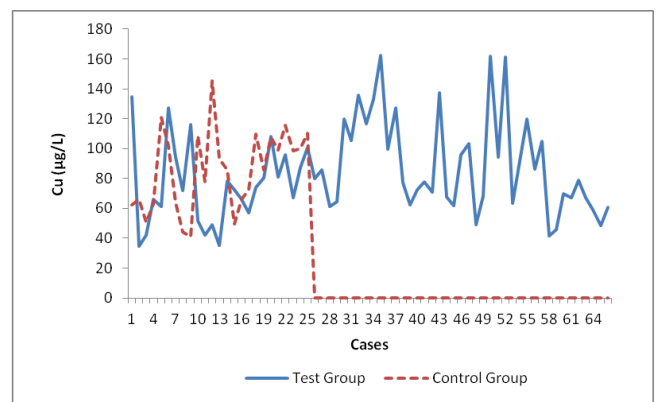


Figure 1: Different levels of copper (Cu) in the subjects. The test group showed significantly higher Cu levels than the controls.

There was no correlation between the levels of trace elements and the age or gender of the participants. A positive correlation existed between Li and Pb ($p=0.023$) (Table 4).

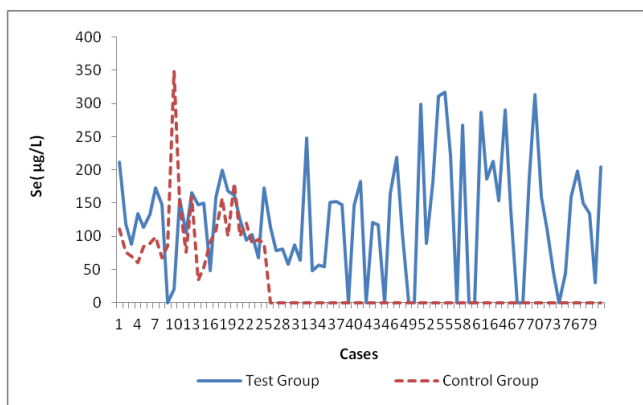


Figure 2: Selenium (Se) levels in all the subjects. Significantly higher levels were observed in the test group compared to the controls.

Discussion

Mean Cu level of the Case group was 766 µg/L and the control group was 855 µg/L. Values obtained for the Case group were statistically insignificant compared to the control group. Similarly, in the study of Nechifor et al. [35] no significant Cu differences were observed between cases and controls. On the contrary, the mean value of Cu for schizophrenic cases was 720 µg/L while that of the Control group was 590 µg/L in another study. Differences were statistically significant [9]. Yanik et al. [11] also reported of similar values; 980 µg/L (Cases) and 760 µg/L (control group) (p<0.05). However, higher values of 1174 µg/L for Schizophrenic cases and 1056 µg/L for controls with differences that were statistically significant have been reported. Altered levels of trace elements in schizophrenic patients may be a consequence of antipsychotic treatment [10].

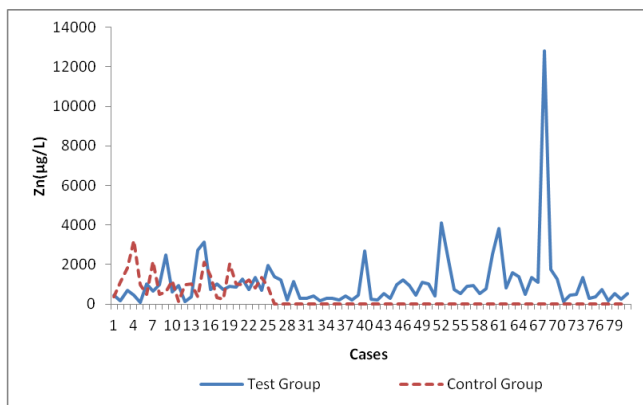


Figure 3: The levels of zinc (Zn) in the test group and the controls. Zn levels were significantly higher in the test group than the control. A high level was observed for case 69 which was complemented by very low selenium levels (Figure 2).

Normal brain functioning depends on the role of the Cu-containing enzyme dopamine -b-hydroxylase converting dopamine into norepinephrine. Other enzymes such as lysyl oxidase (responsible for

collagen synthesis) or monoamine oxidase A and B (the enzyme that eliminates dopamine) all contain copper. Therefore diminished copper impair these functions. On the contrary excess Cu will also reduce Zn-thionein and CuZnSOD, and other enzymes required for serotonin secretion as a result of competitive absorption with Zn, resulting in diminished Zn absorption. These aforementioned antioxidants are relevant in the maintenance of Cu-Zn homeostasis and failure to regulate excess Cu will lead to oxidative stress and damage. Dysmetabolism of these metals as well as disturbances of the dopamine/norepinephrin pathway have been implicated in schizophrenia [36].

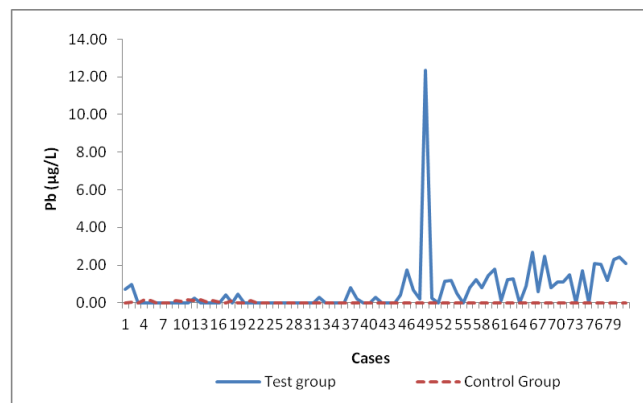


Figure 4: The levels of lead (Pb) in the test group and the controls. Pb levels were significantly higher in the test group than the control. Case 49 had very high Pb levels accompanied by very low selenium levels (Figure 2).

Se was 149 µg/L (cases) and 108 µg/L (control). Thus, Se was higher in the Schizophrenic group above the reference interval and statistically significant when compared to the control group (p= 0.001). The mean value obtained for the control group was similar to that obtained in a previous study (123 µg/L) [37]. Significantly similar higher Se levels in schizophrenics were reported by Kharb et al. [38]. In that study Se levels of cases and controls were 134 µg/L and 101 µg/L, respectively.

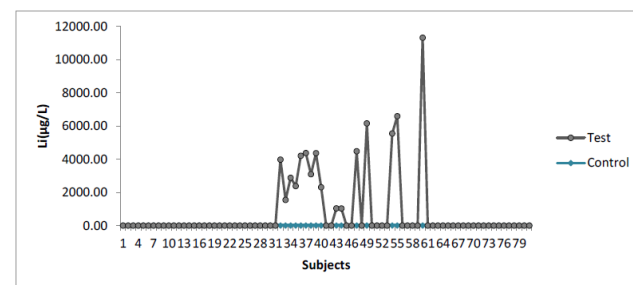


Figure 5: The levels of lithium (Li) in the test group and the controls. Most of the control levels were below the detection limit. However, case 60 showed very high Li levels accompanied by very low selenium levels (Figure 2).

Differences were attributed to geographical locations and soil Se levels. Yanik et al. [11] also

reported of a higher Se level in Schizophrenics (71.65 µg/L) compared to Controls (69.65 µg/L). Differences however, were insignificant. Low blood Se concentrations in schizophrenic patients on clozapine have been reported; 101µg/L (cases) versus 116 µg/L (control), with differences being significant [39]. That study however, could not attribute the low Se to clozapine.

Some authors have pointed out that the geography of schizophrenia may have possible links with Se and calcium deficiencies [40, 41]. However, it is unknown whether schizophrenics display selenium deficiency prior to diagnosis. Tada et al. [42] for example, discovered elevated selenium in the hair of male but not female patients. In contrast, Alertsen et al. [43] found no difference between the levels of selenium in the serum and blood of Norwegian schizophrenics and controls.

In this study, the mean Zn level was 1007 µg/L for the control group compared to 702 µg/L for the case group. Similar low Zn level was obtained in the schizophrenic group (600µg/L) in another study [35] compared to 890 µg/L for the control group. In that study differences were significant and treatment raised the Zn levels appreciably. Zn plays a very complex role in the brain and Zn deficiency is postulated in the pathogenesis of schizophrenia [44]. Pfeiffer and Ilier [45] showed a lower plasma Zn level coupled with excess Cu in schizophrenic patients and Zn loss in the hippocampus of schizophrenic patients has also been demonstrated [46]. On the contrary, another study did not observe any significant difference between Zn levels of cases and controls [11]. In a similar study, serum Zn was lower among schizophrenic criminals compared to schizophrenic non-criminals (680 µg/L and 810 µg/L, respectively) with significant differences [47]. Thus, low serum Zn in major depression is a sensitive marker of treatment resistance and of the immune/inflammatory response in mental illness [48]. Furthermore, Zn deficiency exacerbates loss in blood-brain barrier integrity induced by hyperoxia [49].

Pb levels in the case group and control group were 1.38 µg/L and 0.10 µg/L, respectively. Statistically, differences were significant ($p = 0.001$). Furthermore, case 49 (Figure 4), demonstrated very high Pb levels (12 µg/L), with corresponding low Cu and Se values (Figure 1 and 2, respectively). Prenatal Pb exposure and schizophrenia has been argued [50, 51]. The possible neurobiological connection with Pb exposure could be due to the fact that *N*-methyl-d-aspartate receptor (NMDAR) may be affected by the antagonistic effect of Pb on NMDA thereby reducing the function of NMDAR which is involved in the pathophysiology of the disease [52]. Generally, exposure to such insults from the environment and damage or alteration to the developing central nervous system may be associated with schizophrenia and other mental disorders [53-55].

Li levels in this study were below the detectable limit in all control subjects and majority of the cases. However, very high Li observed in case 60 (Figure 5) corresponded to a very low Se level (Figure 2). Much as Li may act as mood stabilizer, information on its efficacy is inconsistent [56]. Adjunctive Li and anticonvulsants for the treatment of schizophrenia seem to lack the necessary evidence [57]. The dearth of Li levels of schizophrenic patients is also problematic.

In conclusion, Se, Pb and Li levels were significantly higher in schizophrenic patients compared to controls. Zn and Cu levels were significantly lower in the cases than the controls. Female Se levels were extremely high approaching toxic levels while male Zn levels were extremely low approaching deficiency levels. TE dysmetabolism exist among schizophrenics and monitoring of TE is essential to avoid the adverse effects of metal deficiency and/or overload in tandem with mental disease. To the best of our knowledge this data is the first on schizophrenics and trace elements in Ghana.

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