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# Elemental composition of blood and hair of mentally–ill patients using ICP-OES techniques

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## ABSTRACT

Elemental concentrations of blood and hair of 40 mentally-ill patients and 40 controls (healthy subjects) were determined by Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) in order to find out the metal burden of the patients with their level of significance and possible relationship of such elements with mental illness. Generally, higher concentrations of trace elements were obtained in the hair than in the blood. A concentration range of 90 - 400 µg/g was obtained for Mg, K and Fe in hair of patients and controls, 80-7400 µg/g was obtained in their blood; other elements range 0.001-30 µg/g in both blood and hair of patients and controls. Comparative study of statistical significance of median values between different groups was determined by applying a non parametric test (Mann-Whitney) showed that concentrations of Ba, Be, Cr, Li, Mg, Fe and K were significantly higher in patients' blood, while Al, Ba, Be, Na, Cd, Cr, Li, K, Mn, Mg and Sr were significantly higher in patient's hair. Concentrations of most elements are higher in the hair and blood of the mentally ill patients than in the healthy controls. Deficiency of Cu and Zn might be causally related to the illness.



**Keywords**: Schizophrenic patients; trace-metals, controls, ICP-OES, nutrients.

## INTRODUCTION

Toxic and persistent substances are increasing in the environment, continuously due to anthropogenic activities. The concentration of harmful substances in human biological materials may depend on the extent of a person's exposure to environmental conditions, his or her genetic characteristics and individual habits (Dona et al., 2006). Some elements are required in the body to maintain the normal physiological function of the organism. Such elements are key components of metallo-enzymes or are involved in crucial biological functions, such as oxygen transport, free radical scavenging or hormonal activity (Parsons and Barbosa, 2007).

It is not uncommon to see toxic levels of copper, lead, mercury, aluminum, arsenic and cadmium in mental illness particularly schizophrenics. Some of the most advanced schizophrenic cases having three or more heavy metals (Raymond et al., 2008). Most heavy metals are free radicals that induce

oxidative stress (lipid peroxidation) and have an affinity for brain tissue (Kelly, 2000).

The relevance of trace metals in biological samples comes from their potentially toxic effect on living beings (Tatiane et al., 2005). Blood and hair as biological tissues had been used for evaluating the deficiency of nutrient elements in the body of patients and at work place (Ojo et al., 1994, Jairo et al., 2008). They also give useful information about short and long-term exposure of subjects to trace elements. There have been some difficulties in establishing normal or reference ranges of elemental composition of hair due to the natural variance of hair composition as a possible consequence of age, sex, hair colour, ethnical and geographic origin or dietary factors (Sharma et al., 2004). Thus, this study set at finding out the metal burden of the patients and possible relationship of such elements with mental illness; and also to determine their level of significance in the mentally ill patients.

## MATERIALS AND METHODS

### Sample collection and treatment

Blood and hair samples were collected from the out patients of the Department of Mental Health of the Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria after an Ethical Committee review and certification. The blood samples were drawn from the cubital vein with 5 ml syringe polyethylene needle and stored in heparinized bottle and later freeze dried. The hair pieces were collected at the nape of the head to prevent contamination with cosmetics and facial secretions. The hair were cut into smaller pieces approximately 0.3 cm and mixed well to allow a representative sub-sampling of the hair specimen. After cutting, each sample was washed four times with 1:200 v/v dilution of Triton X-100. The samples were rinsed thrice with acetone and de-ionized water then dried in an oven at 75 ± 5°C (Ragupathy et al., 1988) and kept in clean standard envelopes before digestion (Gary Farr, 2003).

### Reagents

Analar grade reagents were used and standard solutions were prepared using Ultra pure water (18MΩcm). Polyethylene sample bottles and Teflon beakers were soaked in 10% $HNO_3$ for 48 hours and later rinsed with distilled de-ionized water prior to its use for metals analysis.

### Sample preparation and analysis

A quantity of 0.05g of each blood and hair samples were weighed and digested separately with 6 ml of analytical grade 70% $HNO_3$ using the microwave digester with the running method (User 001 M) and cleaning method (User 002 M) and the following method parameters:

- Power was set at 400 watts and temperature of 170 $^0$C for 25 minutes with holding time of 10 minutes.
- The cleaning time was set at 20 minutes.

The reference material was subjected to the same condition. The resulting solutions were allowed to cool and diluted to 15 ml with Millipore water (18MΩ).

Perkin Elmer Optima 5000DV with axial viewing configuration inductively coupled plasma-optical emission spectrometer (ICP-OES) was used with the following operation conditions: Optical system– Echelle; Power-1300 W, Plasma gas flow -15 L/min; Auxiliary gas flow - 0 L/min Nebulizer. 8L/min; View distance -15 mm; Sample flow rate -15 mL/min; Wash rate -1.5 mL/min; Wash time-30s.

### Analytical validation

The multi-elements standard solution used for calibration was prepared by diluting the stock solution of 10 mg/l of the given elements supplied and stored in polyethylene bottles. The calibrations of elements were found to be linear with correlation coefficients above 0.99 (Miller and Miller, 2000) for most of the elements as shown in Table 1. The accuracy of the method was studied by analysing the Certified Reference Material (NCS DC 73347) supplied by Industrial Analytical (Pty) Ltd, South Africa with ICPOES. The results obtained showed high

accuracy and precision of the method for multi-elemental samples within the limit of experimental error (Table 2).

**Statistical analysis**

The elemental concentrations in the blood and the hair of mentally-ill patients and control subjects were expressed as geometric mean value in µg/g with the standard deviation to illustrate concentration profile over each group. The statistical significance of the median values between different groups were determined by applying a non parametric test (Mann-Whitney) $H_o$: median for group A= median for group B, $H_1$: median for group A ≠ median group B. The level of significance was set at 95% and 99% confidence intervals.

**RESULTS**

Multi-elemental analysis of whole blood and head hair of 40 schizophrenic patients and 40 healthy controls were validated for 15 elements. Table 3 shows the geometric mean and standard deviation of elements in the blood and hair of the mentally-ill patients and normal control. Generally, the concentrations of heavy metals are particularly higher in the hair than in the blood of the considered patients. The Mann-Whitney test showed a significant difference at 95% and 99% confidence intervals for more elements in the hair than in the blood.

Nutrient elements determined in blood of patients and controls viz: Ca, Mg, K, Fe, Cu and Zn have high concentrations. Among them, K has the highest concentration (above 3000 µg/g) followed by Fe (800-1000 µg/g) while Ca, Cu and Mg have concentrations that are less than 500 µg/g. For Zn and Cu, the values were below detection level in the blood of patients, while Cu level was below the detection limit in the hair of patients. Pb and Hg were below the detection limit in all samples. Other elements such as Al, Ba, Cr and V were present at levels below 5.00 µg/g. Be, Cd, Mn and Sr were detected at concentrations below 1.00 µg/g.

In the hair, Zn, Mg, Na and K were between 25-400 µg/g, Ba, Fe and Ca ranges between 10- 120 µg/g, Al, Cr, Cu, Mn and Sr were less than 15 µg/g, while Be, Cd and Li were less than 1.00 µg/g.

The general approach to studying elemental interactions was undertaken by finding the Pearson's product moment correlation coefficients (two tailed) at P= 0.01 and 0.05 level. Tables 5a, 5b, 6a and 6b show the interactions of the elements and their correlation coefficient values.

**Table 1:** Calibration curve of elements in ICP with correlation coefficients.

| Element | Correlation coefficient |
|---|---|
| Li | 0.9998 |
| Mg | 0.9749 |
| Mn | 0.9998 |
| Sr | 0.9989 |
| Ni | 0.9919 |
| Al | 0.9875 |
| Ba | 0.9965 |
| Cr | 0.9996 |
| Be | 0.9999 |
| Cu | 0.9995 |
| Cd | 0.9945 |
| Fe | 0.9807 |
| Ca | 0.9937 |
| Na | 0.9995 |

**Table 2:** Hair standard (NCS DC 73347) analysis with ICP-OES.

| Elements | Observed concentration (µg/g) | Expected concentration (µg/g) | % Observed |
|---|---|---|---|
| Ba | 16.830 ± 1.860 | 17.00 ± 0.200 | 99.0 |
| Be | 0.055 ± 0.001 | 0.063 ± 0.002 | 87.3 |
| Cr | 0.430 ± 0.001 | 0.370 ± 0.060 | 116.2 |
| Cu | 13.60 ± 1.400 | 10.600 ± 1.200 | 128.3 |
| Fe | 49.80 ± 6.200 | 54.00 ± 0.100 | 92.2 |
| Li | 1.90 ± 0.0200 | 2.00 ± 0.010 | 95.0 |
| Sr | 25.80 ± 0.200 | 24.00 ± 1.00 | 107.5 |
| Zn | 189.90 ± 3.00 | 190.00 ± 2.900 | 99.9 |

**Table 3:** Geometric means and standard deviation of the elements in the patients and control blood and hair.

| Element | Hair patients | Hair controls | Mann-Whitney U Test | | Blood patients | Blood controls | Mann-Whitney U Test | |
|---|---|---|---|---|---|---|---|---|
| µg/g | Geo-mean / Std. Dev | Geo-mean/ Std. Dev | P = 0.05 | P= 0.01 | Geo-mean/ Std. Dev | Geo-mean/ Std. Dev | P= 0.05 | P= 0.01 |
| Al | 12.51± 2.22 | 11.69±2.69 | 0.014 | 0.007 | 3.43±0.55 | 3.01±0.47 | 0.078 | 0.039 |
| Ba | 27.43± 3.74 | 10.68±1.76 | 0.000 | 0.000 | 1.29±0.09 | 1.47±0.89 | 0.026 | 0.013 |
| Be | 0.13± 0.05 | 0.10±0.04 | 0.000 | 0.000 | 0.29±0.16 | 0.22±0.04 | 0.000 | 0.000 |
| Cd | 0.21±0.21 | 0.12±0.97 | 0.002 | 0.001 | 0.12±0.05 | 0.09±0.01 | 0.181 | 0.0905 |
| Cr | 2.15±2.00 | 0.92±0.65 | 0.024 | 0.012 | 0.50±0.97 | 0.23±0.03 | 0.021 | 0.0105 |
| Li | 0.04±0.02 | 0.02±0.01 | 0.000 | 0.000 | 0.03±0.02 | 0.04±0.01 | 0.028 | 0.014 |
| Mn | 5.84±1.70 | 1.20±0.85 | 0.000 | 0.000 | 0.13±0.02 | 0.10±0.01 | 0.163 | 0.0815 |
| Sr | 3.61±0.05 | 1.67±0.35 | 0.000 | 0.000 | 0.27±0.19 | 0.23±0.09 | 0.041 | 0.020 |
| Zn | 92.56±6.75 | 100.74±8.9 | 0.001 | 0.0005 | BDL | 20.56±3.86 | - | - |
| Mg | 110.42±6.23 | 33.73±4.05 | 0.000 | 0.000 | 137.29±23.83 | 88.52±8.45 | 0.000 | 0.000 |
| K | 93.22±8.14 | 24.16±3.70 | 0.000 | 0.000 | 3990.01±54.86 | 7396.69±74.39 | 0.000 | 0.000 |
| Na | 375.77±37.23 | 139.49±24.93 | 0.000 | 0.000 | NA | NA | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Fe | 37.25±2.31 | 34.85±5.11 | 0.322 | 0.161 | 1598.88±42.99 | 809.40±44.79 | 0.000 | 0.000 |
| Cu | BDL | 14.98±4.03 | - | - | BDL | 9.81±4.36 | - | - |
| V | BDL | BDL | - | - | 3.67±0.72 | 2.61±0.63 | 0.00 | 0.00 |
| Ca | BDL | 117.03±10.82 | - | - | 301.17±6.24 | NA | - | - |

**Table 4a:** Elemental concentrations of blood of patients and control subjects in this work compared with literature values.

| Element | Controls (µg/g) | Patients (µg/g) | Literature (µg/g ) |
|---|---|---|---|
| Al | 0.01-7.11<br>3.01±0.47 | 0.00- 12.13<br>3.43±0.55 | 1.23±0.18[a]<br>1.28 – 6.35[e] |
| Ba | 0.05-5.30<br>1.47±0.89 | 0.00-35.59<br>1.29±0.09 | 46.4 –77.6e |
| Be | 0.04-0.34<br>0.22±0.04 | 0.00-1.27<br>0.29±0.16 | 0.02 - 0.09[e] |
| Cd | 0.00-1.45<br>0.09±0.01 | 0.00-0.32<br>0.12±0.05 | 0.15–2.04[e] |
| Cr | 0.00-0.2<br>0.23±0.03 | 0.05-9.84<br>0.50±0.97 | 0.36±0.006[a]<br>0.028-0.42[C] |
| Cu | 6.02-3525.00<br>9.81±4.36 | BDL | 3.17±0.14[a]<br>8.2±0.27[b]<br>4.0-6.0[C],<br>653±81[d] |
| Fe | 90.00-1879.00<br>809.40±44.79 | 991.07-3090.73<br>1598.88±42.99 | 0.21±0.005[b]<br>536±32[d] |
| Mn | 0.02-0.78<br>0.10±0.01 | 0.03-1.02<br>0.13±0.02 | 5.0–12.8[e] |
| Sr | 0.02-0.48<br>0.23±0.09 | 0.02-1.27<br>0.27±0.19 | 9–41[e] |
| Zn | 8.01-30.66<br>20.56±3.86 | BDL | 32.70±2.2[a]<br>20.70±0.3[b]<br>17.0-37.20[C] |

Fathi et al., 2008[a]; Zhuk et al., 1994[b]; Iyengar et al., 1978[C]; Teresa et al., 1997[d;] Jean-Pierre G, et al., 2005[e.]

**Table4b:** Comparison of elemental concentrations of hair of patients and control subjects in this work with literature values.

| Element | Controls (µg/g) | Patients (µg/g) | Literature (µg/g) |
|---|---|---|---|
| Al | 11.69±2.69 | 0.05-42.64 | 14.94±29.40[a] |
| | | 12.51± 2.22 | 9.48-210.0[c] |
| | | | 9.00±0.30[g] |
| Cd | 0.00-5.60 | 0.00-1.32 | 0.114-0.14[a] |
| | 0.12±0.97 | 0.21±0.21 | 0.00-12.10[b] |
| | | | 2.81±2.46[b] |
| | | | 0.61±1.13[d] |
| Zn | 5.00-225.00 | 0.65-311.55 | 156.48±74.5[a] |
| | 100.74±8.9 | 92.56±6.75 | 128.04±60.24[d] |
| | | | 159-265[e] |
| Cu | 14.98±4.03 | BDL | 9.0-61.3[a] |
| | | | 7.96±9.12[d] |
| | | | 9.3-71[e] |
| | | | 9.0–61.3[f] |
| Mn | 0.00-18.95 | 0.05-69.10 | 0.601±0.59[a] |
| | 1.20±0.85 | 5.84±1.70 | 2.41±2.24[d] |
| | | | 0.10-2.7[e] |
| Li | 0.00-0.07 | 0.00-0.14 | 0.003–0.042[f] |
| | 0.02±0.01 | 0.04±0.02 | |
| Be | 0.00-0.17 | 0.00-0.22 | 0.003–0.012[f] |
| | 0.10±0.04 | 0.13± 0.05 | |
| Ba | 0.00-96-96 | 0.05-305.55 | 0.05–1.58[f] |
| | 10.68±1.76 | 27.43± 3.74 | |
| Cr | 0.92±0.65 | 0.17-206.72 | 0.568±1.04[a] |
| | | 2.15±2.00 | 0.60±1.13[c] |
| | | | 0.12-0.90[e] |
| | | | 0.11–0.52[f] |
| Sr | 0.00-11.8 | 0.06-25.62 | 0.17–4.63f |
| | 1.67±0.35 | 3.61±0.05 | |
| Fe | 20.01-160.48 | 0.46-528.39 | 45.70±34.74[d] |
| | 34.85±5.11 | 37.25±2.31 | 29-84[e] |
| Na | 27.72-632.40 | 37.51-2707.68 | 217.33±268.78[a] |
| | 139.49±24.93 | 375.77±37.23 | 242.16±147.35[d] |
| | | | 165.9±165.9[c] |
| K | 13.07 -312.02 | 1.00- 464.17 | 71.77±60.96[d] |
| | 24.16±3.70 | 93.22±8.14 | |
| Ca | 2.00-322.93 | BDL | 825.91±880.14[d] |
| | 117.03±10.82 | | 316-1324[e] |

Chojnacka et al., 2005 [a] ; Popko et al., 2003 [b] ; Oluwole et al., 1994[c] ; Nowak 1998[d]; Teresa et al., 1997[e], Jean-Pierre G, et al., 2005[f] , Pacheco et al., 2008.

**Table 5a:** Correlation of elements in patients' hair.

| Element | Al | Be | Cd | Cr | Fe | Li | Mn | Sr | Mg | K | Na |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Al | 1.000 | | | | | | | | | | |
| Be | .396** | 1.000 | | | | | | | | | |
| Cd | .528** | .206 | 1.000 | | | | | | | | |
| Cr | .380** | .244 | .406** | 1.000 | | | | | | | |
| Fe | .560** | .381** | .500** | .918** | 1.000 | | | | | | |
| Li | .476** | .612** | .554** | .543** | .580** | 1.000 | | | | | |
| Mn | .424** | .300* | .497** | .187 | .254 | .551** | 1.000 | | | | |
| Sr | .244 | .227 | .278* | -.019 | .057 | .385** | .689** | 1.000 | | | |
| Mg | .133 | .009 | .272 | -.063 | -.014 | .328* | .647** | .800** | 1.000 | | |
| K | .244 | .123 | .580** | .204 | .238 | .492** | .480** | .196 | .371** | 1.000 | |
| Na | .256 | .130 | .486** | .066 | .121 | .516** | .702** | .430** | .651** | .749** | 1.000 |

**Correlation is significant at the 0.01 level (2-tailed). $r \geq 0.37$ , *Correlation is significant at the 0.05 level (2-tailed). $r < 0.371 \geq 0.272$

**Table 5b:** Correlation of elements in controls' hair.

| Element | Al | Ba | Be | Cd | Cr | Cu | Li | Mn | Sr | Zn | Ca | Mg | K | Fe | Na |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Al | 1.000 | | | | | | | | | | | | | | |
| Ba | .812** | 1.000 | | | | | | | | | | | | | |
| Be | .320* | .065 | 1.000 | | | | | | | | | | | | |
| Cd | .626** | .799** | .108 | 1.000 | | | | | | | | | | | |
| Cr | .131 | .162 | .299 | .189 | 1.000 | | | | | | | | | | |
| Cu | .314 | .274 | .568** | .152 | .204 | 1.000 | | | | | | | | | |
| Li | .729** | .682** | .415** | .712** | .300 | .532** | 1.000 | | | | | | | | |
| Mn | .355* | .533** | .287 | .211 | .114 | .749** | .513** | 1.000 | | | | | | | |
| Sr | .459** | .629** | .287 | .285 | .111 | .489** | .541** | .809** | 1.000 | | | | | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Zn | .430** | .492** | .322* | .540** | .343* | .111 | .478** | .130 | .309 | 1.000 | | | | | |
| Zn | .430** | .492** | .322* | .540** | .343* | .111 | .478** | .130 | .309 | 1.000 | | | | | |
| Ca | -.001 | -.120 | .134 | -.120 | .474** | -.055 | -.018 | -.048 | .106 | .034 | 1.000 | | | | |
| Mg | .009 | .293 | -.248 | .016 | -.159 | .116 | .044 | .378* | .388* | -.134 | -.139 | 1.000 | | | |
| K | -.086 | -.061 | -.013 | -.106 | -.113 | .283 | .082 | .254 | .065 | -.363* | -.163 | .466** | 1.000 | | |
| Fe | -.065 | .121 | -.034 | .137 | -.201 | -.133 | .149 | -.024 | .042 | .094 | -.088 | -.101 | -.214 | 1.000 | |
| Na | -.024 | .099 | .102 | -.055 | -.096 | .523** | .250 | .629** | .415** | -.279 | -.109 | .520** | .587** | -.048 | 1.000 |

** Correlation is significant at the 0.01 level (2-tailed). r≥0.4 , * Correlation is significant at the 0.05 level (2-tailed). r < 0.4 ≥ 0.32

**Table 6a:** Correlation of elements in patients' blood.

| **Element** | **Al** | **Ba** | **Be** | **Cd** | **Cr** | **Fe** | **Li** | **Mn** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Al | 1.000 | | | | | | | | | | |
| Ba | .496** | 1.000 | | | | | | | | | |
| Be | .532** | .787** | 1.000 | | | | | | | | |
| Cd | .221 | .157 | -.013 | 1.000 | | | | | | | |
| Cr | .390** | .591** | .627** | .046 | 1.000 | | | | | | |
| Fe | .367** | .379** | .639** | .276* | .244 | 1.000 | | | | | |
| Li | .476** | .880** | .668** | .219 | .532** | .377** | 1.000 | | | | |
| Mn | .465** | .907** | .685** | .291* | .563** | .411** | .920** | 1.000 | | | |
| Sr | .464** | .805** | .825** | .173 | .458** | .590** | .743** | .760** | 1.000 | | |
| V | .484** | .674** | .931** | -.071 | .592** | .569** | .570** | .599** | .729** | 1.000 | |
| Ca | .242 | .398** | .530** | .183 | .293* | .413** | .423** | .443** | .544** | .428** | 1.000 |
| Mg | -.077 | -.093 | .112 | .140 | -.019 | .322* | .012 | -.028 | .168 | .035 | .321* |
| K | -.090 | -.237 | -.057 | .062 | -.050 | .150 | -.125 | -.115 | -.069 | -.035 | .520** |

** Correlation is significant at the 0.01 level (2-tailed). r≥0.35, * Correlation is significant at the 0.05 level (2-tailed). r< 0.35 r ≥ 0.276

**Table 6b:** Correlation of elements in controls' blood.

| Element | Al | Ba | Be | Cd | Cr | Cu | Fe | K | Li | Mg | Mn | Sr | Zn | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Al | 1.000 | | | | | | | | | | | | | |
| Ba | .460** | 1.000 | | | | | | | | | | | | |
| Be | .491** | .447** | 1.000 | | | | | | | | | | | |
| Cd | .233 | -.064 | .170 | 1.000 | | | | | | | | | | |
| Cr | .564** | .317* | .154 | .005 | 1.000 | | | | | | | | | |
| Cu | -.075 | .284 | .089 | -.057 | -.047 | 1.000 | | | | | | | | |
| Fe | .501** | .536** | .778** | .131 | .149 | .060 | 1.000 | | | | | | | |
| K | .473** | .424** | .679** | .074 | .086 | .023 | .919** | 1.000 | | | | | | |
| Li | .602** | .350* | .737** | .255 | .125 | -.038 | .598** | .555** | 1.000 | | | | | |
| Mg | .510** | .518** | .743** | .040 | .161 | .003 | .931** | .930** | .610** | 1.000 | | | | |
| Mn | .563** | .256 | .264 | -.017 | .944** | -.050 | .214 | .165 | .170 | .245 | 1.000 | | | |
| Sr | .719** | .730** | .579** | .036 | .271 | .149 | .675** | .618** | .692** | .696** | .266 | 1.000 | | |
| Zn | .618** | .549** | .649** | .163 | .314* | -.004 | .844** | .821** | .476** | .815** | .334* | .620** | 1.000 | |
| V | .540** | .445** | .971** | .195 | .201 | .109 | .728** | .644** | .711** | .703** | .301 | .568** | .634** | 1.000 |

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed). $r<0.4$, $\geq 0.314$

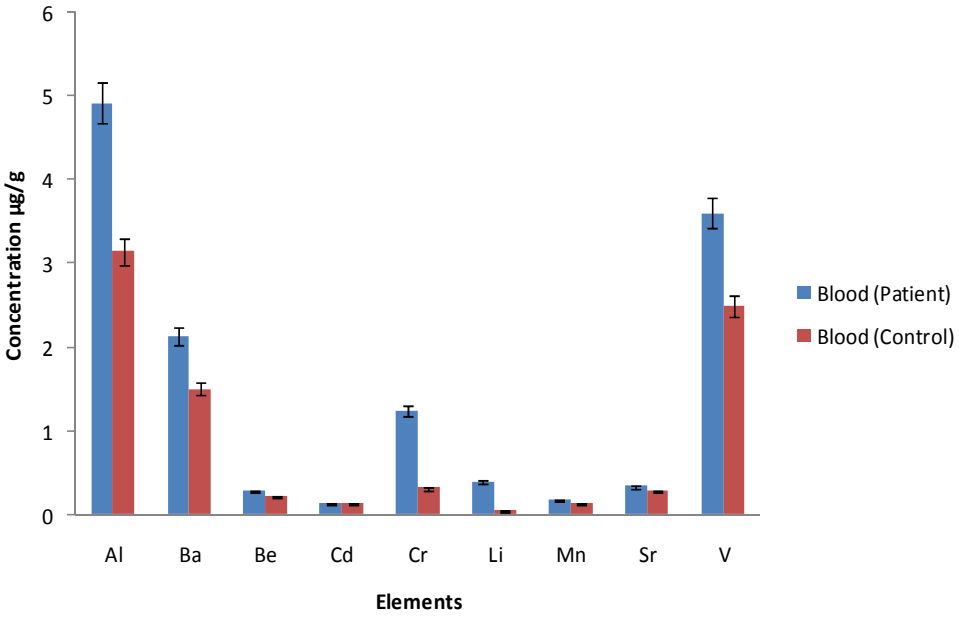


**Figure 1a**: Concentration of trace elements in blood of the patients and controls.

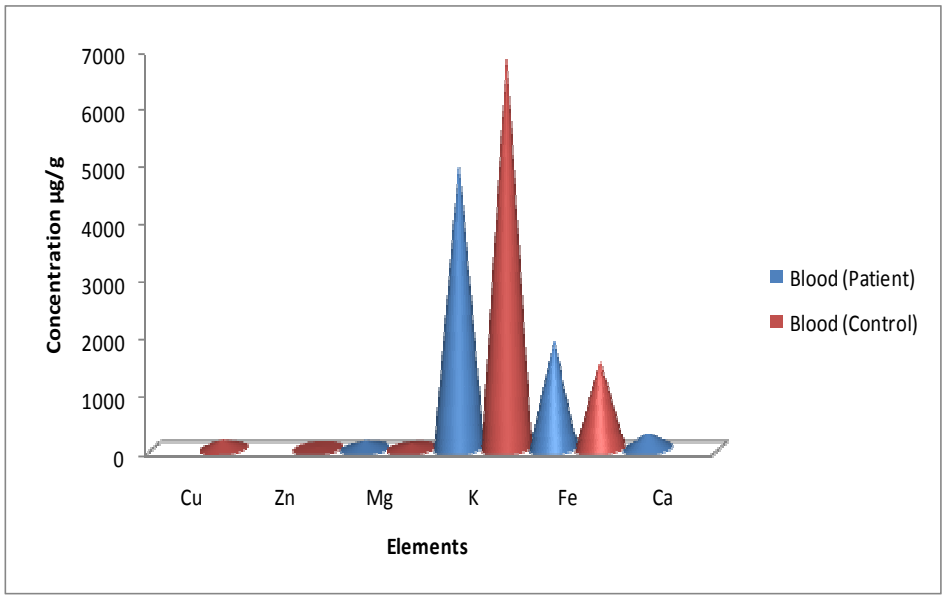


**Figure 1b**: Concentration of nutrient elements in blood of the patients and controls.

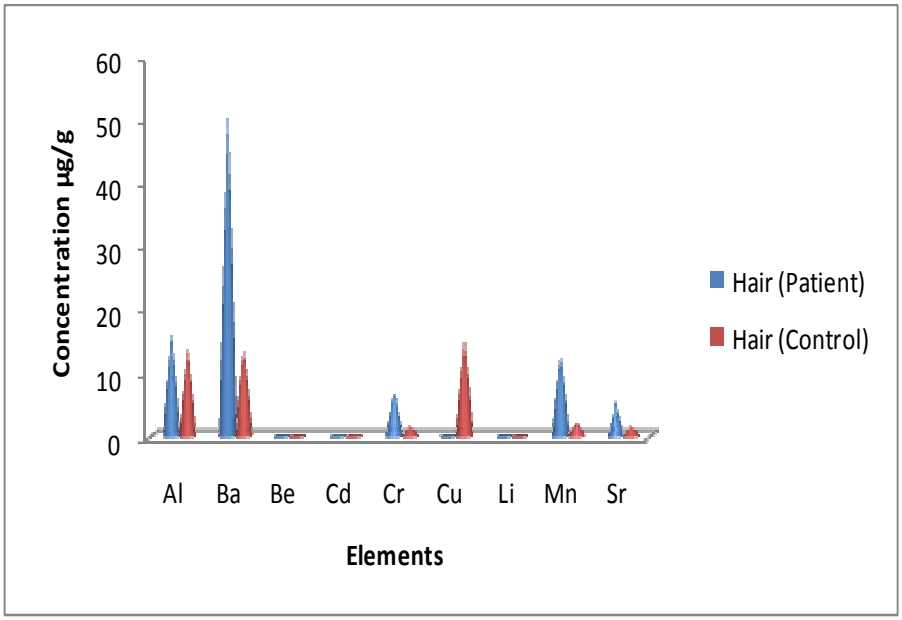


**Figure 2a***:* Concentration of trace elements in hair of patients and controls.

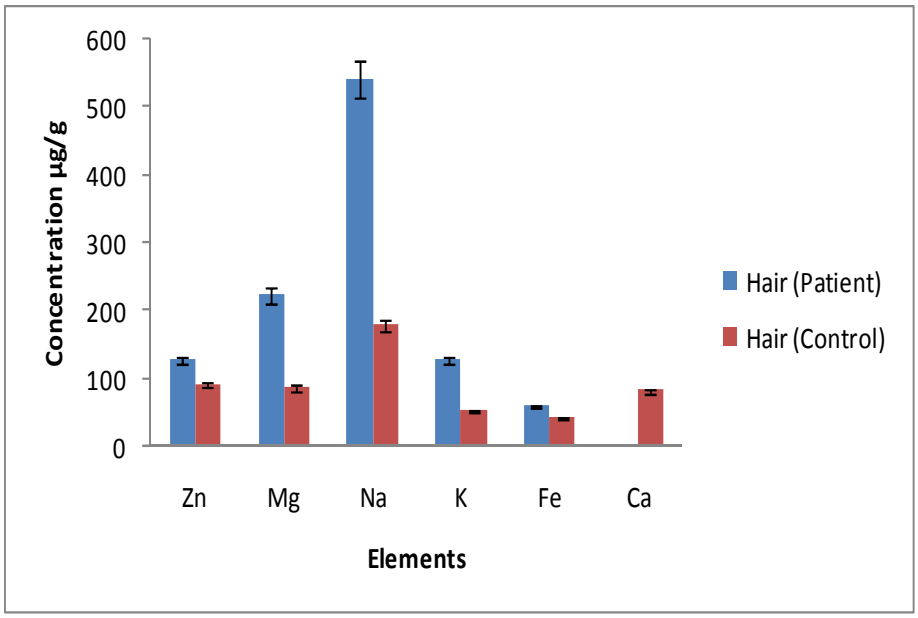


**Figure 2b**: Concentration of nutrient elements in hair of patients and controls.

## DISCUSSION

Generally, concentrations of most elements in the hair were observed to be higher than in the blood except potassium, vanadium, iron and calcium that appeared to have more resident time in the blood. This is a reflection of the fact that the elements accumulate more and stay longer in the hair than in the blood. The levels of the elements in the hair reflect the long term exposure of the individuals to the metals (Dean et al., 2001). The levels detected for most elements in the control subjects were all relatively lower than those found in the patients; this was supported by Kelly (2000); that elevated heavy metal levels are associated with schizophrenic pathology.

The mean concentration of aluminium in the pooled blood samples was 3.01 µg/g for the control subjects and 3.43 µg/g for the patients. The aluminium concentration in the hair of the patients was 12.51 µg/g, while that for the control subjects was 11.69 µg/g. In the study of aluminium levels in urine, saliva and hair of school children, it was reported that behavioural difficulties in schools have positive correlation with levels of Al (Pacheco et al., 2008). The base value for aluminium in healthy individual in that study was recorded as 19 µg/g. The range of 9.48-210 µg/g of Al in hair of healthy Nigerian subjects was reported by Oluwole et al., 1994. Our result falls within this range, which to us could mean that there was no abnormal concentration of aluminium either in the mentally ill patients studied or in the controls, but, Foster (2004) stated that aluminum can be toxic in patients with schizophrenia, mood disorders, Alzheimer's disease and digestive system pathologies.

Manganese and Group II elements such as Be, Mg, Sr, and Ba are significantly higher in the hair and blood of the patients compared to blood and hair of the control subjects at 0.01 probability level. These elements are highly electropositive and can easily loose electrons; this reductive property may increase the electro- activities and since they are higher in patients' blood and hair, such may be responsible for the manifestation of symptoms like hallucination and depression. In many human and animal tissues, manganese concentrations were found to be less than 1 µg/g wet weight (Sumino et al., 1975). This is in agreement with what was found in the blood of the patients and the controls in this study. However, the average concentration of manganese in the controls' hair (1.20 µg/g) and that of patients (5.84 µg/g) is worrisome. The values of manganese in hair of patients in this study are higher than those of some earlier workers (Nowak, 1998; Teresa et al., 1997; Chojnacka et al., 2005) (Table 4b). The higher values may be responsible for mental disorder. Although manganese is known to be essential for the development and functioning of the brain, it could also be a toxicant. If this metal is highly concentrated in the brain, especially in the basal ganglia, it will result in neurological disorders similar to Parkinson's disease (Ono et al., 1995).

Manganese supplementation reportedly lowers the elevated level of copper in schizophrenia. An important effect of manganese supplementation may be the lowering of elevated copper levels as manganese caused a 3-fold increase in copper excretion in patients with copper overload and low histamine levels. Copper reduction is even more pronounced if manganese is given together with increased zinc (Pfeiffer et al., 1972). If Mn supplement was given as part of treatment procedure, it may be rapidly utilised to stabilise the patients and this may be the reason why the mean concentration is not significant in the blood. The constant use of manganese containing drugs over time could result in its high concentration in hair and could account for the detection of Cu below detection limit in the hair and blood of the

patients. Similar report of low copper concentrations were found in the plasma of retarded dwarfs and of male micro-cephalic subjects (Bruhl et al., 1987)

Zinc was below the detection limit in the blood of patients, this was quite worrisome; it depicts that it was extremely low in the patients' blood and low level of zinc in the body is associated with schizophrenia and depression (Raymond, 2008). Zn indirectly protects tissues and cells from being damaged by free radicals (antioxidant property). This element is crucial in the production of metallothioneins (MTs) which are free radical scavengers due to their high contents of cysteine (Tapiero et al., 2003). Several research works reviewed by Cherian et al., (2003) indicated 15 associations of MTs with protection against DNA damage, oxidative stress and apoptosis. Zn prevents Fe and Cu ions to initiate the lipid peroxidation on cells because Zn in the state of $Zn^{2+}$ has no unpaired electrons which could allow it to participate in redox reactions. On the other hand, $Fe^{2+}$ and $Cu^{+}$ due to their redox properties, play a big role in the initiation and propagation of lipid peroxidation. Lipid peroxidation is a process whereby a free radical such as $HO^{\cdot}$ removes electrons from the lipids in cell membranes. The $HO^{\cdot}$ radical is formed when a single electron is transferred to hydrogen peroxide (H2O2).

$e^{-} + H_2O_2$ ---------- $HO^{\cdot} + OH$ Since $Fe^{2+}$ and $Cu^{+}$ have multiple oxidation states, they can easily donate the required electron to oxidize $H_2O_2$.

$Fe^{2+} + H_2O_2$ ---------- $HO^{\cdot} + OH^{-} + Fe^{3+}$

$Cu^{+} + H_2O_2$ ---------- $HO^{\cdot} + OH^{-} + Cu^{2+}$

Thus, by competing for binding sites with Fe and Cu, Zn antagonises their abilities to promote formation of $HO^{\cdot}$ from $H_2O_2$. Therefore, the deficiency of Zn reduces the activities of MTs and Cu/ZnSOD hence, increasing the levels of lipid peroxidation in mitochondria and microsomal membranes which might damage the cells and result into the development of chronic disease (Mohammed, 2008). The mean concentration in the controls was in agreement with the work of Zhuk et al., (1994), Table 4a. However it was significantly higher in the patients' hair and this value fell within the literature value of control subjects in the work of Chojnacka et al., (2005) (Table 4b). Levels of zinc found in this study supported literature findings, environmental factors, diet, geographical location and genetic components of individual differ which might contribute significantly to the illness.

Lithium concentration was lower in the patients' blood. Low levels of lithium cause abnormal cell imbalance and neurological disturbances (Kerbeshiaan et al., 1987). Lithium is a major constituent of the drugs used for treating some mental disorders. One may therefore expect it to be present at significant levels in the blood and hair of the patients. However, the results obtained in this study showed significant difference only in the hair. A possible reason for this observation could be that, they were metabolised and stored in the hair, since hair is an excretory tissue, resulting in higher concentrations of lithium in the hair.

The mean value of vanadium was found to be significantly higher in the blood of the patients than in the control subjects. Vanadium has been implicated in the literature as causing nervous depression (Barceloux, 1999). It may be playing a major role in mental-illness as experienced by the patients studied. Its presence in ascidians, cereals, vegetable and milk (Goyer and Clarkson, 2001) could serve as a major route to humans.

Figures 1 and 2 show that elemental concentrations in the blood and hair of mentally ill patients were found to be higher than those of the controls in many elements

considered in this study and their cumulative synergistic effects might have been responsible for the various levels of mental imbalances suffered by the patients.

Fe has a high significant correlation with these metals: Al, Be, Cd, Cr and Li in the patients' hair at P= 0.01 (Table 5a) whereas it has no correlation in the controls hair. This may suggest that they could be introduced through trace components in the drugs (Li based) of patients, this was not proven scientifically. Similar results were obtained in the hair analysis of Nowak (1998), in which Fe correlated with Cd and Cr. At P=0.01, Li correlated with Al, Be, Cd, Cr, Sr, Fe, Mg, Mn, Na and K (Table 5b). Some of the elements that correlated in the patients' blood also correlated in the controls (Tables 6a and 6b). The relationships were not with high positive r values in the patients' blood, as it was with the controls. The correlation of Al with implicated elements in patients' blood, such as, Li, Mn, V, and Fe have r values of 0.476, 0.465, 0.484 and 0.367 (Table 6a) whereas in the controls blood the r values were 0.602, 0.563, 0.540 and 0.501 respectively (Table 6b). The r values of these correlations in the controls' blood were higher, pointing to a better interaction of these elements and their stronger dependence on one another. A high correlation between two variables may really be a manifestation of strong dependence of both variables on the same causal factor (Miller et al., 2000). Elements such as Al, Be, Ba, Li, Fe, Sr and V correlated in both controls and patients' blood but the patients' blood had low negative correlation with nutrient elements such as Mg and K and high positive correlations in the controls' blood. These correlations reflect the well-known interrelationships between trace elements in biological metabolism. Positive correlations point at reinforcement of one element by the presence of the other, whereas negative correlations point at competition (possibly substitution or blockage) between the pair of elements (Ojo et al., 1994) and this could result to sickness.

In the blood of the patients, Mn had a low negative correlation with Mg. This implies competition for sites. However, the action of manganese is not manganese-specific. Because Mn resembles Mg in some physicochemical properties, a number of enzymes can substitute magnesium for manganese in their activation (Wedler, 1993), this is evident in patients' blood as r value of Mn and Mg was –0.028, suggesting substitution and/or competition for binding sites.

The elements that correlated have similar properties such as lustrous nature, chemical similarities; most of them are highly electropositive, such as Ca/Be in patients' blood and Sr/Be in control subjects. The elements: Al, Zn, V, and Mn correlated strongly in the control blood and hair (V not determined in the hair) in agreement with the baseline study of hair analysis of Nigerian subjects (Oluwole et al., 1994). Zn, Fe, K are well positively correlated in the whole blood of the control at $p < 0.05$. This is in agreement with literature (Ojo et al., 1994). The reason(s) for the presence of Pb and Hg below the detection limit could not be ascertained, although the catchments area is agrarian, rural and semi urban and therefore non- industrialized.

**Conclusion**

The results show that; the concentrations of most elements are higher in the hair and blood of the mentally-ill patients than in the healthy control. Higher concentrations of elements are found in the hair than in the blood. This is as expected since hair as an excretory tissue and showed exposure over a longer time than blood. Significant concentration of manganese in the mentally-ill patients' hair may be responsible

for the low concentration of Cu (an essential element for normal growth and development) below the detection limit in the blood and hair of the patients, as manganese inhibits uptake of copper. The lower concentrations of potassium and zinc in the blood of the patients could result into mental problem. It is believed that careful regulation of the concentrations of certain elements in the body system and identification of culprit foods and culprit environmental compound can be useful in reducing / removal of metal burden in the body of the mentally-ill patients. Prospective, controlled, larger studies designed to study individual elements with elimination of confounders will further clarify the role, causal relations and therapeutic implications of these elements in relations to mental illness in our population.

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