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Effect of metal poisoning and the implications of gender and age on the elemental composition in patients with mental behavioural disorders

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The objective of this work was to investigate the possible correlation between the exposure to selected toxic metals and the behavioural disorder of mentally ill patients. The study also sought to establish if gender and age of the patient had an effect on the pattern of the elemental distribution in their head hair and blood samples. To achieve this, the concentrations of a number of selected toxic metal elements were determined in 60 mentally ill patients and 43 healthy individuals (control) in Ile-Ife area, in Nigeria, using inductively coupled plasma spectrophotometer-optical emission spectrometer (ICP-OES). The behavioural disorder cases investigated were 8 bipolar, 2 post partum psychosis, 43 schizophrenia and 7 non-specific cases. The concentration ranges of Cu, Zn, Ca, Li, V, Be (for both males and females), Cd and Sr (for females only) as analyzed from the patients' head hair with behavioural disorders, were found to be similar with those of the controls. However, the concentration ranges of Al, Ba, Mg, Cr and Cd, Sr (for males only) were higher in patients than in the controls, while those for K and Fe were found to be higher in the controls than in the patients for both males and females. Blood samples analysis showed that, nearly all the elements were higher in the female (patients and control) than in the males; a possible indication that women may be at greater risk than men. It was also shown that, age may have an influence on the accumulation of some specific elements. The accuracy of the analytical results was experimentally demonstrated by NCS DC 73347 certified reference material that was analyzed along the standards while the significance of the data obtained was tested statistically at both $p = 0.01$ and 0.05 .

Key words: Toxic metals, behavioural disorder, gender, age, inductively coupled plasma-optical emission spectrometer.

INTRODUCTION

Exposure to toxic metals is known to have adverse effects on the central nervous system (CNS) functions and on an individual's mood which may translate into depression, insomnia and other neurodegenerative disorders such as autism and bipolar disorder (Watts, 1990; Ibrahim et al., 2006; Eck and Wilson, 1989; Pfeiffer 1975;

Bowler et al., 2006). Aluminum and lead exposure for example, has been reported to be responsible for behavioral problems and learning deficiency in children which persists for a long time after the initial exposure (Yuan et al., 2006). Other disorders such as delays and/or lack in intellectual ability, academic achievement and psychomotor development have all been associated with the exposure of children to these toxic metals (Yuan et al., 2006). Other toxic metals such as antimony, uranium, arsenic, beryllium, mercury, cadmium and aluminum are also known to cause autism and other behavioural problems

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(Adams et al., 2006). On the other hand, the bioaccumulation tendency of these toxic metals tend to aggravate the situation further because even at trace concentrations, they can gradually build up in the body and reach levels where they can cause serious health problems and behavioural disorders.

Once in the body, the biological fluids such as blood or plasma tend to shape the distribution pattern of the toxic metal elements. Therefore, considerable variations in terms of elemental distribution patterns can occur between specific population subgroups which may be factored in gender and/or age (Christensen, 1995; Cornelis et al., 1994; Gil et al., 2006; Goullé et al., 2005; Kucera et al., 1995; Minoia et al., 1990). Besides investigating the link between psychiatric disorders and the presence of some toxic metals in the blood and head hair samples of patients in this study, the effect of gender and age on the elemental distribution pattern among the patients, was determined.

Elemental analysis typically utilizes the atomic absorption spectrometry (both flame FAAS; and graphite furnace, GFAAS or electrothermal, ETAAS) (Parsons and Barbosa, 2007; Barbosa et al., 2004; Loureiro et al., 2007; Barbosa et al., 2001; Parsons and Slavin, 1993; Zanão et al., 2002). However, nowadays many laboratories are switching towards methods based on inductively coupled plasma interfaced to either optical emission (ICP-OES) or mass spectrometry (ICP-MS) (Parsons and Barbosa, 2007; Batista et al., 2008; Rodrigues et al., 2008; Palmer et al., 2006).

The increasing preference to these techniques is based on the fact that, ICP methods have a number of advantages over AAS based techniques which include the ability for simultaneous multi-element measurement capability, much lower detection limits and a wider linear dynamic range which allow the determination of major and trace elements in the same sample aliquot (Parsons and Barbosa, 2007).

In this work, the results of the analysis of toxic metal elements from blood and head hair samples of mentally ill population selected from Ile-Ife Nigeria was reported. The technique used was ICP-OES, after a microwave-digestion sample preparation step. For convenience, the patients with the behavioural disorders investigated (8 bipolar, 2 post partum psychosis, 43 schizophrenia and 7 non-specific cases) will be referred to as 'patients' throughout this paper.

MATERIALS AND METHODS

Sample collection

The sampling was conducted from 18/06/ 2007 to 30/08/2007. Samples were collected from the out-patients of the Department of Mental Health of the Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria, with the permission of the institution's Ethical Committee Review and Certification (The Consent and Ethics Certificate Number given is - IRB/IEC 00005422).

Design and setting

The design for sample collection and analysis was carried out on a prospective clinical and laboratory study of mentally-ill patients and was conducted on patients at the Department of mental health, Obafemi Awolowo University Teaching Hospital, Ile-Ife; which included in-patients and out patients.

The Obafemi Awolowo University Teaching Hospital is an affiliate of Obafemi Awolowo University with major hospital at Ile-Ife and Ilesa. Catchments area for patients included Osun, Ekiti, Ondo and parts of Kwara and Oyo States.

Inclusion and exclusion criteria

Patients with confirmed cranio-cerebral/head injury and organic psychosis were excluded.

Hair collection criteria

Hair sample was collected at the nape of the head with scissors to avoid contamination with facial secretions and cosmetics after permission from the patients or their responsible relatives, having endorsed the consent form.

Location and general features of the study area

Ile-Ife is the locus of the research work and is situated in Osun State, south western Nigeria. It lies approximately between latitudes 7°26'N to 7°33'N of the equator and longitude 4°30'E to 4°35'E of the prime meridian on a general elevation of about 350 m above the mean sea level. It is about 30 km to Ilesa and about 35 km south of Osogbo, the Osun State capital and 610 km south west of Abuja, the Nigerian capital.

The catchments areas for patients included Osun, Ekiti, Ondo and parts of Oyo States. All were located within the south western Nigeria and there are no large industrial concentrations but mostly agrarian communities with increasing tendencies towards the use of pesticides and fertilizers.

Reagents, chemicals and materials

The Analar grade HNO₃ (70%) used for digestion was purchased from Merck (Darmstadt, Germany). The standard solutions were prepared using ultra pure water (18 MΩcm) purified using a Mill-Q® reagent water system (Millipore, Molsheim, France). The accuracy of the method was ascertained by examining the certified reference material (CRM) - NCS DC 73347 supplied by Industrial Analytical (Pty) Ltd, South Africa. For quantitative analysis, the multi-elements standard solutions used for calibration purposes were prepared by diluting the stock solution of 10 mg/l of the given elements and these were stored in polyethylene bottles, supplied by PolyChem, Congella, Durban 4013, South Africa. Polyethylene sample bottles and Teflon beakers were soaked in 10% HNO₃ for 48 h and later rinsed with distilled de-ionized water prior to metals analyses.

Instrumentation and apparatus

Inductively coupled plasma- optical emission (ICP-OES)

The ICP-OES used was a Perkin Elmer Optima 5000 DV model with Echelle optical system. The following features and settings were utilized; axial viewing configuration with RF power 1300 W, plasma argon gas flow of 15 L/min, auxiliary gas flow of 0.5 L/min,

Table 1. Analysis of CRM (NCS DC 73347) with microwave digestion (n = 3).

Element	Microwave digestion ($\mu\text{g/g}$) (X)	Expected ($\mu\text{g/g}$) (Y)	Difference
Ba	16.830 \pm 1.860	17.00 \pm 0.200	0.17
Be	0.055 \pm 0.001	0.063 \pm 0.002	0.08
Cr	0.430 \pm 0.001	0.370 \pm 0.060	0.06
Cu	13.60 \pm 1.400	10.600 \pm 1.200	3.00
Fe	49.80 \pm 6.200	54.00 \pm 0.100	0.20
Li	1.90 \pm 0.0200	2.00 \pm 0.010	0.20
Sr	25.80 \pm 0.200	24.00 \pm 1.00	1.80
Zn	189.90 \pm 3.00	190.00 \pm 2.900	0.10

nebulizer flow of 8 L/min, view distance of 15 mm, sample uptake flow rate of 15 ml/min, wash rate of 1.5 ml/min and wash time of 30s.

Microwave digester

The microwave digester used for the sample preparation was Auto Paar multiwave 300 with pressure/temperature sensor supplied from Austria. An analytical Mettler AE240 balance was used for all weights measurements. Digestion was carried out under the following settings; power = 400 W at 170°C for 25 min and at a holding time of 10 min.

Sample preparation

Certified reference material (CRM)

CRM weights of 10, 20, 30, 40 and 50 mg were weighed in clean PTFE crucibles and digested in a closed-vessel high-pressure microwave digestion unit to minimize sample contamination and analyte losses. To avoid incomplete sample digestion, an oxidizing acid mixture was used which composed 6 ml nitric acid, 3 ml hydrogen peroxide and 2.4 ml hydrogen fluoride.

Head hair samples

The hair samples (50 mg) were cut into smaller pieces approximately 0.3 cm and were thoroughly mixed to allow a representative sub-sampling of the head hair specimen and approximately 50 mg of the head hair samples were weighed into 25 ml Teflon vessels and then, concentrated HNO₃ (70% w/v, 6 ml), hydrogen peroxide (30% w/v, 3 ml) and hydrogen fluoride (2.4 ml) were added to each vessel and digested using microwave digester. The cleaning time was set at 20 min, after which it was made up to 15 ml with ultra pure water (18 M Ω cm), filtered and was analyzed on ICP-OES.

Blood samples

The blood samples were drawn from the cubital vein using 5 ml syringe and polyethylene needle and were stored in pre-treated heparinized bottle and later freeze dried. The hair pieces were collected at the nape of the head to prevent contamination from cosmetics and facial secretions. After cutting, each sample was washed four times with 1:200 v/v dilution of Triton X-100. The samples were rinsed with acetone and allowed to drain. This was followed by three rinses with de-ionized water and two rinses with acetone. The samples were then, dried in an oven at 75 \pm 5°C and

kept in a clean standard envelopes or plastic papers, for the extraction step.

Statistical analysis

For the statistical analysis, data were analysed using the SPSS for windows software, version 15.0.1 (SPSS Inc., Chicago, USA). Statistically significant differences between groups were compared using analysis of independent t-test. The data are displayed as means \pm standard deviation (SD) and p values of 0.01 or 0.05 were considered significant.

RESULTS AND DISCUSSION

Analytical validation

In order to ensure validity of results in any quantitative analytical work, the following aspects must be addressed; the correctness in the preparation of the sample and avoidance of errors due to sample loss or contamination (Becker and Dietze, 1999). In this work the use of certified reference material (NCS DC 73347) was employed, so as to provide a measure of accuracy and precision for the experimental data obtained in the analytical measurements. For quantitative analysis, calibration curves of different elements (prepared from a multi-element standard solution of 10 mg/l) were found to be linear with correlation coefficients above 0.99 as shown in Table 1 (curves not included). From Table 1, it is evident that by comparison with the CRM observations, the concentration values for Fe were lower than expected while for Cu, the values were higher than expected. The higher values for Cu may be due to a considerable contamination during sample preparation because of the small sample size used. Moreover, the lower results for Fe could either be as a result of losses in the washing step of sample preparation or inadequate digestion of the sample.

Choice of sample specimens for analysis

Human hair has been extensively studied in clinical prac-

Table 2. Gender based elemental distribution pattern in patients' blood samples.

Element	Patient ($\mu\text{g/g}$)			Control ($\mu\text{g/g}$)		
	Male	Female	Difference	Male	Female	Difference
Al	5.05 \pm 0.072	4.85 \pm 0.084	0.100	3.25 \pm 0.040	2.76 \pm 0.02	49.00
Ba	2.22 \pm 0.089	2.09 \pm 0.038	0.13	1.67 \pm 0.002	1.09 \pm 0.02	0.58
Be	0.28 \pm 0.007	0.29 \pm 0.008	0.010	0.22 \pm 0.005	0.19 \pm 0.001	0.03
Cd	0.39 \pm 0.005	0.11 \pm 0.004	0.28	0.15 \pm 0.005	0.105 \pm 0.005	0.045
Cr	1.24 \pm 0.009	1.24 \pm 0.009	0.00	0.36 \pm 0.008	0.21 \pm 0.006	0.15
Li	0.04 \pm 0.007	0.037 \pm 0.002	0.3	0.04 \pm 0.001	0.037 \pm 0.001	0.003
Sr	0.324 \pm 0.01	0.330 \pm 0.001	0.0006	0.28 \pm 0.008	0.24 \pm 0.001	0.004
Mg	140.13 \pm 2.75	131.05 \pm 3.51	9.08	86.13 \pm 1.19	74.83 \pm 3.27	11.30
K	5606.02 \pm 16.35	4781.20 \pm 14.08	824.82	7127.23 \pm 15.42	6443.29 \pm 16.56	683.94
Fe	2025.11 \pm 12.25	1847.00 \pm 15.02	178.11	1640.71 \pm 13.50	1342.18 \pm 15.57	298.53
V	3.42 \pm 0.07	3.73 \pm 0.89	0.31	2.63 \pm 0.07	2.22 \pm 0.02	0.41
Zn	19.11 \pm 0.40	26.09 \pm 1.08	6.98	10.73 \pm 1.12	15.29 \pm 1.05	4.56

tices because it serves as a good non-invasive medium for the determination of metal elements in the body as it gives an average concentration of trace elements over cumulative longer periods of time. In the case of body fluids, blood is usually preferred as the most appropriate specimen for elemental determination. In this study, head hair and blood samples were used for the elemental analysis.

Elemental patterns in patients versus healthy persons' head hair and blood samples

The elemental composition of blood, plasma and head hair samples is known to be altered by some diseases (Dombova'ri et al., 1999). The main pattern changes as far as the elemental distribution was concerned were some elements that exhibited an increase in levels and others that showed decreased levels. In this study, it was observed that, the concentration ranges of Cu, Zn, Ca, Li, V, Be (for both males and females), Cd and Sr (for females only) as analyzed from hair samples from patients with behavioural disorders, were found to be similar with those of the healthy persons (controls). However, the concentration ranges of Al, Ba, Mg, Cr and Cd, Sr (for males only) were higher in patients than in the controls, while those for K and Fe were found to be higher in the controls than in both male and female patients. The increased elemental composition in patients may be due to the fact that some proteins expressed in the body due to natural defensive mechanisms provided additional binding sites for these metal elements. The trend shown for K and Fe is in line with what was reported in a clinical practice (Fischbach, 1988); their concentrations in the patient's body deviated from the normal range.

Influence of gender on the elemental composition in the patients' blood and head hair

In order to study the effect of gender in elemental distribution among the group of people with behavioural disorder, a group of male and female was selected randomly from both patients as well as the healthy group (control). For the blood samples analyzed, the concentrations of most elements were found to be higher in males than in females with the exception of Zn, Ca and V (Table 2). Tables 3 shows that, majority of the elements had higher levels in female hair samples than in male hair samples, an indication that women may be at greater health risk than men because hair specimens give a measure of long term toxic effect. (Schuhmacher, 1996) This trend observed with hair specimen may be due to increased gastrointestinal uptake of metals such as lead and cadmium at low iron stores; a common scenario with women at childbearing age (Chen et al., 2006; Moon et al., 2007; Ashraf et al., 1994; Barany et al., 2002; Vahter et al., 2004; Sharma et al., 2004). The observed variation in the metal concentration patterns occurred mainly among the patients, pointing to the possibility of the involvement of these metals in the sickness.

Influence of age on the metal elemental composition in the patients' blood and hair

The results pertaining to the influence of age on the concentration pattern of the elements in the hair are depicted in Table 4. The studied population (for patients and controls) was divided into two age groups; (under 30 years old and 31 to 60 years old). The results in Table 4 reveals that, for most of the elements analyzed, higher levels were recorded in the 31 to 60 years old group ($n =$

Table 3. Gender based elemental distribution pattern in patients' head hair samples.

Element	Patients ($\mu\text{g/g}$)			Control ($\mu\text{g/g}$)		
	Male	Female	Difference	Male	Female	Difference
Al	12.47 \pm 6.00	17.70 \pm 0.70	5.23	14.31 \pm 1.89	14.58 \pm 3.13	0.27
Ba	41.60 \pm 4.00	62.29 \pm 2.12	20.69	14.13 \pm 1.90	15.17 \pm 1.08	1.04
Be	0.12 \pm 0.05	0.12 \pm 0.06	0	0.09 \pm 0.07	0.06 \pm 0.06	0.03
Cd	0.17 \pm 0.01	0.36 \pm 0.07	0.19	0.38 \pm 0.06	0.16 \pm 0.04	0.22
Cr	4.34 \pm 4.91	8.32 \pm 0.20	3.98	1.85 \pm 0.30	1.11 \pm 0.06	0.74
Li	0.03 \pm 0.002	0.04 \pm 0.001	0.01	0.02 \pm 0.01	0.014 \pm 0.001	0.006
Sr	5.10 \pm 0.78	6.09 \pm 0.37	0.99	1.35 \pm 0.04	2.48 \pm 0.79	1.13
Zn	132.16 \pm 4.40	123.09 \pm 6.58	9.07	95.73 \pm 1.52	78.29 \pm 3.65	17.44
Mg	156.57 \pm 5.33	253.75 \pm 3.83	97.18	40.31 \pm 2.63	199.04 \pm 2.17	158.73
K	77.73 \pm 4.56	149.46 \pm 3.18	71.73	25.43 \pm 1.46	117.02 \pm 3.65	91.53
Fe	49.46 \pm 3.90	62.37 \pm 0.54	12.91	49.45 \pm 0.95	19.51 \pm 0.89	19.94
Na	318.99 \pm 4.36	646.12 \pm 4.24	327.13	136.39 \pm 1.21	282.15 \pm 4.41	0.24

Table 4. Age based elemental distribution pattern in the patients' head hair samples.

Element	Patients head hair ($\mu\text{g/g}$)		Control head hair ($\mu\text{g/g}$)	
	Below 30 years	31 to 60 years	Below 30 years	31 to 60 years
Al	17.20 \pm 1.70	15.46 \pm 1.40	17.09 \pm 2.20	12.88 \pm 1.28
Ba	45.25 \pm 4.70	57.66 \pm 5.05	11.98 \pm 1.20	16.15 \pm 1.27
Be	0.11 \pm 0.005	0.12 \pm 0.006	0.59 \pm 0.006	0.09 \pm 0.005
Cd	0.24 \pm 0.018	0.33 \pm 0.02	0.14 \pm 0.01	0.44 \pm 0.01
Cr	4.01 \pm 0.61	8.80 \pm 0.32	1.34 \pm 0.04	1.87 \pm 0.07
Fe	56.95 \pm 4.50	59.04 \pm 2.91	41.52 \pm 1.01	45.53 \pm 2.50
Li	0.03 \pm 0.002	0.04 \pm 0.002	0.13 \pm 0.02	0.16 \pm 0.017
Sr	6.10 \pm 0.90	5.59 \pm 1.11	1.80 \pm 0.08	1.66 \pm 0.070
Zn	156.57 \pm 4.38	112.68 \pm 5.01	77.63 \pm 5.49	98.45 \pm 3.80
Mg	220.78 \pm 2.17	223.44 \pm 2.05	99.80 \pm 1.00	77.16 \pm 1.29
K	100.34 \pm 1.99	141.83 \pm 1.53	36.23 \pm 3.60	62.14 \pm 5.28
Na	420.36 \pm 3.29	612.36 \pm 5.15	41.52 \pm 1.01	182.94 \pm 2.29

24) than under 30 years interval ($n = 14$) with the exception of Al, Be Sr, Ca and Mg. Similar results were previously reported by other researchers (Schuhmacher et al., 1996; Nowak, 1998). The low levels of Ca and Mg is attributed to these elements being mostly responsible for bone formation which normally takes place in individuals who are still growing (under 30 years old). For the older generation, there is almost no active bone formation, hence, no accumulation of Ca and Mg.

Aluminum was found to be higher in the hair samples within the 31 to 60 age group (Table 4) while levels of Al in blood samples were higher in the younger generation (under 30 years old) than in the older one (Table 5) and the correlation of its accumulation to the normal neurological function is supported by the work of Capel et al. (1981). The concentration levels of some elements such as Sr in the blood seemed to be independent of age bracket, since the concentration levels assessed in the patients and in the control (of all age groups) were almost

similar.

In general, the younger age group (below 30 years old) represents a group that is known to be more susceptible to the effects of some toxic metals because they accumulate several times the percent ingested compared with adults (Pamphlett et al., 1997; Kristiansen et al., 1997). Tables 6 and 7 give the comparison of the data obtained in this work with the reported literature values. There are some differences in terms of the reported levels of metals accumulated that are obvious in the comparison. This may be due to the fact that, elemental composition of head hair samples or body tissue may vary as a result of natural variance of head hair composition such as hair colour and texture. With respect to other tissues of body fluid such as blood, the variations may emanate from dietary related factors.

The data obtained in this work were also validated statistically at confidence levels of $p = 0.01$ and at $p = 0.05$ and then, based on the data obtained the paired t -

Table 5. Age elemental distribution pattern in patients' blood samples.

Element	Patients blood ($\mu\text{g/g}$)		Control blood ($\mu\text{g/g}$)	
	Below 30 years	31 to 60 years	Below 30 years	31 to 60 years
Al	4.83 \pm 0.35	4.97 \pm 0.10	2.57 \pm 0.64	3.35 \pm 0.60
Ba	1.16 \pm 0.06	2.69 \pm 0.10	1.04 \pm 0.07	1.72 \pm 0.10
Be	0.27 \pm 0.01	0.29 \pm 0.07	0.19 \pm 0.02	0.2 \pm 0.06
Cd	0.13 \pm 0.02	0.127 \pm 0.03	0.20 \pm 0.03	0.10 \pm 0.05
Cr	0.59 \pm 0.06	1.61 \pm 0.10	0.21 \pm 0.03	0.36 \pm 0.03
Fe	1913.15 \pm 16.96	1898.02 \pm 15.62	1456.64 \pm 16.58	1595.70 \pm 13.21
K	5714.65 \pm 15.49	4656.87 \pm 22.94	6181.38 \pm 26.89	7277.24 \pm 22.66
Li	0.03 \pm 0.01	0.04 \pm 0.01	0.038 \pm 0.002	0.043 \pm 0.005
Mg	135.52 \pm 6.54	133.02 \pm 3.26	74.00 \pm 5.31	86.92 \pm 8.62
Sr	0.33 \pm 0.01	0.33 \pm 0.10	0.23 \pm 0.01	0.29 \pm 0.02
V	3.46 \pm 0.15	3.73 \pm 0.84	2.24 \pm 0.15	2.62 \pm 0.08

Table 6. A comparison of some of the elemental concentration ranges in whole blood (n = 3) obtained in this work and from the literature for similar elements.

Element	This Work ($\mu\text{g/g}$)	Literature ($\mu\text{g/g}$)	Reference
Al	2 - 5	1.23	El-Amri et al. (1994)
Be	0 - 0.3	-	-
Cr	0.1 – 1.6	0.36 0.028	El-Amri et al. (1994) Iyengar et al. (1984)
Fe	1400 - 1900	536 32.70	Teresa et al. (1997) El-Amri et al. (1994)
Zn	10 - 26	20.70 17.0	Zhuk et al. (1997) Iyengar et al. (1984)

Table 7. A comparison of some of the elemental concentration ranges in head hair samples (n = 3) from this work and from the literature.

Element	This work ($\mu\text{g/g}$)	Literature ($\mu\text{g/g}$)	Reference
Al	12 - 17	14.94 9.48 0.114	Chojnacka et al. (2005) Oluwole et al. (1994) Chojnacka et al. (2005)
Cd	0.1 – 0.4	12.10 2.81 0.61	Popko et al. (2003) Nowak (1995) Teresa et al. (1997)
Zn	77 - 78	156.48 128.04 159.00	Chojnacka et al. (2005) Nowak (1995) Teresa et al. (1997)
Cr	1- 9	0.568 0.60 0.12	Chojnacka et al. (2005) Oluwole et al. (1994) Teresa et al. (1997)
Fe	19 - 62	45.70 29-84 217.33	Nowak (1995) Teresa et al. (1997) Chojnacka et al. (2005)
Na	41 - 641	242.16 165.9	Oluwole et al. (1994) Nowak (1995)
K	25 - 149	71.77	Nowak (1995)

Table 8. Statistical correlation among elements in patients and control samples at p = 0.01 and 0.05.

Element	Correlating elements: Statistically significant (p = 0.01)				Correlating elements: Statistically significant (p = 0.05)			
	Hair (control), r ≥ 0.4	Blood (control), r ≥ 0.4	Hair (patients), r ≥ 0.38	Blood (patients), r ≥ 0.38	Hair (control) r < 0.35 ≥ 0.314	Blood (control), r < 0.4 ≥ 0.314	Hair (patients), r < 0.37 ≥ 0.272	Blood (patients), r < 0.35 ≥ 0.276
Al	Ba,Be,Cr,K,Sr,Fe,Li, V, Mg, Zn	Ba,Be,Cr,Fe,K,Li,Mg,, Sr,Zn,V	Be, Cd , Cr, Fe, Li,	Ba,Be, Cr, Fe ,Li, Sr,V	NC	NC	NC	NC
Ba	Al ,Fe,Be,K, Mg Sr,Zn,V	Al,Be,Fe,K,Mg,Sr,Zn, V	ND	Al, Ba,Cr, Fe , Ca ,Li, Sr,V	Cr,Li	Cr, Li	ND	NC
Be	Al Fe,Ba,K,Li,Mg,Sr, Zn,V	Al,Ba,Fe,K,Li,Mg , Sr, Zn,V	Al , Fe, Li	Al,Ba,Cr,Fe,Li,, Sr,V,Ca	NC	NC	ND	NC
Cd	NC	NC	Al,Cr,Fe,Li, K, Na	NC	NC	NC	Sr,Mg	Fe,
Cr	Al	Al,	Al, Cd,Fe,Li	Al , Ba,Be, Li, Sr,V	Ba , Zn	Ba ,Zn	NC	Ca
Cu	NC	NC	ND	ND	NC	NC	ND	ND
Fe	Al,Ba,Be,K,Li,Mg, ,Sr,Zn,V	Al,Ba,Be,K,Li,Mg,Sr, Zn,V	Al, Be , Cd,Cr,Li	Al Ba ,Be, Li , Sr ,V,Ca	NC	NC	NC	Cd
K	Al, Ba ,Be, Fe, Li , Mg, Sr, Zn,V	Al, Ba ,Be,,Fe,Li, Mg, Sr, Zn, V	Cd, Li, Na, Mg	Ca	NC	NC	NC	Mg
Li	Al,Be,Fe,K,Mg,Sr, Zn,V	Al,Be,Fe,K,Mg,Sr,Zn, V	Al,Be,Cd,Cr,Fe, Sr, K,Na	Al,Be,Ba,Cr,Fe, Sr,V,Ca	Ba	Ba	Mg	NC
Mg	Al,Ba,Be,Fe,K,Li,Sr, Zn,V	Al,Ba,Be,Fe,k,Li,Sr,Zn ,V	,Sr,Na	NC	NC	NC	Li	Fe , Ca , K
Sr	Al,Ba,Be,Fe,K,Mg, Zn, V	Al,Ba,Be,Fe,K,Li,Mg, Zn,V	Mg,Na, Li	Al,Ba,Be,Cr,Fe,Li,V, Ca	NC	NC	Cd	NC
Zn	Al,Ba,Be,Fe,K,Li, Mg,Sr,V	Al,Ba,Be,Fe,K,Li,Mg, Sr ,V	ND	ND	Cr	Cr	ND	ND
V	Al,Ba,Be,Fe,K,Li, Sr ,Mg ,Zn	Al,Ba,Be,Fe,K,Li,Mg ,Sr,Zn	ND	Al,Ba,Be,Cr,Fe,Li, Sr,Ca	NC	NC	ND	NC
Na	ND	ND	Cd, Li, Sr, Mg ,K	ND	ND	ND	NC	ND
Ca	ND	ND	ND	Ba ,Be ,Fe ,Li , Sr, V,K	ND	ND	ND	Cr , Mg

ND = not determined; NC = not correlating.

test and analysis of variance (ANOVA) statistical analyses using SPSS was utilized as data reduction techniques to generate a visual data for qualitative evaluation on the link between metal presence and the behavioural disorder as well as the influence of gender and age for the tested the samples. The results in Table 8 indicate that the levels of a number of elements were significant at the probability levels tested. However, although, the differences of the content between the

samples from different groups (gender and age) for some metals were obvious, it may be difficult still to directly link their toxicity to the mental behavioural disorder, as more other factors such as metal analysis of the diet taken by the individuals, environmental (air and water) analysis of metals in areas they live are not included in this work. Never-theless, one may still theorize that the link may exist based on the data obtained for the patients versus the healthy individuals.

Conclusions

The study revealed that, some toxic metals such as cadmium and aluminum may be linked to some forms of behavioural disorder as the elemental distribution of these toxic metals was shown to vary between the patients and the healthy individuals from the same locality. Gender and age appeared to have a strong influence on the distribution pattern for some elements and for the

type of specimen; blood and hair.

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