Comparative Metal Distribution in Scalp Hair of Pakistani and Irish Referents and Hypertensive Patients

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Abstract

The abnormal metabolism of metal ions plays an important role in health and disease conditions, and studies about them have been attracting significant interest. The aim of our study was to assess the heavy metals (cadmium (Cd), nickel (Ni), lead (Pb), and zinc (Zn)) in scalp hair samples of 50 Irish and 78 Pakistani hypertensive patients of an urban population together with 50 fish and 96 Pakistani non-hypertensive male subjects in the age group of 30-50 years. The concentrations of trace and toxic elements were measured by inductively coupled plasma—atomic emission spectrophotometer and atomic absorption spectrophotometer before microwave-assisted acid digestion. The validity and accuracy of the methodology were checked using certified reference materials, and by the conventional wet acid digestion method on the same certified reference materials and on real samples. The recovery of all the studied elements was found to be in the range of 97.5-99.7% in certified reference material. The results of this study showed that the mean values of cadmium, nickel, and lead were significantly higher in scalp hair samples of both Pakistani and Irish hypertensive patients than in referents ($p<0.001$), whereas, the concentration of zinc was lower in the scalp hair samples of hypertensive patients of both genders. The deficiency of zinc and the high exposure of trace and toxic metals may be the risk factors associated with hypertension.

Introduction

Hypertension (HT) is an increasingly important medical and public health issue. The prevalence of HT increases with advancing age (60-90 years) [1]. But nowadays, the age criteria have been changed and even people below 30 years of age have HT problems because of the lack of exercise, fast foods, smoking, coffee, and alcohol consumption [2]. Genetic effect may also be a factor [3]. The essential trace element zinc (Zn) is an important component of biomembranes and an essential cofactor in a variety of enzymes [4]. Zinc has antioxidant-like properties; thus, it can stabilize macromolecules against radical-induced oxidation in vitro as well as limit excess radical production [5]. Zinc deficiency is associated with an increase in Cd, as a result of the antagonistic relationships between these elements [6].

Toxic metals play a vital role in diseases of unknown etiology. Many researchers have studied the relationship of cadmium (Cd), lead (Pb), zinc, and nickel (Ni) to hypertensive status using hair as a biopsy material [7-9]. The exposure of these toxic metals occurred

mainly through cigarette smoking, inhalation of airborne cadmium, lead and nickel in ambient air (usually higher near coal-fired power plants and municipal waste incinerators), or consumption of some foods (highest levels in shellfish, and liver and kidney meats).[10] In urban areas, the use of concrete and the combustion of oil, coal, and gasoline represent the major potential sources of environmental pollution for toxic metals [11] and toxic metals containing particles may thus easily find their way into the respiratory tract [11]. Certain metals may promote hypertensive and atherosclerosis by increasing oxidative stress (e.g., by catalyzing the production of reactive oxygen species or inhibiting their degradation) and increasing the blood pressure levels [12, 13].

The intake of trace and toxic elements may promote hypertensive and atherosclerosis disorders by increasing oxidative stress (for example, by catalyzing the production of reactive oxygen species or inhibiting their degradation) due to the deficiency of an antioxidant element (Zn) and by increasing blood pressure levels [13]. The deficiency of essential nutrients, lack of homeostatic control or an excess intake of some TEs causes chronic physiological disorders, such as HT and cardiovascular disease [14].

Determinations of trace elements in human tissues and fluids were used to obtain information on nutritional status for diagnosis of diseases, indication of systemic intoxication, and to obtain information on environmental exposure. In the majority of cases, whole blood, serum, plasma, and urine were analyzed [15]. Hair can provide a more permanent record of trace and toxic elements associated with normal and abnormal metabolism as well as toxic elements assimilated from the environment. In addition, hair is easily collected, conveniently stored, and easily treated. Therefore, the analysis of human hair has become an important way to understand any quantitative change in certain elements inside the body [16]. One of the most widely used analytical technique for different elements determination in biological and environmental materials is inductively coupled plasma—atomic emission spectrometry (ICP-AES) due to its advantages over other analytical methods: above all, a possibility of simultaneous determination of many elements of interest, freedom from different chemical interferences, and high detection power. ICP-AES also offers rapid, multi-element determinations. The sensitivity of ICP-AES is lower than that of either inductive coupled plasma—mass spectrophotometer (ICP-MS) or atomic absorption graphite tube atomizer (AA-GTA), but ICP-AES can handle higher levels of total dissolved solids (TDS) than ICP-MS and is much faster than AA-GTA. Since ICP-AES is able to analyze samples with higher TDS, more concentrated solutions can be prepared allowing trace elements to be measured. The main advantage of microwave-assisted samples pretreatment is its requirement of small amount of mineral acids and a reduction in the production of nitrous vapors. Microwave systems keep blank levels low because only small volumes of reagents are required and allow more samples to be processed per hour than conventional digestion systems [17].

Accordingly, the present study envisaged a comparative distribution of selected metals (Cd, Ni, Pb, and Zn) in the hair of a segment of Pakistani and fish referents and hypertensive patients of age group (30-50) years. The donors of both gender from both countries, with matched age groups and belonging to a matched typical urban residence, were selected for the study. The metal levels were examined for a possible mutual correlation also, and were

compared with reported metal levels for donors from other regions of the world. It was anticipated that the comparative metal levels in hair of Pakistani and Irish referent segments would bring out distinct sources responsible for the distribution of selected metals to help assess the nutritional status and environmental exposure of the two categories of subjects compared with those from other nations where different environmental and living conditions prevail.

Materials and Methods

Apparatus

The analysis of elements in Ireland was carried out by means of Varian Liberty 220 (Mulgrave, Victoria, Australia) inductively coupled plasma—atomic emission spectrometer with the axially viewed plasma was used for the analysis. The Liberty Series II ICP features a 40-MHz free running RF generator, a 0.75-m Czerny-Turner monochromator with 1,800 grooves per millimeter holographic grating used in up to four orders. The resolution of the spectrometer is typically 0.018 nm in the first order, 0.009 nm in the second order, 0.007 nm in the third order, and 0.006 nm in the fourth order. The instrument was controlled with a Digital Equipment Corporation Venturis computer with an Intel Pentium processor and Varian Plasma 96 software running under Microsoft Windows 95 operating system. The instrumental conditions are shown in Tables 1 and 2. A Hinari Life style (Elstree, Hertfordshire, England) domestic microwave oven (maximum heating power of 800 W) was used for digestion of the scalp hair samples.

The analysis of elements in Pakistan was carried out by means of a double beam PerkinElmer atomic absorption spectrometer model 700 (Norwalk, CT, USA), equipped with a flame burner and graphite furnace HGA-400, a pyrocoated graphite tube with an integrated platform and an autosampler AS-800 (PerkinElmer). The instrumental parameters are shown in Tables 3 and 4. Zn was measured under optimized operating conditions using FAAS with an air—acetylene flame, whereas Cd, Ni, and Pb were determined using ETAAS. Signals were measured as absorbance peaks in the flame absorption mode, whereas integrated absorbance values (peak area) were determined in the graphite furnace. A Pel (PMO23, Osaka, Japan) domestic microwave oven (maximum heating power of 900 W) was used for digestion of the biological samples. Acid-washed polytetrafluoroethylene (PTFE) vessels (Kartell, Milan, Italy) and flasks were used for preparing and storing solutions.

Reagents and Glasswares

Ultrapure water obtained from ELGA Lab Water system (Bucks, UK) was used throughout the work. Concentrated nitric acid (65%) and hydrogen peroxide (30%) were obtained from Merck (Darmstadt, Germany), and checked for possible trace metal contamination. Working standard solutions of Cd, Ni, Pb, and Zn were prepared immediately prior to their use, by

stepwise dilution of certified standard solutions (1,000 ppm) Fluka Kamica (Buchs, Switzerland), with 0.5 M HNO3.

Table 1 Measurement conditions for inductively coupled plasma–atomic emission spectroscopy Liberty 220 ICP-AES

Parameters	Cd	Ni	Pb	Zn
Wavelength (nm)	226.502	231.604	220.553	213.8
Height (mm)	3	5	3	5
Windows (nm; above the coil)	0.027	0.027	0.027	0.027
Scan (nm)	0.040	0.040	0.040	0.040
Integration (s)	3	3	3	3
Replicates	3	3	3	3
Sample uptake (s)	30	30	30	30
PMT (V)	650	650	650	650
Power (kW)	1.10	1.10	1.10	1.10
Plasma flow (L/min)	15.0	15.0	15.0	15.0
Auxiliary flow (L/min)	1.50	1.50	1.50	1.50
Pump speed (rpm)	15	15	15	15
Background mode	Dynamic	Dynamic	Dynamic	Dynamic
Max curve order	1	1	1	1
C.C. limit	0.995	0.995	0.995	0.995

Table 2 Liberty 220 common parameters

Nebulizer type	V-groove
Nebulizer pressure	150 kPa
Stabilization time	10 s
Sample delay time	30 s
Rinse time	10 s
Pump–tube	Orange–orange (inlet)
	Blue–blue (outlet)
Snout purge	Off
Fast pump	On

Table 3 Measurement conditions for electrothermal atomization AAS 700

Parameters	Cd	Ni	Pb
Lamp current (MA)	6.0	3.5	8.0
Wave length (nm)	228.8	232.0	283.3
Slit width (nm)	0.7	0.2	0.7
Dry temperature (°C)/ramp/hold (s)	140/15/5	140/15/5	140/15/5
Ashing temperature (°C)/ramp/hold (s)	850/10/20	1,000/10/20	700/10/20
Atomization temperature (°C)/ramp/hold (s)	1,650/0/5.0	2,300/0/5.0	1,800/0/5.0
Cleaning temperature (°C) (ramp/hold) (s)	2,600/1/3	2,600/1/3	2,600/1/3
Chemical modifier	$Mg(NO_3)_2$+$Pd(NO_3)_2$	$Mg(NO_3)_2$	$Mg(NO_3)_2$

Sample volume (10 µl), cuvette=cup, carrier gas=(200 ml/min) background correction (D_2 lamp) used for all elements

Table 4 Measurement conditions for Zn

Wave length (nm)	214
Slit width (nm)	0.7
Lamp current (mA)	7.5
Burner height (mm)	7.5
Oxidant (air) l min^{-1}	17.0
Fuel (acetylene) l min^{-1}	2.0

All solutions were stored in polyethylene bottles at 4°C. For the accuracy of methodology, the certified reference materials (CRMs), human hair NCSZN 81002b (Beijing, China), and certified reference materials of human hair BCR 397 (Brussels®, Belgium) were used (Table 5). All glassware and plastic materials used were previously soaked for 24 h in 5 M nitric acid, washed with distilled and finally rinsed with ultrapure water, dried, and stored in a class 100 laminar flow hoods.

Table 5 Determination of trace elements in certified sample of human hair (CRM) by conventional (CDM) and microwave digestion method (MWD) (n=10)

Elements	Conventional digestion method CDM	Microwave digestion method MWD	T value[a]	% Recovery[b]	Certified values
Certified human hair reference material (NCS ZC 81002b) (μg/g)					
Cd	0.0716±0.003 (4.19)	0.0714±0.006 (8.40)	0.305	99.7	0.072±0.010
Ni	5.71±0.51 (8.93)	5.67±0.43 (7.58)	0.339	99.4	5.77[c]
Pb	3.80±0.37 (9.74)	3.72±0.35 (9.41)	0.081	98.05	3.83±0.18
Zn	191±7.28 (3.81)	187±9.53 (5.09)	0.648	97.9	191±16
Certified human hair material CRM 397 (μg/g)					
Cd	0.53±0.025 (4.72)	0.524±0.024 (4.58)	0.2256	98.87	0.52±0.024
Ni	46.07±1.41 (3.06)	45.75±1.38 (3.02)	0.9242	99.3	46.0±1.4[#]
Pb	33.29±1.21 (3.63)	32.56±1.18 (3.62)	0.096	97.8	33±1.2
Zn	197±12.8 (6.2)	194±11.3 (5.7)	0.0345	98.6	199±5

Values in parentheses are RSD. DF=9, T (critical) at 95% CI=2.262, p=0.05

[#] Indicative value

[a] Paired t test between CDM and MWD

[b] Calculated according to: $\frac{[MDM]}{[CDM]} \times 100$

[c] Means in percentage

Experimental

Collection of Scalp Hair Samples

The study was completed in two phases. Phase 1 was completed during June 2005 to December 2005 and phase 2 during July to October 2010. The sampling locations were Hyderabad, Pakistan, and Dublin, Ireland. The donor ages of both genders ranged between 30-50 years from each location. The details related to a donor's identity, residence, food habits, health status, socio-economic status, and education were recorded on a regular proforma at the time of sampling. Before the start of this study, all the control subjects and HT patients were informed about the aim of the study by being administered a form, and all agreed to participate and signed the form. A questionnaire was administered to them in order to collect details concerning physical data, ethnic origin, health, and dietary and smoking habits. Physical examinations were performed to measure participants' weight, height, blood pressure, and biochemical data. There were no statistically significant differences between normal and hypertensive patients regarding height, weight, or comparable aspects of family. The total number of normal and hypertensive subjects is shown in Table 6. The hypertensive patients who had blood pressure exceeding 130/95 mmHg (systolic/ diastolic) were admitted for their uncontrolled HT and had earlier histories of high blood pressure. The criteria of

healthy subjects included no history of symptoms of hypertension and any coronary disease documented in their medical notes, and no family history of heart disease was defined by a first-degree relative with a myocardial infarction, or cardiac death before the age of 55 years. All control subjects underwent a routine medical examination including myocardial infarction test. All patients and controls/referents were requested to complete an interviewer-administered questionnaire, concerning their demographic characteristics, age, health history, lifestyle habits, and diet. They gave written consent to participate in the study.

Table 6 The number of subjects as control and hypertensive patients of age group (30–50) years

Countries	Male		Female	
	Referents	Hypertensive patients	Referents	Hypertensive patients
Pakistan	51	40	45	38
Ireland	27	24	23	26

Collection of Scalp Hair Samples

The hair samples (-1.0 g each) were taken from the nape of the neck. Hair samples were put into separate plastic envelopes for each participant, on which the identification number of the participant was indicated. The plastic envelope of each subject was tightly sealed and attached to a questionnaire. Before analysis, each individual hair sample was cut into approximately 0.5-cm-long pieces and mixed to allow a representative subsampling of the hair specimen. After cutting, each sample was washed with diluted Triton X-100: samples were then rinsed with distilled water and then with deionized water. The samples were then rinsed three times with acetone [17]. The samples were then dried in an oven at 75±5°C for 2 h. Dried samples were stored separately in polyethylene bags.

Microwave-Assisted Acid Digestion

A microwave-assisted digestion (MWD) procedure was carried out, in order to achieve a shorter digestion time. Duplicate samples of scalp hair (200 mg) of each hypertensive patients and control individuals were directly placed into Teflon PFA flasks. Two milliliters of a freshly prepared mixture of concentrated HNO_3—H_20_2 (2:1, v/v) were added to each flask and kept for 10 min at room temperature then placed in a covered PTFE container. This was then heated following a one-stage digestion program at 80% of total power (800 W). Complete digestion of scalp hair samples required 5-8 min. After the digestion, the flasks were left to cool and the resulting solution was evaporated to semidried mass to remove excess acid. About 5 ml of 0.1 M nitric acid was added to the residue and filtered through a Whatman no. 42 filter paper and diluted with deionized water up to 10.0 ml in volumetric flasks. Blank extractions were carried through the complete procedure. Blanks and standard solutions were prepared in a similar acid matrix. The validity and efficiency of the MWD

method was checked with certified values of human hair NCSZC 81002b and certified human hair CRM 397 and with those obtained from conventional wet acid digestion method [15].

Analytical Figures of Merit

Statistical analyses were performed using computer program Excel XL State (Microsoft Corp., Redmond, WA, USA) and Minitab 13.2 (Minitab Inc., State College, PA, USA). Calibration was performed with a series of Cd, Ni, Pb, and Zn standards. Sensitivity (m) was the slope value obtained by least-square regression analysis of calibration curves based on peak area measurements. The linear range of the calibration curve ranged from the quantification limit up to 100μg/1 was used for all trace and toxic elements. The limit of detection, equal to 0.0003, 0.01, 0.0003, and 0.01 ng/mg for Cd, Ni, Pb, and Zn, respectively, was defined as 3 s/m, "s" being the standard deviation corresponding to ten blank injections and "m" the slope of the calibration graph. The quantification limits, defined as 10 s/m were calculated as: 0.0009, 0.05, 0.001, and 0.04 ng/mg for Cd, Ni, Pb, and Zn, respectively.

Result

The analytical results of hair provide a more permanent record of the trace elements than blood and urine analysis [18]. Its values, therefore, are valuable in forensic studies, environmental investigation, nutritional status, and clinical diagnosis. Analytical results of the CRM 397 and NCS ZC 81002b obtained by both digestion methods were close to that of the certified values, which confirmed the reliability of the methods (Table 5). The analysis of normal and patient scalp hair samples is reported as mean values with standard deviation (SD) and p values reported for each element (Tables 7 and 8).

Table 7 Concentrations of trace and toxic metals in scalp hair samples of Irish referent and hypertensive subjects (μg/g)

Elements	Male			Female		
	Referents	Hypertensive patients	*p* Value	Referents	Hypertensive patients	*p* Value
Cadmium	0.72±0.08	2.4±0.19	0.001	0.62±0.13	1.99±0.16	0.001
Nickel	1.86±0.34	3.97±0.23	0.001	1.65±0.27	3.59±0.31	0.001
Lead	3.36±0.45	5.18±0.11	0.001	3.25±0.39	5.09±0.17	0.001
Zinc	278±9.62	185±19.3	0.001	269±7.53	193±14.6	0.001

Table 8 Concentrations of trace and toxic metals in scalp hair samples of Pakistani referent and hypertensive subjects (μg/g)

Elements	Male			Female		
	Referents	Hypertensive patients	*p* Value	Referents	Hypertensive patients	*p* Value
Cadmium	1.68±0.3	2.98±0.2	0.009	1.6±0.25	2.5±0.5	0.021
Nickel	4.3±1.2	7.8±1.62	0.001	3.9±1.15	7.5±1.21	0.001
Lead	6.24±0.5	8.35±1.27	0.014	5.58±1.2	7.56±1.25	0.016
Zinc	230±17.1	165±18.9	0.003	250±19.3	178±15.3	0.009

Average metal concentrations (µg/g, dry weight) along with some basic statistical parameters pertaining to the distribution of the selected metals in scalp hair of Irish and Pakistani hypertensive and referent donors are given in Tables 7 and 8, respectively.

The concentrations of Zn in the scalp hair samples of male and female Irish referents were significantly higher at 95% confidence interval (CI) (266, 289) and (260, 280)μg, respectively, compared with those in male and female Irish hypertensive patients, (CI, 164, 205) and (CI, 178, 209) µg/g, respectively, with $p<0.001$. The Zn levels in the scalp hair samples of male and female Pakistani referents, (CI, 213, 248) and (CI, 230, 271) ['gig, respectively, were found to be higher than those in male and female Pakistani hypertensive patients, (CI, 144, 186) and (CI, 163, 195) µg/g, respectively, ($p>0.001$).

For both categories of donors, Cd, Ni, and Pb showed the highest metal levels in scalp hair samples of hypertensive patients. The concentrations of Cd in the scalp hair samples of male and female Irish hypertensive patients were significantly lower at 95% confidence interval (220, 263) and (182, 218) ['gig, respectively, compared with those in male and female referents, (CI, 0.65, 0.81) and (CI, 0.48, 0.79) ['gig, respectively, with $p<0.001$ (Table 7). The Cd levels in the scalp hair samples of Pakistani male and female referents, (CI, 1.29, 1.99) and (CI, 1.35, 1.87) µg/g, respectively, were found to be lower than those in male and female Pakistani hypertensive patients, (CI, 2.79, 3.17) and (CI, 2.05, 3.03) µg/g, respectively, ($p=0.001$; Table 8).

An elevated level of Pb content was observed in the scalp hair of male and female Irish hypertensive patients of both countries. The ranges of Pb in the scalp hair samples of male Irish and Pakistani referent were (CI, 3.15— 3.58) and (CI, 5.97-6.50) µg/g, respectively, whereas those in Irish and Pakistani hypertensive male patients were (CI, 5.14-5.25) and (CI, 5.01-5.17) µg/g, respectively, ($p<0.002$). The same trend was observed in female cases (Tables 7 and 8).

The levels of Ni in the scalp hair samples of male Irish and Pakistani referent were found to be lower, (CI, 1.69-2.03) and (CI, 3.63-4.93) µg/g, respectively, compared with those in Irish and Pakistani hypertensive patients, (CI, 3.85— 4.06) and (CI, 7.02— 8.55) µg/g, respectively. The same trend was observed in females (Table 7 and 8) ($p>0.001$).

Discussion

The present study brings out data related to the metal distribution in hair in terms of parameters such as spread around mean metal concentrations, possible correlations, origin identification, and comparative evaluation of the two classes of donors. Tables 7 and 8 present basic distribution parameters pertaining to selected metals in hair samples of Irish and Pakistani donors of both genders, respectively.

There are many causes of high blood pressure, such as smoking, obesity, poor diet (lack of cold water fish, fresh fruits, and vegetables), lack of exercise, poor sleep, genetics, stress, and insomnia. Drinking too much alcohol can raise the levels of some fats in the blood (triglycerides). It can also lead to high blood pressure, heart failure, and an increased calorie intake. Excessive drinking and binge drinking can lead to stroke. Other serious problems include fetal alcohol syndrome, cardiomyopathy, and sudden cardiac death.

Toxic elements (Cd, Pb, and Ni) may deplete glutathione and protein-bound sulfhydryl groups, resulting in the production of reactive oxygen species, such as superoxide anion, hydrogen peroxide, and hydroxyl radical [19]. In the past few years, increasing consideration has been given to interactions occurring in the organism between toxic metals and bioelements essential for life.

These interactions are complex and involve biometals such as zinc, copper, iron, selenium, calcium, and toxic elements, including cadmium [20]. The basis of Cd toxicity is its negative influence on enzymatic systems of cells, resulting from substitution of other essential metal ions (mainly Zn, Cu, and Ca) in metalloenzymes and its very strong affinity to biological structures containing —SH groups, such as proteins, enzymes, and nucleic acids [12]. The relevance of Cd, Pb, Ni, Cu, and Fe—Zn interactions should be considered in the light of the general population exposure to toxic metals [21] and the common deficiency of Zn in the world, mainly due to nutritional factors [22]. The concentrations of zinc in the scalp hair samples of the Irish and Pakistani hypertensive patients of both genders were lowered (Tables 7 and 8). Zinc deficiency causes the arteries to become hard, brittle, and often inflamed instead of soft and flexible.

This loss of flexibility will raise the blood pressure, in particular the systolic pressure [23, 24]. In the case of hypertensive patients, vegetarian foods have high phytate, and their intake may result in reduced availability of iron and zinc for intestinal absorption [25]. The Pb, Cd, and Ni in the occupational hypertensive persons from urban areas were significantly higher [26] as compared with normal subjects. Smoking was found to be a contributing factor to higher bioaccumulation of Cd as also reported by other researchers [27]. Workers with chronic headache and dizziness have higher levels of Cr and Pb in the scalp hair samples, such as those working in a fireworks factory [28]. It was observed in our studies that the level of nickel was significantly higher in hypertensive subjects as compared with normotensive age-matched subjects (Table 8). Significant Ni levels in smokers compared with nonsmokers have also been reported at significant level of $p>0.001$ [29]. Besides this, the inhalation of vapors of nickel carbonyl certain occupations (welding, fitting, etc.) also causes elevated Ni levels in biological samples [30,31].

This is the first study with comprehensive data on toxic and essential elements in the scalp hair samples of hypertensive and referent subjects of two countries (Pakistan and Ireland). The concentrations of essential trace and toxic elements in scalp hair samples of the Irish referent subjects were close to those reported from other European [32-38], American [39-42], and Australian [43] countries (Table 9). The elemental concentrations of Cd, Pb, and Ni

in Pakistani referents were almost higher than in European countries, which is in agreement with the studies which were done in Asian [44-58] and African [59-63] countries (Table 9).

Table 9 Comparison of different elemental contents (μg/g) in scalp hair of people from various parts of the world

Europe				
Sweden				
Authors	Elements	Age (years)	*N*	$x \pm s$ (μg/g)
Rodushkin and Axelsson 2000 [32]	Pb	1–75	114	0.22–7.26
	Cd	1–75	114	0.010–0.356
	Zn	1–75	114	68–198
	Ni	1–75	114	0.11–1.60
England				
Reilly and Harrison 1979 [33]	Zn	16–25	215	210–235
Poland				
Nowak and Chmielnicka 2000 [34]	Pb	25–39	624	4.8–5.7
	Cd	25–39	624	0.56±2.3
	Zn	25–39	624	132.7±135.7
	Ni	25–39	624	1.1±5.4
Trojanowski et al. 2010 [35]	Pb	26–50	109	3.71±0.29
	Cd		109	0.401±0.035
	Pb	51–75	121	3.88±0.35
	Cd		121	0.260±0.022
Italy				
Sturaro et al. 1993 [36]	Ni	21–60	50	1.4–3.2
	Zn	21–60	50	171–314
	Pb	21–60	50	6.5–8.7
France				
Goulle et al. 2005 [37]	Ni	40–60	45	0.08–0.90
	Zn	40–60	45	129–209
	Pb	40–60	45	0.13–4.57
Netherlands				
7Iyengar and Wolttlez 1988 [38]	Zn	21–60	50	176±38
South America				
Nagra et al. 1992 [39]	Cd	22–59	50	31.6±38
Ryan et al. 1978 [40]	Zn	20–55	42	108–357
North America				
Saiki et al. 2008 [41]	Zn	50–70	50	45–162
	Zn	71–87	50	30–202
DeAntonloet al. 1982 [42]	Zn	15–35	67	90–294
Australia				
McKenzie 1979 [43]	Zn	16–56	118	189±24
Asia				
Pakistan				
Pasha et al. 2008 [44]	Pb	15–94 year	86	14.62±8.01 (0.577–31.8)
	Cd	15–94 year	86	2.13±1.74 (0.196–9.17)
	Zn	15–94 year	86	154.2±117.1 (12.4–729.2)
	Ni	15–94 year	86	3.82±2.85 (0.068–11.8)
Pasha et al. 2010 [45]	Pb	37–65	37	15.50±8.11

Table 9 (continued)

Europe				
	Cd	37–65	37	1.675±1.13
	Zn	37–65	37	140.7±79.5
	Ni	37–65	37	4.309±2.63
Shah et al. 2006 [46]	Pb	3–54	62	15.97±5.56
		3–54	62	24.95±8.69
	Cd	3–54	62	0.38±0.186
		3–54	62	0.53±0.26
	Zn	3–54	62	226±53.7
		3–54	62	190±34.0
	Ni	3–54	62	2.46±1.95
		3–54	62	1.99±1.64
Khalique et al. 2005 [47]	Cd	41–50	18	0.300±0.140
	Zn	41–50	18	270±46.5
	Ni	41–50	18	5.45±1.03
India				
Vishwanathan et al. 2002 [48]	Pb	36±1.23	25	24.8±5.92
	Cd	36±1.23	25	5.12±3.41
	Zn	36±1.23	25	265.2±17.3
	Ni	36±1.23	25	6.48±1.09
Sukumar and Subramanian 2003 [49]	Pb	31–45	17	8.9±1.9
		46–60	11	4.5±2.8
	Cd	31–45	17	1.5±0.3
		46–60	11	1.9±0.5
	Zn	31–45	17	87.0±1.9
		46–60	11	112.8±25.3
	Ni	31–45	17	0.8±0.2
		46–60	11	1.0±0.5
Mehra and Juneja 2005 [50]	Pb	1–30	50	7.60±6.44
	Cd	1–30	50	0.32±0.21
	Zn	1–30	50	182.4±45.2
	Ni	1–30	50	25.3±15.7
Rao et al. 2002 [51]	Cd	17–60	20	0.12–0.61
	Ni	17–60	20	0.72–1.61
	Zn	17–60	20	45.44–123.5
	Pb	17–60	20	0.75–4.1
Bangladesh				
Rahman et al. 2009 [52]	Zn	–	30	199.16±27.85
	Cd	–	30	0.47±0.32
Turkey				
Sasmaz et al. 2003 [53]	Pb	–	26	3.06±1.42
	Cd	–	26	0.67±0.33
Ulvi et al. 2002 [54]	Zn	47.76±13.11 years	29	176.96
Iran				
Faghihian and Rahbarnia 2002 [55]	Zn	14–67 years	100	36–329

Table 9 (continued)

Europe				
Hong Kong				
Man and Zheng 2002 [56]	Pb	20–50 years	30	12.04±7.0
	Zn	20–50 years	30	184.85±60.89
Man et al. 1996 [57]	Zn	30–69 years	95	355–503
China				
Sandstead et al. 1998 [58]	Zn	7–25 years	662	109–155
Africa				
Nigeria				
Nnorom et al. 2005 [59]	Pb	1–30 years	46	63.6
	Cd	1–30 years	46	1.0
	Zn	1–30 years	46	128.6
	Ni	1–30 years	46	19.5
Saudi Arabia				
Hashem and Abed 2007 [60]	Cd	20–25 years	20	0.035±0.007
Syria				
Khuder et al. 2008 [61]	Pb	21–59 years	281	10.7±8.9
	Zn	21–59 years	281	260±113
	Ni	21–59 years	281	2.58±1.19
Sudan				
Eltayeb and Van-Grieken 1989 [62]	Zn	30–50 years	35	89–170
	Pb	30–50 years	35	3–17
Egypt				
Mortada et al. 2002 [63]	Pb	28–40 years	93	1.8–9.7
	Cd	28–40 years	93	0.08–0.82

There are a number of factors contributing to the higher levels of cadmium and lead in congested areas of Pakistan and other Asian countries. In Asian countries, there are many populated areas which represent a typical urban environment with heavy traffic load, high population density, and industrial units. In addition, open burning of plastics and brick making among other activities contribute to this higher level of toxic elements. Our study revealed that the levels of cadmium and lead in hair were higher in Hyderabad City, suggesting that dust containing these heavy metals is attached to hair samples due to a typical urban environment with heavy traffic load, high population density, and industrial activities. Cadmium, which enters the environment from mining, industry, vehicles, and household waters, binds strongly to soil particles or dissolves in water [64]. Once taken up by fish, plants, and animals, cadmium stays in the body for a long time [65]. Humans are also affected by cadmium through smoking and consumption of foods and beverages. Rice is the main source of cadmium in rice-eating countries. Human lead exposure is mainly through air and food. The presence of lead in fuels has contributed much of the current human exposure [66]. In most developed countries, the fuel content of lead has been controlled but still remains an issue of immediate consideration in developing countries, including Pakistan. Other sources of lead exposure include lead-based paints, lead pipelines in water supply systems, and ceramics. Lead-based products, including paints and food containers, are not completely banned in Pakistan [67].

The accumulation of cadmium in the human body may replace zinc in the arteries, which contributes to arteries becoming brittle and inflexible. Cadmium accumulates in the kidneys, resulting in high blood pressure and kidney disease. Therefore, cadmium is known to cause arterial hypertension [68]. Death rates in hypertensive heart disease are closely correlated with cadmium in air and milk [68]. Cadmium causes significant increases in blood pressure [69]. High levels of lead have been identified in significant numbers of patients with high blood pressure [70]. Lead may also replace zinc and calcium, contributing to this cause of hypertension [70]. Once the arteries become inflamed and brittle, the body may coat them with calcium and fatty plaques to prevent rupture of the arteries. This plaque unfortunately reduces the interior diameter of the arteries, which in turn raises blood pressure. More pressure is required to force the blood through the smaller diameter arteries. Toxic substances can build up within the kidneys and damage their ability to regulate water balance in the body. This can lead to water retention, salt retention, and high blood pressure.

Conclusion

The present study showed a marked hair metal concentration dependence on geographic location, environmental exposure, and dietary habits of the donors. The observed variations in metal concentrations in hair of referents and hypertensive patients of two donor groups (Pakistan and Ireland) reflected different food habits and geographic location as causatives that collectively affected individual variability and metabolic activity. In addition, environmental exposure emerged as a critical covariate that was found to overload the city atmosphere of Hyderabad, Pakistan, due to Cd, Ni, and Pb pollution arising from automobile exhaust emissions. In general, the local habitants of Dublin, Ireland, were not critically exposed to the adverse effects of environmental trace metal pollution, as has been observed for mega cities of the world. The present data could act as baseline information for relevant futuristic studies. The results of this study revealed that hypertensive patients have a different pattern of essential trace and toxic elements in their scalp hair samples than do controls/referents. However, higher levels of Cd, Cu, Fe, Pb, and Ni, as well as a lower level of Zn, correlated well with the consequences of HT. The deficiency of the essential element, Zn, which is replaced by trace and toxic elements (Cd, Pb, and Ni), may result in abnormal physiology disorders, and, in addition to other factors, this may have a role in hypertensive and cardiovascular disease. We propose that essential and toxic elemental measurements may be performed on patients reaching in the emergency department, to test whether the concentration of it may serve not only as markers of hypertension and its remedies but also as predictors of adverse outcomes.

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