Evaluation of Essential Trace and Toxic Elements in Scalp Hair Samples of Smokers and Alcohol User Hypertensive Patients

Hassan Imran Afridi & Dermot Brabazon & Tasneem Gul Kazi & Sumsun Naher.

Abstract

The incidence of hypertension has been associated to cigarette smoking and consumption of alcohol. In the present study, trace and toxic elements were determined in scalp hair of patients diagnosed with hypertension who are smokers and habitual alcohol drinkers living in Dublin, Ireland. These results were compared with age- and sex-matched healthy, nonsmokers, nondrinking controls. The concentrations of trace and toxic elements were measured by inductively coupled plasma atomic emission spectrophotometer after microwave-assisted acid digestion. The validity and accuracy of the methodology were checked using certified reference material (NCS ZC 81002b) and by the conventional wet acid digestion method on the same certified reference material 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, copper, iron, nickel and lead were significantly higher in scalp hair samples of both smoker and nonsmoker hypertensive patients than 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 as a result of cigarette smoking and alcohol consumption may be synergistic with risk factors associated with hypertension.

Keywords Scalp hair . Cigarette smokers . Alcohol drinking . Toxic elements . Copper . Iron . Zinc . Inductively coupled plasma atomic emission spectrophotometer

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]. Smoking, however, is an important source of exposure to toxic elements (TEs) such as cadmium (Cd), nickel (Ni), and lead (Pb), which have been proposed as causative agents of cigarette smoke-induced physiological disorders [4-6]. In fact, a study showed that serious symptoms (strong urges to smoke, feeling anxious, or unsuccessful attempts at not smoking) appeared in youth within weeks or only days after the initial start of smoking [6].

Iron (Fe) is an essential trace element that has been proposed to participate in free radical-producing reactions, in particular the hydroxyl radical-producing Fenton reaction [7]. It has been shown that the pulmonary alveolar macrophages (PAM) of smokers contain increased amounts of Fe and ferritin, the reason for these increases is not known [8]. Although of paramount importance in normal homeostasis, especially with regard to hemoglobin, copper (Cu) is necessary only in minute amounts in comparison with other minerals such as iron (Fe) and zinc (Zn) [8]. The research in rats has been reported by Clegg et al., 1987 [9] focusing on the problems of hypocupremia. The most common manifestation of hypercupremia is hypertension. As early as 1974, the World Health Organization [10] warned that high Cu levels in the tissues are positively correlated with cardiovascular diseases and hypertension. Zinc is an important component of biomem-branes and an essential cofactor in a variety of enzymes [11, 12]. Zinc has antioxidant-like property, thus, it can stabilize macromolecules against radical-induced oxidation in vitro as well as limit excess radical production [13]. Zinc deficiency is associated with an increase in Cd, as a result of the antagonistic relationships between these elements [14].

Cigarette design has evolved considerably over the last few decades with the incorporation of new tobacco processes, papers, filters, and several ingredients (flavor, humectants, and casing materials), which either alone or in combination have the potential to modify the quantity and/or the quality of the smoke yielded [15]. The tobacco plant absorbs TEs most probably from the soil, from fertilizers or from pesticides [16]. Other environmental factors that may influence the uptake of TEs by tobacco plants include the pH of soil, contaminated irrigated water and sewage sludge used as fertilizers. Tobacco smoking delivers 87 organic carcinogens to the lungs, in addition to TEs, [17] which may partition into the smoke phase on combustion [18]. Some of these (Cd, Ni, and Pb) readily pass into the bloodstream and may accumulate in specific organs, such as the kidney and liver [19].

There are a few studies that have reported on the large variations of heavy metal/TEs in the compositions of commercial tobacco products, which have tried to link smoking-related diseases with TEs derived from tobacco combustion [20]. Intake of alcohol is a generally accepted behavior, but it has a significant health implications. Cross-sectional investigations have demonstrated a high prevalence of hypertension in drinkers, suggesting a possible contributory role for alcohol intake in the development of hypertension [21, 22]. Although the strong positive relationship between alcohol consumption and smoking is well established [23], a literature review found limited data on the interactions of smoking and drinking alcohol on atherosclerotic risk factors (e.g., blood pressure, serum lipids). The intake of trace and TEs 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 [24]. 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 [25].

Determinations of trace elements in human tissues and fluids have been used to obtain information on nutritional status for diagnosis of diseases, indication of systemic intoxication,

and to obtain information on environmental exposure [26]. In the majority of cases, whole blood, serum, plasma, and urine were analyzed [27]. Hair can provide a more permanent record of trace and TEs associated with normal and abnormal metabolism as well as TEs assimilated from the environment [28, 29]. In addition, hair is easily collected, conveniently stored, and easily treated [30]. Therefore, the analysis of human hair has become an important method for understanding any quantitative change in certain elements inside the body [31, 32].

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: before 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, multielement determinations. The sensitivity of ICP-AES is lower than that of either Inductively 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 [33, 34]. 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 [35].

The aim and objective of our present study was to assess the concentrations of Cd, Cu, Fe, Ni, Pb, and Zn in the scalp hair samples of smoker and alcohol user hypertensive patients. For a comparative study, 54 nonhypertensive individuals (smoker and alcohol user) of the same age group (range, 30-50 years), socioeconomic status, localities and dietary habits were selected as referents. The understudy elements were analyzed by inductively coupled plasma atomic emission spectrophotometer, after microwave-assisted acid digestion.

Materials and Methods

Apparatus

A 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 Czemy—Turner monochromator with 1,800 grooves/mm holographic grating used in up to four orders. The resolution of the spectrometer is typically 0.018 nm in 1st order, 0.009 nm in 2nd order, 0.007 nm in 3rd order, and 0.006 nm in 4th 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. Acid-washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparing and storing solutions.

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

Parameters	Cd	Cu	Fe	Ni	Pb	Zn
Wavelength (nm)	226.502	327.396	238.204	231.604	220.553	213.8
Height (mm)	3	5	5	5	3	5
Windows (nm) (above the coil)	0.027	0.027	0.027	0.027	0.027	0.027
Scan (nm)	0.040	0.040	0.040	0.040	0.040	0.040
Integration (s)	3	3	3	3	3	3
Replicates	3	3	3	3	3	3
Sample uptake (s)	30	30	30	30	30	30
PMT (V)	650	650	650	650	650	650
Power (kW)	1.10	1.10	1.10	1.10	1.10	1.10
Plasma flow (L/min)	15.0	15.0	15.0	15.0	15.0	15.0
Auxiliary flow (L/min)	1.50	1.50	1.50	1.50	1.50	1.50
Pump Speed (rpm)	15	15	15	15	15	15
Background mode	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic	Dynamic
Max curve order	1	1	1	1	1	1
C.C. limit	0.995	0.995	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

Reagents and Glass Wares

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, Cu, Fe, 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. All solutions were stored in polyethylene bottles at 4°C. For the accuracy of methodology, the certified reference material, human hair NCSZN 81002b (Beijing, China), was used (Table 3). 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 3 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
Cd	0.0716±0.003 (4.19)	0.0714±0.006 (8.40)	0.305	99.7	0.072±0.010
Cu	33.5±1.92 (5.73)	33.0±1.58 (4.79)	0.193	98.5	33.6±2.3
Fe	158±6.73 (4.26)	154±8.72 (5.66)	0.182	97.5	160±16
Ni	5.71±0.51 (8.93)	5.67±0.43 (7.58)	0.339	99.4	5.77*
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

Sample Collection and Pretreatment

Before the start of this study, all referents and hypertension patients of both genders, age range 30-50 years, were informed through a consent form by the administration about the aim of study, and all agreed to participate and signed the form. A questionnaire was also administered to them to collect details regarding physical data, ethnic origin, health, duration of smoking and drinking the alcohol, frequency of smoking and drinking alcohol, dietary habits, age, and consent. The patients were grouped according to their habits, not smoking or alcohol users patients (PNACS), cigarette smoking patients (PCS), alcohol users patients (PAD), and alcohol consumption with cigarette smoking patients (PACS). While control group are also divided into four groups: first group, not smoking or alcohol users referents (RNACS), second cigarette smoker referents (RCS), third alcohol users referents (RAD) while last group had alcohol consumption with cigarette smoking (RACS) as shown in Table 4.

Table 4 Characteristics of study subjects (30–50) age groups

Parameters	Referents		Hypertensive patients	
	Male	Female	Male	Female
Occupation				
Labor	32	25	35	28
Office workers	29	12	23	15
Not working	09	21	13	13
Habits				
Smoking tobacco	11 (15.7%)	10 (17.2%)	20 (28.2%)	15 (26.8%)
Alcohol drinkers	19 (27.1%)	14 (24.1%)	16 (22.5%)	16 (28.6%)
Smoking tobacco+alcohol drinkers	15 (21.5%)	11 (19.0%)	13 (18.3%)	12 (21.4%)
Nonsmoking tobacco and alcohol drinkers	25 (35.7%)	23 (39.7%)	22 (31%)	13 (23.2%)

Physical examinations were carried out to measure participant's weight, height and blood pressure. For all patients and referents, anthropometric parameters including weight, height and waist circumference were measured using the standard protocols (Table 5).

Table 5 Clinical and biochemical characteristics of hypertension patients and controls

Parameters	Referents				Patients			
	RNACS	RACS	RCS	RAD	PNACS	PACS	PCS	PAD
Male								
Height (cm)	180.3±2.5	182.9±1.4	181.6±0.9	180.8±1.6	179.6±1.32	184.4±2.32	179.8±0.83	181.2±1.7
Weight (kg)	79.4±1.3	83.7±2.42	82.1±1.13	84.9±2.26	80.3±2.54	83.5±1.35	82.6±2.68	84.3±1.12
Waist circumference (cm)	76.4±1.43	80.3±1.52	79.5±1.36	83.1±1.07	86.2±1.43	82.5±1.28	87.6±1.22	88.3±1.38
BMI (kg/m^2)	24.4±2.0	25.0±1.5	24.9±0.85	25.9±2.2	24.9±1.6	24.6±1.3	25.5±1.34	25.7±1.51
Systolic BP (mmHg)	119.3±3.1	126.2±1.2	122.7±2.4	124.5±1.5	152.2±2.8	156.7±2.4	160.5±1.06	163.5±1.52
Diastolic BP (mmHg)	79.9±2.7	89.2±1.48	83.2±0.92	85.3±1.74	96.5±1.42	98.5±2.35	99.2±0.62	101.3±1.25
Female								
Height (cm)	164.2±1.28	163.4±0.8	165.2±1.0	164±1.02	165.1±0.95	164.2±0.76	164.3±0.82	164.5±0.87
Weight (kg)	60.5±1.26	64.5±1.42	62.3±0.72	63.1±1.31	62.2±1.73	67.8±0.78	64.7±0.55	65.9±2.16
Waist circumference (cm)	63.3±1.38	65.2±1.21	64.6±1.41	65.8±1.54	64.4±1.04	65.9±1.21	65.2±0.49	65.6±0.82
BMI (kg/m^2)	22.4±1.57	24.2±1.93	22.8±1.65	23.5±2.34	22.8±1.57	25.2±0.85	23.9±1.74	24.3±2.16
Systolic BP (mmHg)	119.1±1.2	123.5±1.3	120.7±0.5	122.1±1.3	150.4±1.92	153.2±1.42	158.5±1.21	160.3±1.62
Diastolic BP (mmHg)	80.1±0.93	86.4±1.35	83.5±0.61	84.9±1.56	95.2±1.25	97.2±1.24	96.5±0.47	99.2±1.18

BMI body mass index

There were no statistically significant differences between both groups of patients and referents with regard to height and weight. The 62% hypertensive patient used antihypertensive drugs. This research project was evaluated and approved by the Higher Education Commission, Pakistan.

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 (MI), or cardiac death before the age of 55 years. All control subjects underwent a routine medical examination including MI 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.

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 (ID) 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, then samples were rinsed with distilled water and then with deionized water. The samples were then rinsed three times with acetone [35]. 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 HNO3—H202 (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 with those obtained from conventional wet acid digestion method [31].

Analytical Figures of Merit

Statistical analyses were performed using computer program Excel XL State (Microsoft Corp., Redmond, WA) and Minitab 13.2 (Minitab Inc., State College, PA). The Student's t test was used to assess the significance of the differences in concentrations of elements among study subjects.

Calibration was performed with a series of Cd, Cu, Fe, Ni, Pb, and Zn standards. Sensitivity (m) was the slope value obtained by least-square regression analysis of calibration curves based on absorbance signals. The equation (n=5) for the calibration curves was as follows:

$$Y = (1.28 \times 10^{-3} \pm 8.60 \times 10^{-5})(\mathrm{Cd}) + (1.30 \times 10^{-3} \pm 1.23 \times 10^{-4}), r = 0.999$$
$$Y = (4.38 \times 10^{-2} \pm 7.11 \times 10^{-3})(\mathrm{Cu}) + (4.39 \times 10^{-2} \pm 7.25 \times 10^{-3}), r = 0.999$$
$$Y = (1.38 \times 10^{-3} \pm 9.41 \times 10^{-4})(\mathrm{Fe}) + (1.40 \times 10^{-3} \pm 8.07 \times 10^{-4}), r = 0.999$$
$$Y = (1.66 \times 10^{-2} \pm 2.24 \times 10^{-3})(\mathrm{Ni}) + (1.73 \times 10^{-2} \pm 2.92 \times 10^{-3}), r = 0.999$$
$$Y = (1.875 \times 10^{-2} \pm 7.40 \times 10^{-4})(\mathrm{Pb}) + (1.91 \times 10^{-2} \pm 5.83 \times 10^{-3}), r = 0.999$$
$$Y = (7.83 \times 10^{-2} \pm 1.18 \times 10^{-2})(\mathrm{Zn}) + (8.51 \times 10^{-2} \pm 1.03 \times 10^{-2}), r = 0.999$$

where Y is the integrated absorbance, r is the regression and the concentration range of As, Cd, and Pb for calibration curve reached from the detection limits up to 100 µg/L. The limit of detection, equal to 0.0003 ng/mg, 0.01 ng/mg, 0.01 ng/mg,0.01 ng/mg, 0.0003 ng/mg and 0.01 ng/mg for Cd, Cu, Fe, Ni, Pb, and Zn, respectively, was defined as 3 s/m, "s" being the standard deviation corresponding to ten blank injections and 'In' the slope of the calibration graph. The quantification limits, defined as 10 s/m were calculated as: 0.0009, 0.03, 0.03, 0.05, 0.001, and 0.04 ng/mg for Cd, Cu, Fe, Ni, Pb, and Zn, respectively.

Results

In the study population, ~48% of HT patients and ~37% of referents were smokers, whilst 50% HT patients and 49% referents were alcohol drinkers. Blood pressure was measured in the population under study in the sitting position after a 5-min rest. A patient was diagnosed as having HT if systolic blood pressure was >150 mmHg, diastolic pressure was >90 mmHg and if the patient was receiving drug treatment for HT. The other physical parameters of both groups of patients and referents were obtained by a standard method as shown in Table 5. The weight, body mass index, and blood pressure (systolic and diastolic blood pressure) levels of HT patients were significantly higher than those in healthy referents ($P<0.05$). The smoker and alcohol drinker referents weighed more than nonsmoker referents ($P=0.042$).

The elemental contents in the scalp hair samples varied widely among individuals; thus, a significantly large number of samples were required for statistical interpretation of the data to achieve a meaningful correlation between physiological disorders and concentrations of trace and TEs. The mean concentrations with standard deviations for each element in biological samples, as shown in Table 6, indicate that the concentrations of the essential trace element (Cu, Fe, and Zn) and TEs (Cd, Ni, and Pb) were altered in the scalp hair samples of smoker and alcohol drinker-hypertensive patients, whereas in the case of RCS, there is no significant difference for Zn, although the levels of other trace elements were higher in their scalp hair samples.

The concentrations of Zn in the scalp hair samples of male RNACS and RCS were significantly higher at 95% confidence interval (CI, 263 and 275) and (221 and 232) μg/g, respectively, compared with those in PNACS and PCS, (CI, 203 and 232) and (CI, 157 and 172) μg/g, respectively, with $P<0.001$. The Zn levels in the scalp hair samples of RACS and RAD, (CI, 175 and 190) and (CI, 196 and 210) μg/g, respectively, were found to be higher than those in PACS and PAD, (CI, 97.4 and 120) and (CI, 122 and 129) μg/g, respectively, ($P=0.001$-0.002).

Table 6 Concentrations of trace and toxic metals in scalp hair samples of referent and hypertensive subjects (μg/g)

Elements	Referents				Patients			
	RNACS	RACS	RCS	RAD	PNACS	PACS	PCS	PAD
Male								
Cadmium	0.64±0.12	2.45±0.20	1.45±0.16	1.60±0.27	2.1±0.11	3.86±0.54	2.58±0.21	2.71±0.38
Copper	11.7±1.46	18.3±3.5	14.8±2.15	15.4±2.74	18.5±1.40	33.5±1.66	28.0±3.46	30.9±1.59
Iron	18.4±3.02	25.6±1.86	19.2±2.92	25.3±0.85	23.5±1.28	38.2±0.83	28.0±5.06	32.1±3.26
Nickel	1.78±0.29	5.02±0.54	3.32±0.34	4.6±1.04	3.89±0.38	8.36±1.39	6.2±0.59	6.4±0.76
Lead	3.29±0.41	5.79±0.26	4.73±0.31	5.15±0.27	5.11±0.30	9.01±1.25	7.37±0.30	8.22±0.70
Zinc	269±11.3	185±9.04	226±10.6	203±13.9	218±31.2	109±21.4	164±16.9	126±5.82
Female								
Cadmium	0.51±0.10	2.33±0.14	1.28±0.11	1.44±0.16	1.94±0.12	3.54±0.26	2.37±0.17	2.71±0.14
Copper	11.1±1.18	17.8±2.19	16.1±2.73	15.5±1.65	18.0±1.41	32.2±1.23	26.9±1.57	29.3±1.12
Iron	19.4±1.54	25.1±0.93	22.6±1.94	24.1±1.21	25.4±1.36	35.6±2.15	30.9±2.51	32.4±1.70
Nickel	1.62±0.41	4.81±0.63	3.50±0.47	4.10±0.35	3.40±0.43	8.74±1.05	5.62±0.43	6.33±1.00
Lead	3.18±0.32	5.43±0.24	4.21±0.15	4.93±0.26	5.16±0.22	8.82±1.42	7.17±0.35	8.21±0.41
Zinc	256±9.88	159±9.96	213±8.69	185±11.1	211±22.0	98.8±5.66	154±10.7	116±4.18

The same trend was observed in female case (Table 6). It was observed that the level of Zn did not vary significantly in the scalp samples of referent smokers and alcohol users, indicating that the alteration of Zn in scalp hair samples of smokers and alcohol drinker-hypertensive patients was mainly because of the disease state of the patients. The levels of Cu and Fe in scalp hair samples were statistically significantly higher ($P< 0.01$) in smoker and alcohol users hypertensive patients of both genders, although RCS and RAD also had high levels of these trace elements in their scalp hair samples (Table 6). An elevated level of Cd content was observed in the scalp hair of female RNACS and RCS. The ranges of Cd in the scalp hair samples of RACS and RAD were (CI, 2.20-2.41) and (CI, 1.37-1.52) µg/g, respectively, whereas those in PACS and PAD were (CI, 3.40-3.59) pg/g and (CI, 2.62-2.80) i.tg/g, respectively, ($P<0.002$).

The same trend was observed in male cases (Table 6). The Pb concentration in the scalp hair samples of male RNACS was found at 95% CI, (3.07 and 3.50) µg/g, whereas in the PNACS, the Pb level was in the range of (CI, 4.94-5.26) gg/g (Table 5). Similarly, a higher level of Pb was observed in PACS (CI, 8.83-9.15) [ig/g, PCS (CI, 7.15-7.52) 4g/g and PAD (CI, 7.76-8.50) Rg/g than in RNACS ($P<0.001$). The same trend was observed in females (Table 6). The levels of Ni in the scalp hair samples of female RNACS and RCS were found to be lower, (CI, 1.41-1.83) and (CI, 3.27-3.74) µg/g, respectively, compared with those in PNACS and PCS, (CI, 3.21-3.62) and (CI, 5.43-5.84) pg/g, respectively. The ranges of Ni concentration in the scalp hair samples of RACS and RAD were (CI, 4.52-5.13) and (CI, 3.92-4.28) µg/g, respectively, compared with those of PACS and PAD, (CI, 8.20-9.11) and (CI, 5.82-6.85) µg/g, respectively.

The same trend was observed in males (Table 6) ($P>0.002$). The unpaired student t test at different degrees of freedom between hypertensive patients and referents of both genders were calculated at different probabilities. Our calculated tvalue exceeds that of tcritical value at 95% confidence intervals, which indicated the significant differences between mean values of understudy elements in referents and hypertensive patients ($p<0.001$). The inter-elemental correlation (r) of Zn versus trace and TEs in hypertensive patients and referents indicates that the values of Zn in scalp hair of PACS and PAD have low correlation ($r=0.05-0.17$; Table 7). The Zn/Cd, Zn/Cu, Zn/Fe, Zn/Ni, and Zn/Pb ratio were varied in the scalp hair samples of referents as compared to the hypertensive patients of both genders in age group (30-50) years (Table 8).

Discussion

This study provides data on the essential trace element (Zn, Cu, and Fe) and TEs (Cd, Ni, and Pb) in scalp hair samples obtained from smoker and alcohol user hypertensive and nonhypertensive referents of both genders of age group (30-50) years. There are many causes of high blood pressure, such as smoking, obesity, poor diet, lack of cold water fish, fresh fruits, vegetables, exercise, poor sleep, genetics, stress, and insomnia. Drinking too much alcohol can raise the levels of some fats in the blood (triglycerides).

Table 7 Linear regression and Pearson's coefficient for zinc versus trace and toxic elements (Cd, Cu, Fe, Ni, and Pb) in referents and hypertensive patients

Elements	Referents				Patients			
	RNACS	RACS	RCS	RAD	PNACS	PACS	PCS	PAD
Male								
Cadmium (*R*)	0.009*x*−1.87 (0.90)	0.007*x*+1.08 (0.33)	−0.008*x*+3.28 (0.51)	−0.01*x*+3.59 (0.50)	−0.0009*x*+2.28 (0.25)	0.0017*x*+3.68 (0.07)	0.0025*x*+2.17 (0.20)	0.0116*x*+1.25 (0.18)
Copper (*R*)	0.109*x*−17.6 (0.84)	0.139*x*−7.55 (0.36)	0.116*x*−11.55 (0.57)	−0.077*x*+30.9 (0.39)	−0.015*x*+21.8 (0.34)	0.009*x*+32.2 (0.13)	−0.054*x*+36.9 (0.26)	0.06*x*+23.4 (0.22)
Iron (*R*)	0.217*x*−39.9 (0.81)	−0.0698*x*+38.5 (0.34)	0.148*x*−14.3 (0.54)	0.026*x*+19.96 (0.42)	−0.0148*x*+26.7 (0.36)	0.0039*x*+37.7 (0.10)	0.0918*x*+12.9 (0.31)	0.073*x*+22.9 (0.13)
Nickel (*R*)	0.023*x*−4.32 (0.88)	0.0164*x*+1.97 (0.28)	0.0178*x*−0.71 (0.56)	0.028*x*−1.17 (0.38)	−0.0031*x*+4.56 (0.25)	−0.004*x*+8.79 (0.06)	0.0086*x*+4.80 (0.24)	0.02*x*+3.81 (0.15)
Lead (*R*)	0.029*x*−4.47 (0.79)	0.0096*x*+3.99 (0.34)	0.014*x*+1.51 (0.49)	0.0069*x*+3.74 (0.35)	0.0022*x*+4.63 (0.22)	−0.009*x*+10.0 (0.16)	0.0017*x*+7.09 (0.10)	0.02*x*+5.63 (0.17)
Female								
Cadmium (*R*)	0.0089*x*−1.78 (0.85)	0.005*x*+1.53 (0.38)	−0.003*x*+1.71 (0.45)	0.0065*x*+0.23 (0.44)	−0.0013*x*+2.20 (0.24)	0.0026*x*+3.29 (0.06)	0.0018*x*+1.96 (0.11)	0.0028*x*+2.39 (0.08)
Copper (*R*)	0.108*x*−16.5 (0.90)	0.0504*x*+9.73 (0.23)	−0.057*x*+25.6 (0.35)	0.0468*x*+6.88 (0.31)	−0.0117*x*+20.4 (0.18)	−0.0145*x*+33.7 (0.07)	−0.021*x*+30.2 (0.14)	−0.02*x*+32.0 (0.09)
Iron (*R*)	0.125*x*−12.5 (0.80)	−0.032*x*+30.2 (0.34)	−0.042*x*+26.3 (0.36)	0.041*x*+16.4 (0.38)	0.0112*x*+23.1 (0.14)	−0.019*x*+37.6 (0.05)	0.025*x*+26.99 (0.11)	−0.0325*x*+36.0 (0.08)
Nickel (*R*)	0.037*x*−7.61 (0.90)	0.022*x*+1.29 (0.35)	−0.012*x*+5.46 (0.42)	−0.0095*x*+5.83 (0.30)	0.0048*x*+2.36 (0.25)	0.0125*x*+7.5 (0.07)	−0.005*x*+6.44 (0.13)	−0.02*x*+8.65 (0.08)
Lead (*R*)	0.029*x*−4.18 (0.90)	0.0085*x*+4.08 (0.36)	−0.0047*x*+4.98 (0.53)	−0.0097*x*+6.73 (0.41)	−0.0015*x*+5.47 (0.15)	−0.0145*x*+10.3 (0.06)	−0.0053*x*+7.99 (0.16)	−0.0085*x*+9.2 (0.09)

Table 8 Zn/Cd, Zn/Cu, Zn/Fe, Zn/Ni and Zn/Pb Mole Ratio in scalp hair samples of referents and hypertensive patients of both genders

Mole ratio	Genders	RNACS	RACS	RCS	RAD	PNACS	PACS	PCS	PAD
Zn/Cd	Male	722	130	268	219	178	48.5	109	79.9
	Female	863	117	286	221	187	50.0	112	73.6
Zn/Cu	Male	22.3	9.81	14.8	12.8	11.4	3.16	5.69	3.96
	Female	22.4	8.67	12.8	11.6	11.4	2.98	5.56	3.84
Zn/Fe	Male	12.5	6.17	10.0	6.85	7.92	2.44	5.00	3.35
	Female	11.3	5.41	8.05	6.55	7.09	2.37	4.25	3.06
Zn/Ni	Male	135.6	33.1	61.1	39.6	50.4	11.7	23.7	17.7
	Female	141.8	29.7	54.6	40.5	55.7	10.1	24.6	16.4
Zn/Pb	Male	259.0	101.2	151.4	124.9	135.2	38.3	70.5	48.6
	Female	255.0	92.8	160.3	118.9	129.5	35.5	68.0	44.8

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. Cigarette smoking is a risk factor that alters low-density lipoprotein (LDL) [36], reducing the endothelium-dependent relaxation induced by acetylcholine, in a manner similar to oxidised LDL, without altering nonendothelium-dependent relaxation. Both active and passive smoking [37] are associated with the development of several clinical disorders. Smoking was the most important risk factor, considering that 48% of all the patients were smokers (Table 3). Some evidence indicated that the influence of smoking is independent but also synergistic with other risk factors such as HT, a high blood concentration of cholesterol and other physiological disorders. Cigarette smokers and people living in contaminated areas have a higher level of Cd in their blood and urine, with smokers having Cd levels more than twice as that of nonsmokers [38]. 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 [39].

Tobacco leaves naturally accumulate and concentrate relatively high levels of Cd, Ni, Pb, Fe, Cu, and therefore smoking of tobacco is an important source of these metals exposure for smokers [40]. The total amount of carcinogens in cigarette smoke ranges from 1 to 3 1.1g per cigarette [40]. The country of origin and type of the product play major roles in determining

the chemical composition of cigarette tobacco [19]. It was investigated that one pack of cigarettes deposits 2-4 Rg Cd and 1-2 Rg Pb and Ni into the lungs of a smoker, whereas some of the smoke passes into the air to be inhaled by smokers and nonsmokers alike [41]. It was also consistent with another study that smokers generally exhibit significantly higher Cd, Ni, Pb, Fe, and Cu body burdens than nonsmokers, while smoking with alcohol consumption enhance the Cd, Ni, Pb, Fe, and Cu absorption and accumulation in all the tissues [33]. The results suggested that although these toxic elements (Cd, Ni, and Pb) pose a hazard to essential trace metal homeostasis of various organs, co-exposure can pose a major threat, while consumption of ethanol may absorb much more Cd and Pb than their unexposed counterparts [42].

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 Zn, Cu, Fe, selenium (Se), calcium (Ca), and TEs, including Cd [43]. 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 affmity to biological structures containing —SH groups, such as proteins, enzymes and nucleic acids [44]. The relevance of Cd, Pb, Ni, Cu, and Fe—Zn interactions should be considered in the light of the general population exposure to TEs [45] and common deficiency of Zn in the world, mainly due to nutritional factors [46].

The concentration of zinc was found to be lower in the scalp hair samples of smokers and alcohol drinker-hypertensive patients as compare to referents of both genders (Table 6). Zinc plays an important role in normal metabolism and assists more than 200 enzymatic reactions [47]. Another biological function of Zn is the maintenance of the integrity of proteins, required for the stability of membrane structures [47]. Our results are consistent with other investigations, which showed that the concentration of Zn in blood, hair and fingernail was significantly lower in the aged patients with HT and cardiovascular diseases than in the aged healthy controls [48]. The low Zn levels may correlate with the intake of antihypertensive medication and also with reduced essential intake of macro and micro-nutrients [49]. An epidemiological study reported that low concentrations of Zn in serum and high concentrations of Zn in urine were found in patients of cardiovascular diseases, possibly because of diuretic medicines [50]. Our results indicated a high level of all TEs in hypertensive patients, with smokers and alcohol drinkers being more prone to accumulate these metals than were referents. The Cd oxides generated during the burning of cigarettes are highly bioavailable, —10% of the inhaled Cd is deposited in lung tissues and —40% is absorbed into the systemic blood circulation of smokers [51]. It was reported in a study that Thai men, who smoked an average of nine cigarettes per day for 9 years, had an approximately twofold greater body Cd load than did nonsmoking men of the same age [52]. In another study on men older than 50 years of age in northern Taiwan, smokers were 2.5 times more likely to excrete higher urinary Cd levels than were nonsmokers [53]. The uptake of Cd following environmental or occupational exposure results in a gradual accumulation in the liver and kidney, eventually resulting in kidney dysfunction and HT; its half-life in the body is not known exactly, but it may be as long as 30 years [54]. The antagonistic effect of

Cd and Zn was investigated for the fact that the accumulation of Cd in the human body may replace Zn in the arteries, which contributes to arteries becoming brittle and inflexible. Once the arteries become inflamed and brittle, the body may coat them with Ca and fatty plaques to prevent their rupture [55].

This plaque unfortunately reduces the interior diameter of the arteries, resulting in more pressure being required to force the blood through the smaller diameter arteries, which in turn raises blood pressure [55]. The concentrations of Cu and Fe were higher in the scalp hair samples of smokers and alcohol drinker-hypertensive patients as compare to referents of both genders (Table 6). A portion of iron from tobacco leaf and cigarette paper is present in mainstream smoke is well established. The Fe content of tobacco has been reported to be 420 Rg/g in 440-1,150 1.tg/g of cigarette [56]. Approximately 0.1% of the cigarette Fe is present in mainstream smoke. Thus, a one pack/day smoker can inhale over 1 pg of Fe per day [57]. Alveolar macrophages of smokers have up to six times as much Fe as in macrophages of nonsmokers [58]. Although about 75% of the intracellular metal is incorporated in insoluble hemosiderin, the remainder is found in ferritin and can be released by poly hydroxybenzenes in tobacco smoke [59]. Among the >4,000 compounds in tobacco, smoke are numerous mutagens and carcinogens [60]. The iron component, however, not only is mutagenic and carcinogenic but also uniquely possesses several additional toxic activities. A salient role of Fe is that of stimulating microbial and neoplasmic cell growth. [61]. Another critical attribute of Fe is its catalytic production of dangerous amounts of hydroxyl(free)radicals. In aminoglycoside antibiotic ototoxicity, the 1:1 drug—iron complex is required for radical generation [62].

Neoplasmic cell initiation, osteoblast suppression, and vascular cell toxicity likewise are attributed to iron-catalyzed hydroxyl radical formation [62]. In the association of smoking with enhanced respiratory tract infection and neoplasms, the role of inhaled Fe is more prominent than that of ingested or injected Fe. On the other hand, ingested and injected routes of Fe entry can be more accountable than inhaled Fe for the development of nonrespiratory tract disorders. Nevertheless, patients at elevated risk for diseases of Fe loading by either ingestion (e.g., hemochromatosis and African siderosis) or by erythrocyte injection (thalassemia and myelodysplasia) should be cautioned about inhaling materials laden with Fe such as tobacco smoke. The dietary heme Fe intake was positively and dietary Zn intake was inversely associated with the risk of cardiovascular disease (CVD) mortality among women who consumed >10 g alcohol/day. Because oxidative stress is implicated as a common mechanism in the pathogenesis of various diseases, including cancer, diabetes, and CVD [63], these similar patterns with different outcomes are not surprising but rather support our hypothesis. The effect of nonheme Fe may depend on other beneficial or detrimental constituents also in the main food sources of non heme Fe: vegetables, grains, or iron-fortified commercial foods such as cold breakfast cereal. Sullivan [64] proposed that Fe depletion attributable to menstruation could explain the low risk of CVD in women. Experimental and animal studies also support a potentially adverse effect of Fe overload on atherosclerosis [65, 66]. Epidemiologic studies have, nevertheless, reported conflicting results concerning Fe and CVD [67]. Although free Fe is a strong oxidant and catalyzes LDL

oxidation in vitro, in vivo Fe is bound to proteins, such as ferritin or transferrin, which effectively prevents tissue damage from Fe-free radicals [68]. However, a trigger such as alcohol consumption can affect human Fe homeostasis, which leads to nontransferrin-bound Fe [69]. Any effect of Fe intake on CVD mortality would be expected among drinkers, as we observed. At the same time, our observed the association of alcohol with CVD mortality is consistent with the literature [70].

Two factors, which substantially contribute to the daily intake of Cu, are multivitamin supplements and cigarette smoking. Cigarette smoking only adds to Cu and Cd poisoning. Public Health Service [71] found a cigarette to contain 0.19 ug of Cu. This accumulates in the body as smoke is inhaled as evidenced by Creason et al.'s [72] documentation of significantly higher levels of Cu in smokers as compared to nonsmokers. Yet another health hazard associated with smoking. Medeiros and Lui [73] have repeatedly demonstrated that rats placed on high Cu diets have significantly higher systolic blood pressures than those with low Cu intakes. Ahmed and Sackner [74] documented a similar correlation with sheep receiving infusions of Cu sulfate. Similarly, most hypertensive patients seen at the Princeton Brain Bio Center exhibit elevated serum Cu as well as depressed serum Zn. Melanin, the natural skin pigment, seems to be a factor in the etiology of some forms of hypertension, especially in the darker skinned populations. Larsson and Tjalve [75] reported the physical properties of melanin cause it to bind heavy metal cations. This osmotically inactive pool of heavy metals was substantiated by epidemiological data, collected by Creason et al. [72], which documented significantly higher blood Cu and Pb levels in black military recruits as compared to white military recruits. Thus Cu, through its strong correlations to hypertension and its ability to be sequestered by melanin, may well lie at the heart of the high incidence (25%) of hypertension in the black population. Lead may also be present in high concentrations in cigarette smokers and alcohol drinker-hypertensive patients of both genders. Smokers have considerably higher levels of Pb in their blood than do nonsmokers [76]. Other possible health consequences of Pb accumulation are HT and peripheral arterial diseases [77]. Workers with chronic headache and dizziness have higher levels of Cd and Pb in the scalp hair samples, such as in those working in a fireworks factory [29]. Lead and Cd may also replace Zn and Ca, contributing to the severity of HT problems. Toxic substances can accumulate in kidneys, which damage their ability to regulate the water balance in the body. This can lead to water retention, salt retention, and high blood pressure [78].

It was observed in our study that the level of Ni was significantly higher in smokers and alcohol drinkers hypertensive patients than in normotensive age-matched referents (Table 5). Significant Ni levels in referents smokers compared with those in smokers alcohol drinkers hypertensive patients have also been reported at $P<0.003$. Besides this, the inhalation of vapors of Ni carbonyl obtained from burning of tobacco and from certain occupations (welding, fitting and so on) may also cause elevated Ni levels in biological samples [79]. As is the case with Cd, tobacco plants absorb Ni from the soil and concentrate it in the leaves [76]. Nickel has long been known to produce nasal and lung cancers [80].

The amount of Ni in tobacco plant lies between 0.640 and 1.15 mg/g, and varies greatly in cigarettes of different brands [80]. The possibility of trace and TEs contamination of various

medications and its effects on the metabolism of TEs are still unknown. Experimental studies show that both metals (Cd and Pb) contribute to oxidative stress by catalyzing the formation of reactive oxygen species [81], increasing lipid peroxidation [82, 83] and depleting the glutathione and protein-bound sulfhydryl groups [75]. The lipid peroxidation in patients, such as HT and cardiovascular disease, may be because of a disturbance in essential trace element metabolism and antioxidant levels [44]. In alcohol drinkers and cigarette smokers hypertensive patients, disturbances in the enzymatic mechanisms of free radicals detoxification lead to an alteration in the antioxidant system and reactive oxygen species. An attack on cell membranes also results in the formation of lipid peroxidation products [82]. In vitro and in vivo studies suggested that Pb-induced oxidation contributes to red blood cell damage [83]. Lead and Cd may also stimulate the production of inflammatory cytokines and may induce endothelial damage by down regulating the production of nitric oxide.

Conclusions

The results of this study revealed that hypertensive patients have a different pattern of essential trace and TEs in their scalp hair samples than do controls/referents, with the prevalence being more in smoker patients and alcohol users. 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 (Cu, Fe, 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. This study provides some support for the hypothesis that dietary intake of essential elements and inhalation of trace (Cu and Fe) and toxic elements (Cd, Pb, and Ni), most probably through drinking alcohol and smoking cigarette, may increase the risk of hypertension and related disorders, which indicates that the causal link may be stronger among cigarette smokers and alcohol users. 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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