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Comparative Distribution, Correlation, and Chemometric Analyses of Selected Metals in Scalp Hair of Angina Patients and Healthy Subjects

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Abstract Numerous epidemiological studies are preponderance of evidences intimating development of coronary artery disease caused by metal imbalance. The present study was aimed to analyze Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, Sr, and Zn in the scalp hair of angina patients and healthy subjects/controls employing HNO_3–$HClO_4$-based wet digestion followed by quantification with atomic absorption spectrophotometry. The average concentrations of Cd, Cu, Cr, Fe, Mn, Pb, and Sr revealed significantly higher levels in scalp hair of patients than controls; however, Na and Zn were appreciably higher in healthy subjects. Dissimilarity in the trace metal distribution was also observed with gender, residence, dietary habits, and smoking habits of both donor groups. The correlation study and multivariate analyses revealed diverse mutual relationships and apportionment of the trace metals in the scalp hair of patients and controls.

Keywords Trace metals · Angina · Scalp hair · Multivariate analysis · AAS

Introduction

Coronary artery disease (CAD) is a disease of civilization, and its dominance has emerged as a major contributor to the total global morbidity and mortality [1]. The disease is usually caused by a condition called atherosclerosis. Its consequence of slow accumulative progression of lipids and fibrous elements in arteries is featured by focal arterial lesions that ultimately block the blood vessels, leading towards angina, myocardial infarction, and even death [2]. In third-world countries, especially in Pakistan, factors associated with angina are hyperlipidemia, hypertension, age, obesity, cigarette smoking, genetic factors, metal imbalance, low income, and low levels of education [3].

Even though some of the causes and mechanisms involved in the onset and development of disease have been identified, the whole picture still remains unclear. In particular, the marked increase in the production of toxic metals and associated emissions to the environment due to industrialization are additional risk factors [4]. It is known that essential and trace metals play major roles in a variety of bio-chemical processes. It has also been recognized that these metals may exhibit beneficial or detrimental effects in the organism, depending on their concentrations. Association of metal contamination and its adverse impact on human health, such as role of toxic metals in atherogenesis, have been well documented. Unlike organic pollutants, toxic metals do not decay and, therefore, have the potential to be accumulated in the environment [5].

The levels of trace metals in the human body are often evaluated by determining their concentrations in body fluids and tissues [6]. In the majority of cases, whole blood, serum, plasma, and urine are analyzed, but hair provides a permanent record about short- and long-term exposure of trace metals as they are permanently incorporated in keratinous structure during formation of hair fiber and are consequently excluded from metabolic processes. In addition, scalp hair presents numerous advantages for human biomonitoring, such as easy collection, low cost, easy transport, storage, and least probability of contamination [7]. Epidemiologically, scalp hair has been used increasingly as a bio-indicator for many metals, both toxic and essential, towards assessing environmental

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exposures and body nutritional status, as well as diagnosis of diseases [8].

Prevention and diagnosis are the most important tools in fighting against the dreadful diseases. Therefore, the present study was aimed to determine the distribution, mutual correlation, and chemometric analyses of selected metals (Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, Sr, and Zn) in the scalp hair of the angina patients in comparison with matched healthy subjects. Viable variations in metal levels with respect to gender, residence, dietary habits, and smoking habits were also assessed.

Materials and Methods

Study Population

Subjects were selected from the patients admitted in cardiology wards of four different hospitals: Pakistan Institute of Medical Sciences, Islamabad; Poly Clinic Hospital, Islamabad; Benazir Bhutto Hospital, Rawalpindi; and Punjab Institute of Cardiology, Lahore, Pakistan on volunteer basis. During the present study, a total of 82 scalp hair samples were collected from the patients aged between 30 and 65 years. Prior to sample collection, the protocol of the study was approved by the human ethical review committees of the hospitals (App. No. QAUC-2012-A51). The diagnosis of cardiovascular disease (CVD) in patients had previously been established by a specialist, a cardiologist, by performing and analyzing the angiograms using routine procedure before scalp hair was taken for biochemical assays. The presence of 1 or more stenoses ≥50 % in diameter of at least in one major coronary artery was considered as the evidence of significant CVD in the patient [9, 10]. The healthy subjects/control (n= 82) were also selected on volunteer basis from the same localities matched with patients for sex, ages, environment, food habits, etc. The details that related to a donor's gender, age, native place, ailment duration, diet, smoking habits, and occupation were recorded on a questionnaire at the time of sample collection from the subjects of both categories.

Sample Collection and Pretreatment Step

The hair sample (~3.0 g) was cut using plastic scissors from the nape of the neck close to the scalp and directly stored in the zip-mouthed polythene bag duly label with the code along with the attached questionnaire [11]. Pretreatment step included washing and drying of hair sample. Firstly, the scalp hair samples were cut into small pieces and mixed thoroughly, followed by the addition of 50-mL detergent solution (5 % *w*/*v*) in the conical flasks which contained the hair sample. Then, the contents were placed on an auto-shaker for about 30 min, shaking at a frequency of 320 vibrations per min. Afterwards, the sample was left undisturbed for at least 3 h and then washed with plentiful tap water and finally with double-distilled water until all the detergents were removed. After this step, 30 mL of 0.5 % *v*/*v* Triton X-100 solution was added and again placed the contents on an auto-shaker for 25 min shaking. The sample was then washed with excess of double-distilled water in order to remove the non-ionic detergent. Finally, the hair sample was dried in an electric oven for overnight at 70 °C [11].

Wet Acid Digestion of Sample

An accurately weighed quantity of hair sample (~1.0 g) was taken in a digestion vessel, followed by the addition of 10 mL of HNO_3, and left the contents for 30 min at room temperature. Afterwards, the sample content was placed on a hot plate for about 30 min, keeping the temperature between 70 and 80 °C and then the sample was allowed to cool at room temperature. It was followed by the addition of 5.0 mL of $HClO_4$ in the digestion flask with subsequent heating to a soft boil until white dense fumes evolved. A blank containing all the reagents in the same sequence and having the same steps except the sample was also processed in the identical manner along with each batch. The prepared digested mixture was transferred to 50 mL volumetric flask, and the final volume was adjusted by double-distilled water [6].

Quantification of Metals

The digested samples were quantified for selected metals (Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, Sr, and Zn) using flame atomic absorption spectrophotometer (Shamidzu AA-670, Japan) under optimum analytical conditions with automatic background compensation. Three sub-samples of each sample were treated and run separately onto the spectrophotometer to pool mean metal concentrations. Parallel routine check on the accuracy of quantified results was ensured through the use of Standard Reference Material (Human hair, Batch GBW-07601), which showed very good recoveries (98–102 %). The samples were also analyzed at an independent laboratory for comparison of the results and a maximum of ±2.5 % difference was observed. All reagents used were of ultrahigh purity (certified >99.99 %) procured from E-Merck. Working solutions were prepared by serial dilution of 1000 mg/L stock standard solutions just before the analysis on the instrument.

Statistical Analysis

Statistical analyses were carried out using STATISTICA software [12]. The data distribution and recognition tools used in this work involved the pretreatment of data in order to achieve normalization by discarding outliers. Univariate analyses of data provided basic statistical parameters, such as range, mean, standard error (SE), and skewness, along with Spearman correlation. Two multivariate methods employed in the present study were principal component analysis (PCA) and cluster analysis (CA), which have been successfully applied to investigate the essential and toxic metals apportionment in human and to distinguish between healthy subjects and diseased persons [6, 13].

Results and Discussion

Demographic Data

The demographic data for the patients and healthy subjects are presented in Table 1, which revealed that the subjects were closely matched for their ages. About 44 % controls were female, while 42 % of angina patients were male. However, 57 % of patients belong to non-vegetarian class while 58 % of controls were vegetarian. Based on the habitat, 55–72 % subjects were selected from the urban areas. A significant number of the patients and controls (70–83 %) were not addicted of tobacco (Table 1). Sixty-five percent of patients were suffering from stable angina disease, while 35 % experience the unstable angina disease.

Table 1 Characteristics of the Subjects

Characteristics	Angina patients (n=82)	Healthy subjects (n=82)
Age (years)		
Range	30–68	30–72
Mean	47.9	44.6
Gender		
Female	48 (58 %)	36 (44 %)
Male	34 (42 %)	46 (56 %)
Diet		
Vegetarian	35 (43 %)	48 (58 %)
Non-vegetarian	47 (57 %)	34 (42 %)
Residence		
Urban	45 (55 %)	59 (72 %)
Rural	37 (45 %)	23 (28 %)
Use of tobacco		
No use	57 (70 %)	68 (83 %)
Use	25 (30 %)	14 (17 %)
Angina types		
Stable angina	53 (65 %)	–
Unstable angina	29 (35 %)	–

Distribution of Metals

The basic statistical parameters related to the distribution of selected metal levels (μg/g, dry weight) in scalp hair of angina patients and healthy subjects/controls were given in Table 2. Dominant mean levels were observed for Ca (2362 μg/g), Mg (315.0 μg/g), Na (224.2 μg/g), and Zn (212.1 μg/g), followed by relatively lower levels of Fe (54.54 μg/g), Sr (40.47 μg/g), K (39.05 μg/g), Pb (25.54 μg/g), and Cu (16.77 μg/g) in case of angina patients. Furthermore, lowest average levels were noticed for Mn (6.349 μg/g), Cr (6.320 μg/g), and Cd (2.009 μg/g). Most of the metals exhibited appreciable randomness in their distribution pattern as manifested by large SE. On the average basis, the decreasing trend of metal levels in the scalp hair of angina patients revealed the following order: Ca>Mg>Na>Zn>Fe>Sr>K>Pb>Cu>Mn>Cr>Cd. Relatively, large dispersion of the concentration was measured in case of Ca, Mg, Na, and Zn, whereas large skewness values for Cr, Fe, K, and Na showed their predominantly asymmetrical distribution in the scalp hair of patients.

The distribution of selected metal levels (μg/g, dry weight) in terms of basic statistical parameters in the scalp hair of healthy subjects/controls was also shown in Table 2. On the mean scale, predominantly higher concentrations were observed for Ca (1827 μg/g), Na (565.9 μg/g), Mg (347.3 μg/g), and Zn (260.4 μg/g), followed by relatively lower levels of K (43.15 μg/g), Fe (33.61 μg/g), Sr (14.03 μg/g), Cu (12.50 μg/g), and Pb (10.45 μg/g). Somewhat lower concentration levels were noted for Mn, Cr, and Cd. The selected metals in the scalp hair of controls revealed the following decreasing order in their average concentrations: Ca>Na>Mg>Zn>K>Fe>Sr>Cu>Pb>Mn>Cr>Cd. Most of the metals exhibited random distribution as evidenced by the corresponding elevated SE values. Nevertheless, Zn, Mg, Cu, Mn, Sr, Cd, Fe, Cr, and Na manifested asymmetrical distribution as shown by relatively higher skewness values.

Two-tailed Student's *t* test ($p<0.05$) of the data showed that there was significant difference between the levels of Cd, Cr, Cu, Fe, Mn, Na, Pb, and Sr in the scalp hair of patients and normal subjects (Table 2). The mean levels of Ca, Cd, Cu, Cr, Fe, Mn, Pb, and Sr revealed significantly elevated levels in scalp hair of the patients than controls. In contrast, mean levels of Na and Zn were appreciably low in patients as compared to healthy subjects. Nevertheless, K and Mg manifested insignificant differences in their average levels in both donor groups. One of the interesting features of this comparative study indicated higher toxic trace metal levels in case of angina patients which showed the adverse effect of these toxic metals on the emergence and development of angina disease.

Significantly higher Cd concentration in scalp hair of the patients compared to healthy subjects was noticed in the present study (Table 2). Several epidemiological studies [14, 15] clearly depicted the relationship between the initiating events of angina

Table 2 Statistical distribution parameters for selected element levels (μg/g, dry weight) in the scalp hair of angina patients and healthy subjects

	Angina patients				Healthy subjects				p value[a]
	Range	Mean	SE	Skew	Range	Mean	SE	Skew	
Ca	205.4–6285	2362	210.8	0.784	590.4–3644	1827	90.34	0.427	<0.05
Cd	0.344–4.713	2.009	0.132	0.69	0.109–3.542	0.875	0.074	1.608	<0.001
Cr	0.783–27.16	6.32	0.676	1.32	0.102–7.541	2.216	0.21	1.178	<0.001
Cu	4.469–30.56	16.77	0.867	0.171	2.951–36.31	12.5	0.605	1.823	<0.001
Fe	9.119–203.9	54.54	4.403	1.759	5.407–135.8	33.61	2.987	1.591	<0.001
K	5.154–178.9	39.05	3.524	2.188	6.821–89.85	43.15	2.331	0.439	NS
Mg	10.63–1083	315	32.65	0.937	97.46–1445	347.3	24.4	2.26	NS
Mn	1.118–17.33	6.349	0.461	0.607	0.254–17.05	3.538	0.432	1.64	<0.001
Na	12.53–886.7	224.2	25.74	1.443	12.17–1830	565.9	46.86	1.069	<0.001
Pb	1.190–68.96	25.54	2.358	0.554	0.689–30.31	10.45	0.824	0.951	<0.001
Sr	7.412–76.70	40.47	2.155	0.185	4.167–50.98	14.03	0.924	1.626	<0.001
Zn	46.54–490.7	212.1	10.59	0.625	66.67–1240	260.4	18.19	3.87	<0.05

[a] Two-tailed *t* test for comparison of mean levels

and high levels of Cd because of two main reasons: Cd has hypertensive nature as well as the ability to cause endothelial cell damage. After inhalation or ingestion of Cd, it is transferred into the bloodstream where it is transported either as a free ion or protein-bound (e.g., attached to metallothioneins or albumin). Such Cd accumulation may accelerate the formation of plaque in the artery. That is why Cd is reported to be an independent risk factor for early atherosclerotic vessel wall thickening in newly diagnosed patients [14, 15].

Likewise, elevated level of Cu in the patients (Table 2) was in good agreement with the reported studies. Although Cu is an essential mineral nutrient that involves in numerous metabolic processes (e.g., hemoglobin synthesis, immune function, as a cofactor for Cu/Zn superoxide dismutase, and ceruloplasmin formation), higher Cu level aggravates inflammation processes contributing to CAD especially atherosclerosis and angina [16]. Furthermore, it is a redox metal, so it catalyzes the production of highly reactive oxygen species (ROS), having the potential of causing oxidative damage to proteins, DNA, lipids, and other molecules, so the Cu overload induces the tissue injuries which later on accelerate the progression of disease [17].

In addition, Table 2 exhibited significantly higher level of Ca in scalp hair of the patients revealing its toxic role in the development of CAD. Hemelrijck and co-workers [18] reported that coronary calcification is being accelerated by other risk factors like cholesterol oxidation, hypertension, and smoking. This Ca deposition in the heart arteries plays a critical role in the atherosclerotic plaque formation [18]. Afterwards, intracellular Ca accumulation may damage the function of endothelial cells, leading towards platelet aggregation at the damage site. Such increase in amount of Ca has been found in noncomplex, lipid-rich fibromuscular plaques and best correlates with severity of stenosis of the artery [19, 20].

In the human body, Fe is an important part of hemoglobin and enzymes, while on the other side higher concentration of Fe involves in oxidative stress, so it is considered as the proverbial two-edge sword [21]. Through Fenton reaction, excess of free Fe is directly involved in the cell oxidative stress by generating hydroxyl radicals and highly reactive intermediates. These ROS are basically involved in the peroxidation of low density lipoproteins (LDL) that consequently produce the oxidized LDL. This oxidized LDL along with other factors leads towards the development of atherosclerosis which is the hallmark of angina disease [22, 23]. The present study revealed that mean level of Fe in scalp hair of the patients was significantly higher, clearly indicating the adverse effect of Fe overload in the patients.

The present study also showed that Pb concentration was significantly higher in scalp hair of the patients suffering from angina disease (Table 2). It is one of the toxic metals that has no beneficial and biological functions [24]. Several studies revealed its negative effect on the activity of enzymes by deactivating antioxidant pools as well as inhibiting absorption of important trace minerals [25]. Numerous animal trials support the fact that Pb promotes the generation of ROS which later on induces the hypertension [26]. Clinical findings on general populations have identified a positive association of Pb exposure with clinical CAD mortality, although the numbers of studies are small [27].

In the light of some publish data, protective effects of Zn intake in the reduction of risk of CAD have been well established [28]. This protective effect may be due to its antiatherogenic effect that reduces the amount of iron in the lesion, which possibly leads to inhibition of iron-catalyzed free radical reactions. This role of Zn expresses its antioxidant and anti-inflammatory properties [29]. Numerous epidemiological studies revealed the association between Zn deficiency and the increase incidence of CAD [30] which is in good agreement with the current results (Table 2), so it has been clearly revealed by the foregoing discussion that the relative distribution of selected essential and toxic metals in scalp hair of angina patients are noticeably diverse compared to healthy subjects which represented specific role of the metals in the development of CAD.

Comparison of the Metal Levels Based on Demographic Characteristics

Variations in the average concentrations of selected essential and toxic metals (±SE) in scalp hair of the angina patients and controls based on gender are shown in Fig. 1a. Based on mean concentrations, Cd, Cr, Cu, Fe, Mn, Pb, and Sr exhibited significantly higher levels in scalp hair of male patients compared

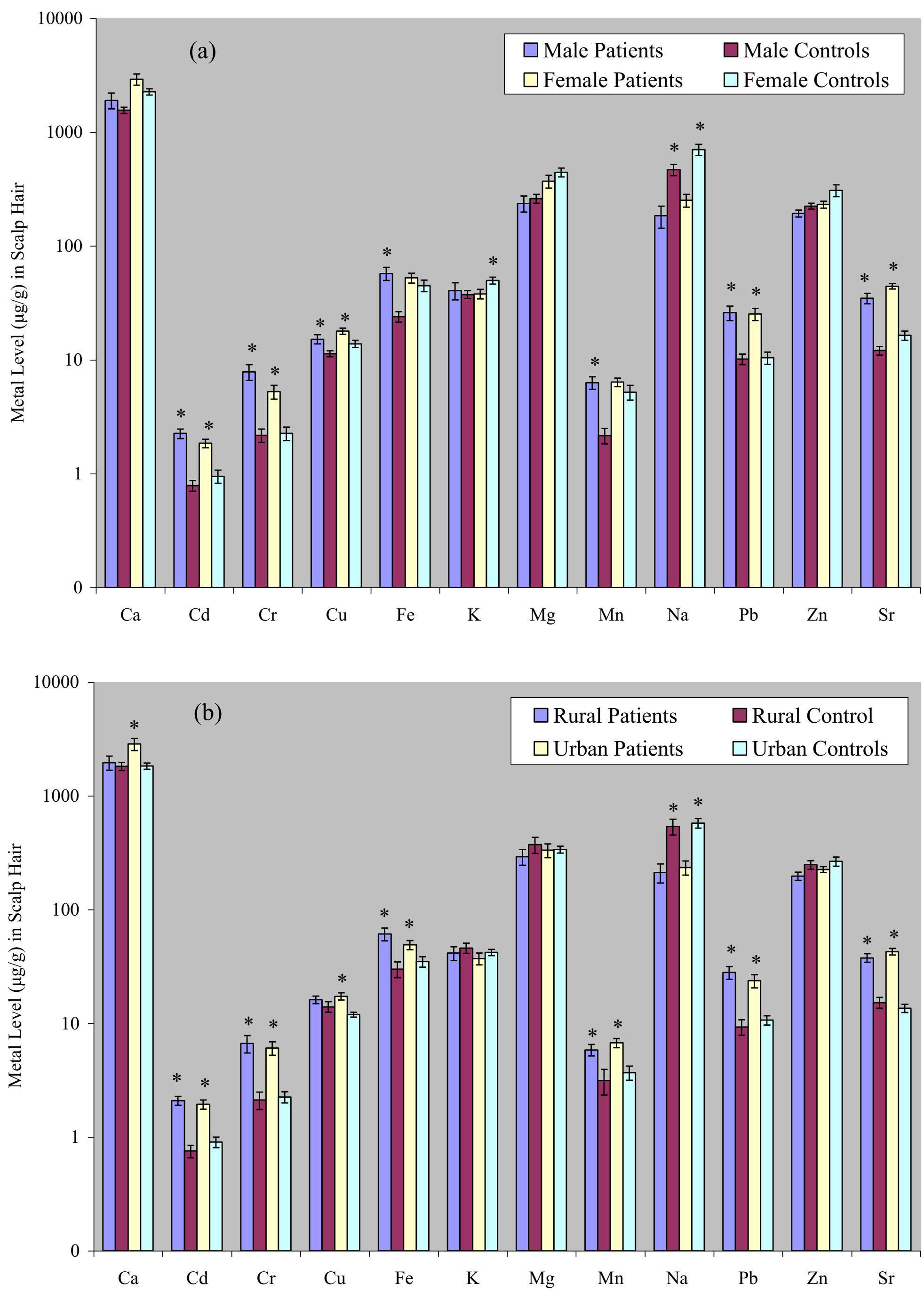

Fig. 1 Variations in the average concentrations (µg/g) of selected metals (±SE) in scalp hair of the angina patients and controls based on **a** gender, **b** abode, **c** food habits, and **d** smoking habits; statistically significant differences at $p \leq 0.05$ are shown with *asterisk*

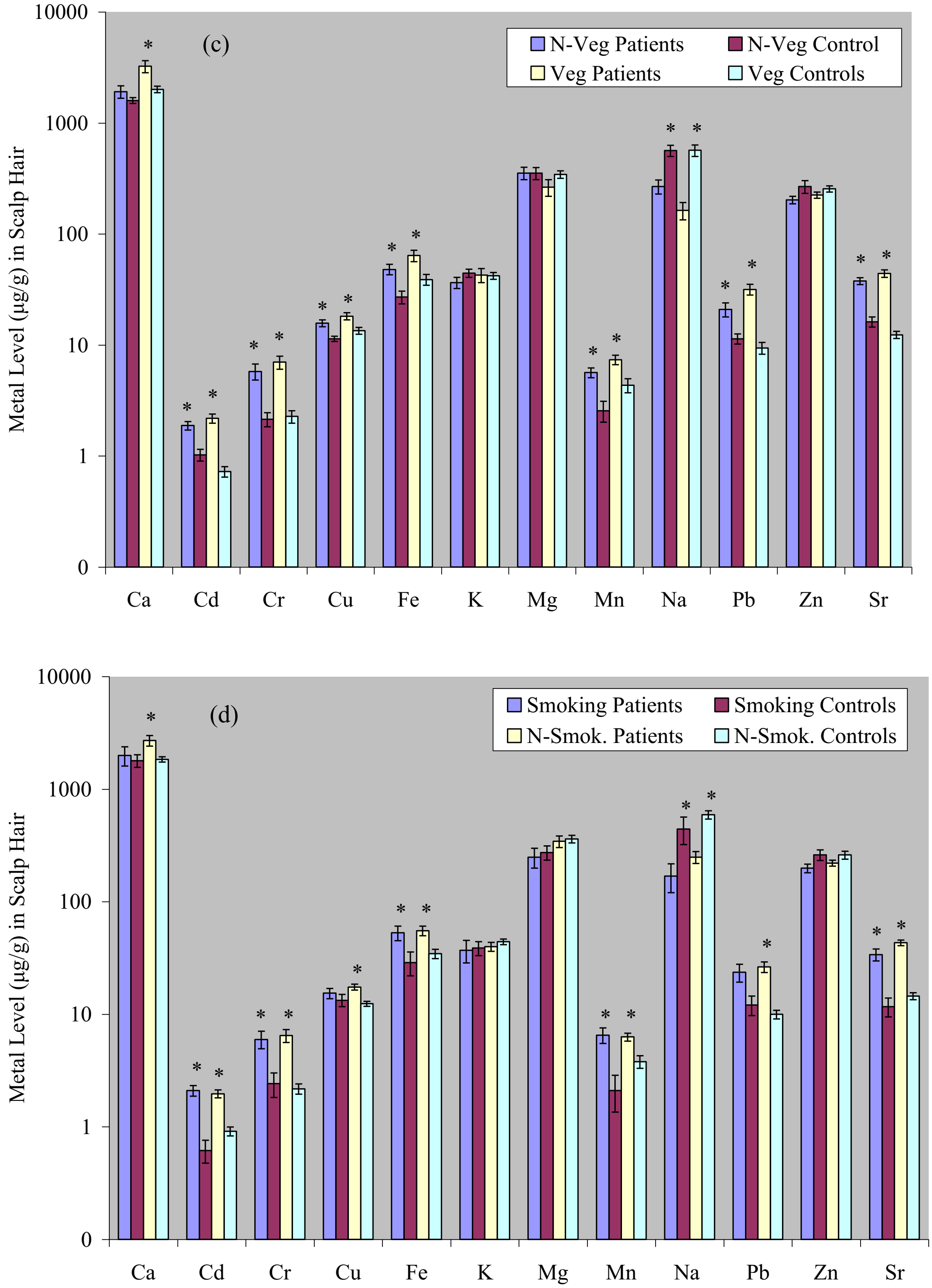

Fig. 1 (continued)

to male controls ($p<0.05$), while on the contrary only Na depicted significantly higher level in case of male controls ($p<0.05$). The remaining metals revealed insignificant differences in male donors. Similarly, Cd, Cr, Cu, Pb, and Sr mean levels were significantly higher in scalp hair of female patients as compared to female controls ($p<0.05$). However, in contrast, Na and K exhibited markedly higher levels in female healthy subjects. The rest of the metals manifested more or less comparable mean levels in scalp hair of both female donors.

Residence-based comparison (Fig. 1b) revealed that average concentrations of Cd, Cr, Fe, Mn, Pb, and Sr were significantly higher in scalp hair of rural patients in comparison with rural healthy subjects ($p<0.05$), while mean level of Na was significantly higher in rural controls. Similarly, urban patients showed significant rise in mean levels of Ca, Cd, Cr, Cu, Fe, Mn, Pb, and Sr in scalp hair of urban patients compared to urban healthy subjects ($p<0.05$). Nevertheless, significantly lower Na level was noticed in scalp hair of urban patients than urban controls. The rest of the metals revealed insignificant differences in their mean levels in scalp hair of the patients and healthy subjects from urban localities.

Figure 1c showed comparative contributions of selected metals with respect to vegetarian and non-vegetarian food habits. Based on the average concentration, Cr, Cu, Cd, Fe, Mn, Pb, and Sr revealed significantly higher levels in scalp hair of non-vegetarian patients than non-vegetarian healthy subjects ($p<0.05$). Only Na exhibited marked elevation in its mean level in case of non-vegetarian controls, while no other metal exhibited any significant variation in their mean levels. Likewise, in the case of vegetarian patients, significantly higher mean levels of Ca, Cd, Cr, Cu, Fe, Mn, Pb, and Sr were observed at $p<0.05$, which revealed appreciable rise in their average concentrations in comparison with vegetarian healthy subjects. The rest of the metals showed almost equivalent levels in case of patients and healthy subjects with vegetarian food habits except Na.

Average levels of Cd, Cr, Fe, Mn, and Sr were significantly higher in scalp hair of the patients with smoking habits than healthy subjects' addict of smoking (<0.05) as shown in Fig. 1d. Nonetheless, mean concentrations of Ca, Mg, K, Zn, Cu, and Pb exhibited insignificant variations in scalp hair of the patients and healthy subjects addicted of tobacco. In case of non-smoker patients and controls, Na exhibited relatively higher content in scalp hair of non-smoking controls, while significantly higher mean levels of Ca, Cd, Cr, Cu, Fe, Mn, Pb, and Sr were noticed in scalp hair of non-smoking patients ($p<0.05$). However, K, Mg, and Zn were measured at almost equivalent levels in scalp hair of non-tobacco user patients and healthy subjects.

Comparison of the Metal Levels Based on the Angina Types

Comparative evaluation of mean metal levels in the scalp hair of various types of angina patients (i.e., stable angina and unstable angina) is displayed in Fig. 2. In the scalp hair of both types of angina patients, mean contents of Zn and K were more or less comparable and exhibited statistically insignificant variations. Relatively higher average concentrations of Ca, Cd, Cr, Cu, Fe, Mn, Pb, and Sr were noticed in the hair of stable angina patients, while average levels of Mg and Na were appreciably higher in the scalp hair of unstable angina patients.

Correlation Study

Spearman correlation coefficients among the metal levels in the scalp hair of angina patients and controls were also calculated to envisage their mutual relationships and the results are shown in Table 3. In case of the patients, statistically significant and strong correlations at $p<0.05$ were observed between Ca–Cu (r=0.573), Cu–Zn (r=0.546), Cr–Fe (r=0.527), and Ca–Mn (r=0.523), while somewhat significant correlations were noticed among Ca–Cd (r=0.488), Mn–Pb (r=0.461), Zn–Sr (r=0.459), Ca–Zn (r=0.446), Ca–Sr (r=0.445), Cu–Pb (r=0.443), K–Zn (r=0.407), Cu–Mn (r=0.403), Cu–Sr (r=0.403), Cd–Cu (r=0.383), Cu–Fe (r=0.380), Ca–Pb (r=0.375), Cd–Mn (r=0.355), Cr–Cu (r=0.354), Fe–Sr (r=0.333), Mg–Na (r=0.326), Fe–Pb (r=0.325), Ca–Fe (r=0.319), Cr–Pb (r=0.317), and Fe–Mn (r=0.311). These mutual relationships among the essential and toxic metals indicated their probable communal variations/sources in the scalp hair of the patients. However, one metal pair Cr–Na (r=−0.412) revealed significant inverse relationship, which

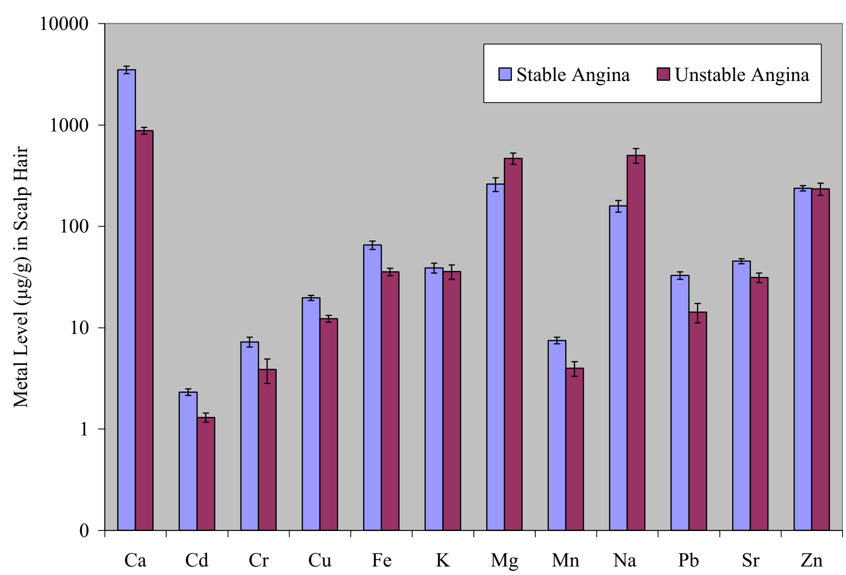

Fig. 2 Comparative average concentrations of selected metals (±SE) in the scalp hair of stable and unstable angina patients

Table 3 Correlation coefficient (*r*) matrix of selected elements in the scalp hair of angina patients (below the diagonal) and healthy subjects (above the diagonal)

	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Pb	Sr	Zn
Ca	1	−0.092	0.218	0.240	0.256	0.083	*0.453*	*0.407*	0.029	0.081	0.373	*0.448*
Cd	*0.488*	1	0.192	−0.034	0.130	0.193	0.226	−0.036	0.059	0.281	0.129	0.031
Cr	0.087	0.117	1	−0.050	−0.076	−0.049	0.045	−0.067	−0.291	0.346	0.237	0.072
Cu	*0.573*	*0.383*	*0.354*	1	0.246	0.057	0.130	0.325	0.128	0.001	0.123	0.131
Fe	*0.319*	0.224	*0.527*	*0.380*	1	0.051	0.292	*0.488*	−0.030	0.024	0.053	0.271
K	−0.011	−0.011	0.250	0.070	0.237	1	*0.414*	0.152	*0.696*	0.299	0.053	−0.075
Mg	−0.190	−0.291	−0.245	−0.260	−0.100	0.152	1	0.190	0.125	0.081	*0.554*	0.242
Mn	*0.523*	*0.355*	0.171	*0.403*	*0.311*	0.094	−0.053	1	0.177	−0.059	−0.062	0.034
Na	−0.251	−0.266	*−0.412*	−0.297	−0.249	0.196	*0.326*	−0.100	1	0.018	−0.053	0.044
Pb	*0.375*	0.288	*0.317*	*0.443*	*0.325*	0.193	0.071	*0.461*	−0.220	1	0.042	0.095
Sr	*0.445*	0.290	0.058	*0.403*	*0.333*	0.268	0.125	0.251	−0.089	0.139	1	*0.459*
Zn	*0.446*	0.199	0.173	*0.546*	0.147	*0.407*	−0.002	0.225	0.047	0.260	*0.585*	1

Italicized *r* values are significant at $p<0.05$

showed the depletion or enrichment of specific metal at the cost of other. Apparently, positive correlations of the toxic metals with the essential metals were evidencing a buildup of the toxic metals in the scalp hair of angina patients.

In present study, Fe and Pb demonstrated a significant positive correlation in case of patients as mentioned above. This positive correlation indicates that these two metals side by side play a crucial role in the development of oxidative stress which leads towards the progression of CAD. Experimental study of Adonaylo and Oteiza [31] elaborated this fact by showing that Pb^{2+} has the ability to stimulate the Fe^{2+} to initiate the membrane lipid peroxidation. During this process, Pb removes the ferrous ion from the membrane-binding site which later on is responsible for the generation of ROS. Thus, Pb–Fe-induced oxidative stress damages the lipids, proteins, and DNA which causes the tissue injury. This tissue injury later on transforms into a degenerative diseases like CAD.

Another important and significant correlation was found between Cu and Zn which pointed out their mutual role in the development and prevention of the CAD. As mentioned earlier, mean level of Zn was found to be lower in the patients while Cu revealed higher levels. Although both Cu and Zn are integral part of superoxide dismutase class of enzymes, these metals showed the antagonistic relationship which is in good agreement with the earlier studies [30, 32]. Due to the antagonistic relationship, Zn removes the Cu from its binding sites because both have the same coordination chemistry; thus, Zn stops the Cu-inducing production of ROS. This pushing off Cu, from the tissue-specific site, by Zn most likely follows the ligand exchange reaction. As a result, Cu is no more available for the production of ROS because it has been washed out of the cell. Results from studies using both in vitro and in vivo models of cardiac ischemic injuries expose to Zn reveal that the cardiac Cu content is significantly decreased [32].

The present study revealed that Ca and Cd exhibited mutual correlation which may be due to the metal's accumulation property. It is well established that under pathological conditions (osteoporosis), Ca leaves the bone and transports via blood stream to the arteries of the heart, which later on causes coronary calcification. Likewise, Cd exhibits higher level in the aorta tissue due to its accumulative nature, so this may be the reason both metals work side by side in the progression and development of the disease [15, 18]. In a similar way, Pb is also released from the bone into the blood, and due to its Ca-like nature, it travels to a large extent inside the body [33]. Consequently, free Pb is available for the initiation of several pathological events such as induced oxidative stress, increased blood pressure, and causes tissue injuries. Most probably, these effects of Pb when coupled with the accumulative Ca in the heart arteries may induce the CAD. This hypothesis is supported by the present study by showing mutual correlation among Ca–Pb pair.

In comparison, the correlation study manifested significantly divergent mutual dependence of the metals in scalp hair of controls (Table 3). Significant and strong positive correlations ($p<0.05$) were noted between Na–K ($r=0.696$), Sr–Zn ($r=0.585$), and Sr–Mg ($r=0.554$), while somewhat significant correlations were shown by Mn–Fe ($r=0.488$), Ca–Mg ($r=0.453$), Ca–Zn ($r=0.448$), K–Mg ($r=0.414$), Ca–Mn ($r=0.407$), Ca–Sr ($r=0.373$), Pb–Cr ($r=0.346$), and Mn–Cu ($r=0.325$). The rest of the metal pairs revealed very weak positive or negative relationships. Interestingly, Cd revealed insignificant relationships with other trace metal, thus manifesting its independent variation in the scalp hair of healthy subjects. The correlation study indicated an apparently mutual variation of essential metals in the scalp hair of normal subjects. Overall, the correlation behavior of the metals in scalp hair of healthy donors remained noticeably diverse compared to

Table 4 Principal component loadings for selected metals in the scalp hair of angina patients and healthy subjects

	Angina patients				Healthy subjects				
	PC 1	PC 2	PC 3	PC 4	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalue	4.240	2.233	1.491	1.052	2.549	2.262	1.808	1.267	1.097
Total variance (%)	35.33	18.61	12.43	8.764	21.24	18.85	15.07	10.56	9.146
Cumulative variance (%)	35.33	53.94	66.37	75.13	21.24	40.09	55.16	65.72	74.86
Ca	0.849	–	0.260	–	0.732	–	–	–	0.385
Cd	0.809	–	0.261	–	–	–	–	–	0.777
Cr	–	–	–	0.887	0.373	0.754	–	–	–
Cu	0.524	0.497	0.321	–	–	–	–	0.845	–
Fe	–	–	0.843	0.277	–	0.681	–	–	–
K	–	0.873	–	–	–	–	0.925	–	–
Mg	0.732	–	–	0.374	0.871	–	–	–	–
Mn	0.485	–	0.745	–	–	–	–	0.791	–
Na	0.408	–	–	0.699	–	–	0.907	–	–
Pb	–	–	0.682	–	–	0.696	–	0.317	–
Sr	–	0.430	0.626	–	0.751	–	–	–	–
Zn	0.405	0.845	–	–	0.260	0.289	–	–	0.721

PC loadings <0.250 are omitted

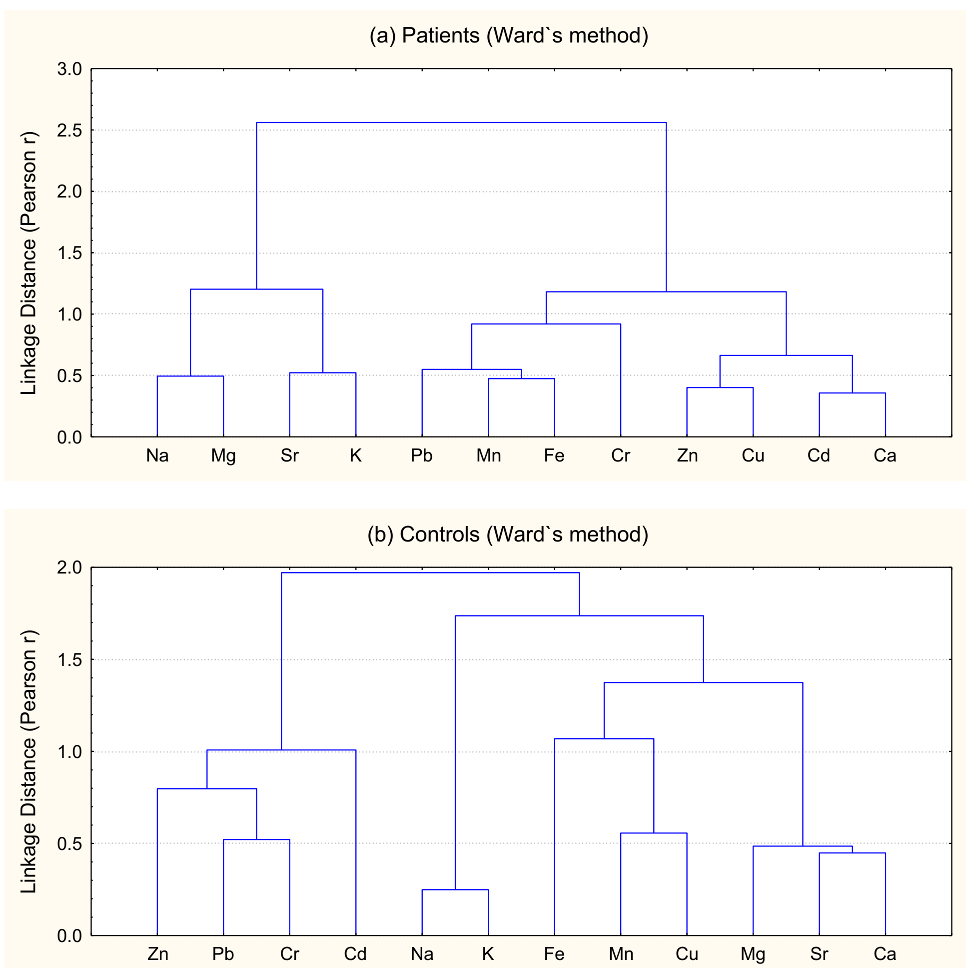

Fig. 3 Cluster analysis of selected metals in the scalp hair of (**a**) angina patients and (**b**) healthy subjects

the angina patients, which may be attributed to the disproportions of the nutrients and selected metals in the patients.

Multivariate Analyses

Metal apportionment and source identification in the scalp hair of the two groups of donors were performed by PCA and CA [13]. The PC loadings of selected metals in the scalp hair samples of angina patients and healthy donors are shown in Table 4. In case of angina patients, PCA yielded four principal components (PCs) of the metals with eigenvalues >1, commutatively exhibiting more than 75 % of the total variance of data. The corresponding CA based on Ward's method is shown in Fig. 3a, which revealed very strong clusters of Ca–Cd–Cu–Zn, Cr–Fe–Mn–Pb, and K–Sr–Mg–Na. Thus, CA showed that toxic trace metals share common clusters with the essential metals, evidencing the imbalances of metals in the scalp hair of the patients. The quantitative information regarding multiple relationships of these metals was assessed by PCA in which PC 1 exhibited dominant loadings for Ca, Cd, Mg, and Cu. This PC indicated that these elements can be originated mostly from the dietary habits as well as from the anthropogenic activities. Similarly, PC 2 exhibited higher loadings for K and Zn which were mainly contributed by the dietary habits. Furthermore, PC 3 for the scalp hair of angina patients revealed the dominant loadings of Fe, Mn, Pb, and Sr, while the last PC exhibited higher loadings for Cr and Na. These associations showed altered body metabolism that may later on participate in the onset and progress of the disease.

In case of healthy subjects, five PCs with eigenvalues >1 were obtained, manifesting more than 74 % of the total variance (Table 4). The corresponding CA in the scalp hair samples of controls based on Ward's method is portrayed in Fig. 3b, which revealed very strong clusters of Ca–Sr–Mg, Cu–Mn–Fe, K–Na, and Cd Cr–Pb–Zn. In case of healthy subjects, PC 1 exhibited higher loadings for Ca, Mg, and Sr, while the second PC showed elevated loadings for Cr, Fe, and Pb which were predominantly linked with the dietary intake (PC 1), anthropogenic exposure, and external environmental factors of the donors (PC 2). Likewise, PC 3 exhibited higher loadings for K and Na, while PC 4 revealed significant loadings for Cu and Mn. The last PC exhibited significant loadings for Cd and Zn. These three PCs were mainly contributed by the dietary intake and food habits of the donors. Overall, CA was in good agreement with PCA findings, and both of these multivariate methods revealed some mutual variations, depletion, and uptake of different trace metals in the scalp hair of angina patients.

Conclusions

In conclusion, the present study showed marked divergences in the distribution of trace metals in the scalp hair of angina patients compared with healthy subjects. The average concentrations of Cd, Cu, Cr, Fe, Mn, Pb, and Sr exhibited significantly higher levels in scalp hair of angina patients than normal subjects; nonetheless, Na and Zn levels were fairly higher in the scalp hair of healthy subjects. Trace metals also exhibited significant disparities with gender, habitat, food habits, and smoking habits of the donors. The correlation study revealed appreciably different mutual variations of trace metals in scalp hair of two donor groups. PCA and CA also manifested diverse apportionment mechanism of trace metals in scalp hair of the patients and healthy donors. These discrepancies were mainly attributed to the buildup of trace metals in the patients and resulted in the alteration of body metabolism which may be associated with the disease.

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