Acta Cardiol Sin 2015;31:518–527
Original Article
doi: 10.6515/ACS20150302A

Coronary Artery Disease

Abnormalities of Selected Trace Elements in Patients with Coronary Artery Disease

Asim Ilyas and Munir H. Shah

***Background*:** Coronary artery diseases are multifactorial, and over the last several decades particular consideration and research have been devoted to investigating the imbalance of patient elemental levels. Our current study aimed to investigate the comparative distribution of Ca, Mg, Fe, Zn, Cu, Co, Mn, Cr, Cd and Pb in the blood of coronary artery disease patients and healthy subjects.

***Methods*:** Blood samples collected from both groups were digested into a HNO_3-$HCLO_4$ (10:1 v/v) mixture in a microwave oven, followed by quantification of the elements by atomic absorption spectrometry.

***Results*:** The average levels of Pb and Cr were markedly higher ($p < 0.001$) while those of Ca, Fe, Cu and Mn were moderately higher ($p < 0.05$) in blood of the patients compared to the controls. However, correlation study showed divergent relationships between various elements in the blood of both groups. Multivariate cluster analysis revealed two major clusters of the elements for patients: Ca-Mg-Mn-Co-Cd and Pb-Cu-Fe-Zn-Cr; whereas three common groups were observed for controls: Ca-Mg-Zn-Cu, Cr-Mn-Fe and Co-Cd-Pb. Variations in the elemental levels were also observed to be associated with gender, habitat, food and smoking habits of the subjects.

***Conclusions*:** Overall, the distribution, correlation and apportionment of elemental data indicated an imbalance of the toxic/essential elements in blood of the patients compared to the controls.

Key Words: AAS • Blood • Cluster analysis • Coronary artery disease • Essential/toxic element

INTRODUCTION

The interaction between humans and trace elements may be described as a love-hate relationship. As the modern world has advanced, a variety of chemical compounds manufactured everyday, along with hazardous chemicals, especially toxic elements, are continuously emitted into the environment. This toxic element exposure may induce some serious ailments in humans, especially the development of coronary artery disease.[1-4] Coronary artery disease can involve paroxysmal thoracic pain, often radiating to the arms, particularly the left, and is most often due to ischemia of the myocardium that arises when one or more of the heart's arteries are narrowed or blocked by low density lipoprotein (LDL) and cholesterol-induced atherosclerosis.[1,5] It is developed by oxidation of LDL molecules by free radicals, particularly reactive oxygen species. Normally, the cell possesses highly efficient protective mechanisms, including metal-binding proteins and antioxidants such as manganese superoxide dismutase, copper-zinc superoxide dismutase and iron containing enzyme catalase, which under normal conditions are designed to prevent the occurrence of free radical-induced injury.[6,7]

There always exists a dynamic balance among various elements in biological systems, which is responsible for many metabolic and physiological processes. Any disorder in the elemental balance is often related to some pathological condition, resulting in the eventual ailments described.[8] Factors such as diet, absorp-

Received: August 11, 2014 Accepted: March 2, 2015
Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan.
Address correspondence and reprint requests to: Dr. Munir H. Shah, Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan. Tel: +92-51-90642137; Fax: +92-51-90642241; E-mail: mhshahg@qau.edu.pk & munir_qau@yahoo.com

tion ability, toxicities and drug-nutrient interactions play a vital role in maintaining a balance of the elements in the body.[9] The selected elements have been proposed to have diagnostic and prognostic value in ischemia heart disease, although there is no direct cause-effect relationship between the development of cardiovascular disease and the prevailing elements status.[10]

Various biological fluids have been used to evaluate the uptake and health effects of chemicals, especially toxic elements.[11] Blood is a widely used specimen because it is the transport medium for the nutrients and elements to and from the tissues, and therefore provides rapid and reliable information about the element's metabolism into the human body.[12,13] Numerous studies have been reported in recent years regarding the essential and toxic element evaluation in the blood due to its natural significance and ease of sampling.[14,15]

The main objective of this study was to assess the disparities in the distribution, correlation and multivariate apportionment of selected trace elements (Ca, Mg, Fe, Zn, Cu, Co, Mn, Cr, Cd and Pb) in the blood of coronary artery disease patients in comparison with healthy subjects. Plausible associations of the blood elemental levels with gender, habitat, food and smoking habits of the subjects were also investigated. Knowledge about the essential and toxic element variations in healthy subjects and differences in coronary artery disease patients might help to elucidate the relationship of the elements to the disease and to decide whether these elements could be used as additional biochemical markers for the diagnosis and/or prognosis of the disease.

MATERIALS AND METHODS

Study population

A total of 80 coronary artery disease patients (newly diagnosed), between the ages of 36-65 years were included in this study, on a volunteer basis. Subjects were selected from the patients admitted in Punjab Institute of Cardiology, Lahore, Pakistan. Prior to the sample collection, the protocol of the study was approved by the hospital's ethics committee and a signed, voluntary consent was obtained from each subject after being thoroughly briefed about the objectives of the study. Healthy donors (n = 79) were also selected on a volunteer basis from the same localities with matched age groups (between 35-66 years of age) and similar socioeconomic status (Table 1). A comprehensive intake form was filled out to record study subject information such as age, sex, residence, nature/duration of ailment, food habits, smoking habits and occupation at the time of sample collection from the subjects of both groups. The diagnosis of coronary artery disease (CAD) in patients had previously been established by a cardiology specialist, by analyzing the angiograms using customary procedures before blood was taken for biochemical assays. The presence of 1 or more stenoses = 50% in diameter of at least one major coronary artery was considered evidence of significant CAD in the patient.[16,17]

Sample collection and preparation

The blood samples were collected from an anticubital vein by using appropriate precautions to prevent exogenous contamination.[18] The samples were immediately transferred to evacuated polyethylene tubes (venoject, 10 mL) and kept in a refrigerator until further

Table 1. Characteristics of the subjects

	Age (yrs)		Gender		Abode		Food Habit		Tobacco use	
	Range	Mean	Female	Male	Urban	Rural	Vegetarian	Non-vegetarian	No use	Use
Coronary artery disease patients (n = 80)	36-65	49.8	31 (39%)	49 (61%)	56 (70%)	24 (30%)	51 (64%)	29 (36%)	35 (44%)	45 (56%)
Healthy donors (n = 79)	35-66	49.3	31 (40%)	48 (60%)	55 (70%)	24 (30%)	47 (59%)	32 (41%)	51 (65%)	28 (35%)
p-value	NS	NS	NS	NS	NS	NS	NS	NS	0.048	0.033

NS, non significant.

processing.[19] A precise and known amount of blood sample was transferred from the storage tube to the digestion flask and was treated with nitric acid-perchloric acid (10:1 v/v) mixture, with subsequent heating to a soft boil until white dense fumes were emitted. The digestion procedure was carried out in a microwave oven, whereafter the sample contents were then cooled down to room temperature and subsequently diluted to the appropriate volume with double distilled water.[12,20] Blanks containing all the reagents in the same sequence were also processed in a similar manner with each batch of the samples and the relative contribution of the elements in blank was generally < 2%. All chemical reagents used during the present study were of analytical grade (certified purity > 99.99%) and procured from E-Merck, Darmstadt, Germany. Working standards of the elements were prepared by serial dilution of 1000 mg/L stock standard solutions, just before the instrumental analysis.

Quantification of the elements

Quantitative analysis of selected essential and toxic elements (Ca, Mg, Fe, Zn, Cu, Co, Mn, Cr, Cd and Pb) was carried out on flame atomic absorption spectrophotometer (Shimadzu AA-670, Kyoto, Japan) under optimum analytical conditions as mentioned in Table 2. Three sub-samples of each sample were treated and run separately onto the spectrophotometer to pool the mean concentrations. Parallel routine check on the accuracy of quantified results was ensured through the use of standard reference material (NIST SRM 1598a) which showed excellent recoveries as shown in Table 2. Some of the samples were also analysed at an independent laboratory for comparison of the results, and a maximum of 5% difference was observed in the results of the two laboratories.

Statistical analysis

Statistical analyses of the elemental data were performed by using STATISTICA software.[21] Basic statistical parameters, such as, range, mean, median, standard deviation (SD) and skewness were computed, along with correlation analysis. In order to evaluate the statistically significant differences (at $p < 0.05$), the two sample t-test was employed for comparison of mean concentrations, while the Wilcoxon rank-sum test was used for the comparison of the median levels. Multivariate analysis in terms of cluster analysis (CA) was carried out for apportionment of the elements in blood samples of the patients and controls.[22] CA involves grouping of the variables into clusters each of which is a combination of objects having similar characteristics, thus resulting in internal homogeneity and external heterogeneity. The purpose of CA is to discover a system of organizing observations where variables share properties in common. The variables are grouped in clusters in terms of their nearness or similarity which is based on the Pearson-r distance. Therefore, it is cognitively easier to predict mutual properties based on an overall group membership.

RESULTS AND DISCUSSION

Characteristics of the subjects

The demographic data related to the coronary artery disease patients and healthy subjects/controls, as shown in Table 1, revealed that the subjects in the two

Table 2. Optimum analytical conditions maintained on AAS for the analysis of selected elements using air-acetylene flame, blank contribution and certified Vs. measured elemental levels in standard reference material (NIST SRM 1598a)

	Ca	Mg	Fe	Zn	Cu	Co	Mn	Cr	Cd	Pb
Wavelength (nm)	422.7	285.2	248.3	213.9	324.8	240.7	279.5	357.9	228.8	217.0
HC lamp current (mA)	6	4	8	4	3	6	5	5	4	7
Slit width (nm)	0.5	0.5	0.2	0.5	0.5	0.2	0.4	0.5	0.3	0.3
Fuel-gas flow rate (L/min.)	2.0	1.6	2.0	2.0	1.8	2.2	1.9	2.6	1.8	1.8
Blank contribution (%)	0.88	1.26	1.14	1.33	0.75	1.2	0.51	0.94	1.37	1.68
SRM certified level (μg/L)	96000	--	1680	880	1580	1.24	1.78	0.33	0.048	--
SRM measured level (μg/L)	95700	--	1672	885	1568	1.22	1.77	0.32	0.046	--
Recovery (%)	99.7	--	99.5	100.6	99.2	98.4	99.4	97.0	95.8	--

groups were closely matched for age (~49 years on the average) and the majority of them (70%) resided in urban areas. About 60% of the samples in both groups were collected from male donors. Sixty-four percent (64%) of cases in the patient group and 59% in the control group were vegetarian in their food habits. A majority of the patients (56%) were using tobacco on a continuous basis, in contrast to the healthy subjects (35%). All patients were newly diagnosed and were not under previous medications.

Distribution of the elements in the blood of coronary artery disease patients and controls

Basic statistical parameters related to the concentrations (μg/L) of Ca, Mg, Fe, Zn, Cu, Co, Mn, Cr, Cd and Pb in blood samples of coronary artery disease patients and healthy subjects are shown in Table 3. Most of the elements exhibited a broad range of concentrations. However, on an average basis, predominantly higher levels were noted for Fe (470000 μg/L), Ca (88900 μg/L) and Mg (39900 μg/L), followed by comparatively lower concentrations of Zn (4550 μg/L), Cu (1170 μg/L) and Pb (58.10 μg/L) in the blood of the patients. Nevertheless, the lowest concentrations were found for Cd and Co. On an average basis, the decreasing trend of elemental levels in the blood of the coronary artery disease patients revealed the following order: Fe > Ca > Mg > Zn > Cu > Pb > Mn > Cr > Co > Cd. The distribution of Fe, Ca and Mg was relatively random as shown by higher SD; however, considerably higher values of skewness for Ca, Mg, Mn and Zn evidenced their asymmetric distribution in blood of the patients.

In the case of controls (Table 3), predominantly higher mean levels were shown by Fe (375000 μg/L), Ca (70000 μg/L) and Mg (35400 μg/L), followed by relatively lower concentration of Zn (4930 μg/L), Cu (902 μg/L) and Pb (39.20 μg/L). The distribution of Fe, Ca and Mg was mostly random, whereas the rest of the elements exhibited a lower degree of randomness. Higher skewness values for Mn, Mg and Ca indicated their asymmetrical distribution in blood of the controls. On the mean scale, a decreasing trend of the elements in blood of controls revealed the following order: Fe > Ca > Mg > Zn > Cu > Pb > Mn > Cr > Co > Cd (Table 3). Overall, a similar decreasing trend of the elemental levels in blood of the patients and controls was observed in this study. A systematic and detailed comparison (two sample t-test) of the mean concentrations of the elements measured in the blood of the patients and healthy donors manifested that average concentrations of Pb and Cr were found to be significantly higher in the blood of coronary artery disease patients ($p < 0.001$), while mean levels of Fe, Ca, Cu and Mn were observed to be moderately higher in blood of the patients ($p < 0.05$). However, statistically non-significant differences at $p < 0.05$ were found for mean values of Zn, Mg, Co and Cd in blood of the patients and controls. Similarly, median concentrations of the elements were also compared by Wilcoxon rank sum test which revealed statistically significant differences ($p < 0.05$) for Cr, Pb, Fe and Cu in the blood

Table 3. Statistical distribution parameters for selected element concentrations (μg/L) in blood of coronary artery disease patients and healthy donors

	CAD patients					Healthy donors					*p-value	#p-value
	Range	Mean	Median	SD	Skew	Range	Mean	Median	SD	Skew		
Ca	11100-582000	88900	56700	51100	3.230	15200-240000	70000	60000	44800	2.140	0.049	0.455
Mg	13700-162500	39900	33600	25300	3.690	13500-133000	35400	30700	20400	3.130	0.492	0.627
Fe	250000-734000	470000	486000	117000	0.045	156000-861000	375000	354000	137000	1.370	0.016	0.001
Zn	1230-11900	4550	4310	2230	1.640	1040-11200	4930	4770	2150	0.840	0.432	0.867
Cu	340-1730	1170	1260	402	-0.440	210-1940	902	820	430	0.790	0.021	0.003
Co	0.113-1.680	0.690	0.633	0.392	0.340	0.050-1.440	0.570	0.513	0.380	0.500	0.127	0.153
Mn	0.380-69.80	7.100	3.740	13.60	3.760	0.330-44.60	5.640	3.720	7.796	3.830	0.041	0.733
Cr	0.070-4.640	1.630	1.687	1.157	0.780	0.033-2.730	0.790	0.684	0.668	1.130	0.0005	0.0004
Cd	0.042-1.480	0.550	0.430	0.381	0.640	0.034-1.290	0.450	0.402	0.273	1.240	0.447	0.732
Pb	0.420-150.0	58.10	53.80	47.14	0.190	2.860-143.0	39.20	19.63	28.68	1.300	0.001	0.0008

* For comparison of mean levels (t-test); # For comparison of median levels (Wilcoxon rank sum test). CAD, coronary artery disease.

samples of two groups.

Several epidemiological studies revealed the fact that the disruption of elemental homeostasis, especially redox-active elements, may lead to uncontrolled elemental-mediated formation of deleterious free radicals participating in the modifications to DNA bases, enhanced lipid peroxidation and altered metabolic activities of enzymatic systems.[23] Iron and copper belong to the category of such redox-active elements. They are vital for life and can be toxic when present in excess. The free redox iron and copper in the body generates damaging reactive free radicals via Fenton chemistry. These deleterious free radicals stimulate the lipid peroxidation, especially LDL, and ultimately lead to subsequent tissue damage. LaMarca et al. in a study showed that elevated iron levels are responsible for the production of reactive oxygen species (ROS) which can predispose to coronary disease and myocardial infarction.[24] Furthermore, a significant correlation between iron catalyzed lipid peroxidation and protein peroxidation/atherosclerosis was reported in the study of de Valk and Marx.[25] These iron and copper generated ROS are involving in the promotion of atherogenesis and prothrombotic events. Along with LDL, cardioprotective high density lipoprotein (HDL) is also susceptible to oxidation. HDL is more sensitive to oxidation by copper than LDL.[3] In the present study, Fe and Cu showed markedly higher levels in the blood of patients, which supported the above toxic role of these two redox-active metals in the development of coronary artery disease (Table 3).

Cadmium is not directly involved in free radicals production, however indirect formation of ROS and reactive nitrogen species (RNS) involving the superoxide radical, hydroxyl radical and nitric oxide has been reported. Cadmium-induced toxicity can be explained on the basis of its ability to displace the copper and iron from their binding sites, and as a result free iron and copper is available for the generation of ROS via the Fenton reaction.[26] This free radical-induced endothelial dysfunction accelerates atherosclerotic plaque formation and interferes with the antioxidant mechanism.[27] It may also contribute to atherosclerosis by increasing blood pressure.[2,28] But the exact role of Cd in the alteration of metabolism is still not clear.

Calcium deposition in the arteries plays a critical role in the atherosclerotic plaque formation which is the main cause of coronary artery disease. Cholesterol and its oxidation products along with other risk factors like hypertension and smoking may accelerate coronary calcification.[29] Furthermore, increased intracellular calcium may damage the function of endothelial cells, resulting in platelet aggregation at the damaged site. This increase in the amounts of calcium has been shown to be present in noncomplex, lipid-rich fibromuscular plaques and is best correlated with severity of stenosis of the artery.[30,31] In the present study, Ca concentration was found to be significantly higher in patients compared to the healthy subjects (Table 3), which is consistent with the above-mentioned hypothesis. In coronary artery disease episodes due to coronary artery spasm, treatment with magnesium has been shown to be considerably efficacious.[32] But epidemiological studies have yielded mixed results about the efficacy of magnesium in the secondary prevention of cardiovascular disease.[33] In this study, Mg levels did not exhibit any considerable difference in both categories of donors (Table 3).

Lead is a toxic element to humans and has multifactorial pathogenetic effects.[34] It directly interrupts the activity of enzymes, competitively inhibits absorption of important trace minerals and deactivates antioxidant sulphydryl pools. It may induce free radical damage via two ways; the first way involves the direct formation of ROS including singlet oxygen, hydroperoxides and hydrogen peroxides, and the second mechanism is achieved via depletion of the cellular antioxidant pool.[35,36] It has been reported that the changes in lipid profile in lead-exposed individuals showed coexistence with other risk factors towards heart diseases.[37] In the present study, Pb level was noted to be higher in patients, which underscores its toxic contribution towards the progression or development of coronary artery disease (Table 3).

Zinc is an essential component of more than 70 different enzymes which are involved in the regulation of several cellular metabolic activities, including the metabolism of different proteins, lipids and carbohydrates in the human body. Antagonistic to the redox-active elements, Zn may serve as an antioxidant agent. Its antioxidant activities involve the protection of sulphydryl groups of proteins against free radical attack and the reduction of free radical formation through prevention mechanisms.[3,38] Furthermore, Zn is an anti-inflamma-

tory and maintains the integrity of endothelial cells which prevent the development of atherosclerosis.[39] Cobalt is rarely associated with the development of heart disease but its acute toxicity may in part manifest through accumulation in the myocardium.[40] In Table 3, a non-significant difference was observed in the Co levels in both donor groups, which revealed that Co is not directly or independently associated with the development of heart disease. Thus, the foregoing discussion clearly showed that the relative distribution of the elements in the blood of patients with coronary artery disease is appreciably different than those observed in blood of healthy subjects, which also pointed out the specific role of the elements in the development of the disease.

Correlation study

Table 4 shows the Spearman correlation coefficients (r) between selected elements in the blood of patients and controls, wherein significant r-values are shown in bold at $p \leq 0.01$. In the case of patients, significantly strong correlations were noted between Mg-Mn (r = 0.903), Ca-Mn (r = 0.860), Ca-Mg (r = 0.776), Mg-Fe (r = 0.678), Mg-Cu (r = 0.660), Zn-Cu (r = 0.651), Cr-Mn (r = 0.601), Zn-Mn (r = 0.579), Pb-Cu (r = 0.565), Co-Cd (r = 0.556) and Fe-Cr (r = 0.532). Thus, toxic elements revealed mutual associations with the essential elements in blood of the patients, which may be associated with the disorder. On the other hand, Pb-Cd showed significant negative correlation (r = -0.481) in the blood of patients. In the cases of healthy subjects, strong mutual correlations were observed between Ca-Mg (r = 0.839), Zn-Mg (r = 0.700), Mg-Mn (r = 0.668), Zn-Fe (r = 0.649), Ca-Cu (r = 0.582), Ca-Mn (r = 0.560), Fe-Mn (r = 0.548), Fe-Cu (r = 0.531), Co-Cr (r = 0.526) and Zn-Ca (r = 0.520). Therefore, unlike the patients, most of the essential elements (Ca, Mg, Zn, Fe and Cu) exhibited significant mutual relationships in the blood of controls. Nonetheless, Cd manifested a significantly inverse relationship with Fe (r = -0.420) in the blood of the controls, while Pb displayed insignificant relationships with other elements. The correlation findings that were observed in the blood of patients were considerably diverse from those observed in blood of the healthy subjects.

Comparison of the elemental levels based on demographic characteristics

Variations in the average concentrations of selected elements [±standard deviation (SD)] in the blood of coronary artery disease patients and controls based on gender, abode, food habits and smoking habits are shown in Table 5. In the case of gender-based comparison, mean concentrations of Mn showed significantly higher levels ($p < 0.001$), along with a considerable rise ($p < 0.05$) in the Ca and Mg mean levels in blood of male patients than female patients. On the contrary, Ca and Mg levels were noted to be appreciably higher ($p < 0.05$) in female controls. However, the average levels of Cd and Pb in male controls exhibited the opposite trend and showed noticeably elevated levels ($p < 0.05$). The

Table 4. Correlation coefficient (r)* matrix of selected elements in blood of coronary artery disease patients (below the diagonal) and healthy donors (above the diagonal)

		Healthy donors									
		Ca	Mg	Fe	Zn	Cu	Co	Mn	Cr	Cd	Pb
CAD patients	Ca	1	**0.839**	**0.409**	**0.520**	**0.582**	-0.201	**0.560**	**0.352**	-0.152	-0.046
	Mg	**0.776**	1	0.097	**0.700**	0.259	0.201	**0.668**	-0.063	0.157	-0.061
	Fe	0.066	**0.678**	1	**0.649**	**0.531**	-0.110	**0.548**	0.109	**-0.420**	0.285
	Zn	-0.113	-0.029	**0.445**	1	0.154	-0.294	-0.131	-0.006	-0.225	0.108
	Cu	0.206	**0.660**	**0.418**	**0.651**	1	0.063	**0.467**	0.046	-0.292	-0.164
	Co	0.186	-0.147	0.276	0.129	0.149	1	-0.027	**0.526**	-0.013	0.163
	Mn	**0.860**	**0.903**	0.117	**0.579**	**0.342**	0.236	1	-0.022	0.051	0.071
	Cr	0.136	**0.420**	**0.532**	**0.495**	**0.467**	-0.073	**0.601**	1	-0.100	0.185
	Cd	0.019	-0.100	0.199	0.237	0.055	**0.556**	0.061	**0.338**	1	0.067
	Pb	-0.033	-0.203	**0.469**	0.029	**0.565**	0.152	0.006	0.212	**-0.481**	1

* Bold *r*-values are significant at $p \leq 0.05$.

Table 5. Comparison of the average concentrations of selected elements (mean ± SD) in blood of the coronary artery disease patients and controls based on gender, abode, food habits and smoking habits

	Ca	Mg	Fe	Zn	Cu	Co	Mn	Cr	Cd	Pb
Female patients	72400 ± 42300	34100 ± 16400	485000 ± 80600	4500 ± 2100	1120 ± 890	0.642 ± 0.510	3.464 ± 1.765	1.641 ± 0.851	0.507 ± 0.210	57.35 ± 30.22
Male patients	99100 ± 53400	43400 ± 19600	460800 ± 78200	4600 ± 1960	1200 ± 940	0.725 ± 0.481	9.407 ± 7.152	1.621 ± 0.789	0.581 ± 0.254	58.61 ± 25.27
*p-value	0.038	0.041	0.824	0.921	0.874	0.751	0.0003	0.867	0.810	0.748
Female controls	79500 ± 52300	42000 ± 17900	418700 ± 76800	5260 ± 2600	893 ± 650	0.602 ± 0.572	5.641 ± 3.970	0.843 ± 0.321	0.381 ± 0.183	34.13 ± 15.22
Male controls	61800 ± 33900	30700 ± 11600	352800 ± 69700	4780 ± 2150	892 ± 360	0.570 ± 0.420	5.575 ± 3.420	0.745 ± 0.304	0.498 ± 0.167	44.29 ± 18.77
*p-value	0.044	0.047	0.624	0.736	0.951	0.685	0.813	0.705	0.046	0.047
Rural patients	110500 ± 74600	44700 ± 21300	493200 ± 112400	4660 ± 1870	1140 ± 820	0.618 ± 0.484	7.000 ± 5.310	1.747 ± 0.752	0.576 ± 0.216	44.59 ± 20.47
Urban patients	79700 ± 52800	37800 ± 17200	460100 ± 106700	4510 ± 1690	1180 ± 730	0.727 ± 0.471	7.147 ± 5.043	1.578 ± 0.410	0.543 ± 0.227	63.93 ± 23.18
*p-value	0.029	0.314	0.442	0.724	0.810	0.773	0.802	0.462	0.754	0.009
Rural controls	86400 ± 45700	40640 ± 18400	349700 ± 95300	5100 ± 2160	1125 ± 760	0.641 ± 0.364	2.481 ± 2.014	0.823 ± 0.542	0.314 ± 0.116	21.50 ± 11.65
Urban controls	65800 ± 30900	34530 ± 12300	388500 ± 98600	4970 ± 1580	842 ± 190	0.572 ± 0.310	6.287 ± 2.837	0.780 ± 0.442	0.476 ± 0.165	43.87 ± 19.20
*p-value	0.028	0.161	0.520	0.791	0.019	0663	0.0007	0.581	0.009	0.0006
N-Veg. patients	107300 ± 69500	36600 ± 20400	487800 ± 124700	4800 ± 1940	1260 ± 500	0.659 ± 0.411	7.771 ± 5.812	1.952 ± 0.950	0.518 ± 0.193	64.62 ± 28.51
Vegetarian patients	78600 ± 46100	41700 ± 21800	460000 ± 116200	4410 ± 1810	1110 ± 480	0.713 ± 0.397	6.715 ± 4.920	1.447 ± 0.641	0.570 ± 0.185	55.35 ± 25.01
*p-value	0.037	0.305	0.427	0.503	0.661	0.390	0.220	0.041	0.916	0.097
N-Veg. controls	73350 ± 35600	36150 ± 16200	369600 ± 88400	4800 ± 2230	855 ± 310	0.480 ± 0.295	3.485 ± 1.823	0.591 ± 0.305	0.382 ± 0.092	36.24 ± 21.05
Vegetarian controls	66840 ± 21600	35260 ± 15700	389900 ± 91300	5120 ± 2530	919 ± 550	0.657 ± 0.250	7.108 ± 4.810	0.925 ± 0.455	0.500 ± 0.152	42.07 ± 20.67
*p-value	0.116	0.317	0.441	0.528	0.160	0.031	0.0005	0.006	0.038	0.137
N-Smok. patients	68000 ± 37200	34300 ± 13500	464500 ± 77600	4380 ± 2050	1120 ± 440	0.738 ± 0.455	3.525 ± 1.760	1.579 ± 0.710	0.566 ± 0.210	57.63 ± 30.20
Smoking patients	105400 ± 61900	44200 ± 16100	474400 ± 81500	4690 ± 2160	1206 ± 450	0.659 ± 0.462	10.02 ± 6.271	1.667 ± 0.682	0.542 ± 0.251	58.52 ± 26.31
*p-value	0.008	0.025	0.350	0.286	0.163	0.521	0.0004	0.273	0.861	0.722
N-Smok. controls	76700 ± 29500	39380 ± 20700	397300 ± 84900	5090 ± 2300	900 ± 370	0.515 ± 0.337	5.639 ± 3.045	0.713 ± 0.341	0.368 ± 0.142	32.03 ± 16.37
Smoking controls	60200 ± 24800	30760 ± 15600	361200 ± 80800	4860 ± 2160	882 ± 330	0.673 ± 0.325	5.559 ± 2.866	0.884 ± 0.382	0.563 ± 0.211	49.69 ± 22.14
*p-value	0.029	0.021	0.273	0.490	0.557	0.043	0.790	0.668	0.008	0.007

* p-value for two sample t-test.

rest of the elements showed non-significant differences in the blood of male and female donors.

In habitat-based comparisons (Table 5), only the average concentration of Ca was considerably higher ($p < 0.05$) in the blood of the rural patients, while the mean level of Pb was markedly higher ($p < 0.01$) in the blood of the urban patients. However, rural controls showed considerable elevation ($p < 0.05$) in mean levels of Ca and Cu, whereas, Mn, Pb and Cd revealed significantly elevated levels in blood of urban controls. The rest of

the elements showed almost equivalent mean levels in both categories.

As shown in Table 5, the mean levels of Ca and Cr exhibited appreciably higher concentrations ($p < 0.05$) in the blood of non-vegetarian patients than vegetarian patients, while other elements exhibited insignificant variation in their mean levels. Likewise, vegetarian controls showed significantly elevated mean levels ($p < 0.01$) of Mn and Cr, followed by considerably higher ($p < 0.05$) Co and Cd concentrations.

A comparison of average elemental concentrations in the blood of patients and controls based on their smoking habits is also displayed in Table 5. The mean levels of Mn and Ca were significantly higher ($p < 0.01$) in the blood of patients with smoking habits, followed by a moderately higher ($p < 0.05$) concentration of Mg. Nonetheless, the mean concentrations of Fe, Zn, Cu, Co, Cr, Cd and Pb were more or less comparable in the blood of the smoker and non-smoker patients. In case of controls, relatively higher contents of Ca and Mg were noted in the blood of non-smoking subjects ($p < 0.05$), while significant elevated mean levels ($p < 0.01$) of Cd and Pb, along with appreciably higher ($p < 0.05$) concentrations of Co were found in the blood of the smoking subjects.

Multivariate apportionment

Another important aspect of the present study was multivariate apportionment of the elements using cluster analyses.[22] The dendrogram of the elements in patient blood samples is shown in Figure 1(A), which showed strong mutual clusters among Mg-Mn-Ca-Co-Cd and Cu-Pb-Fe-Zn-Cr. The former group of elements was mainly contributed by dietary sources whereas the latter was primarily contributed by the anthropogenic activities and regulated by internal body metabolism. One of the advantages of multivariate CA is that it showed multiple mutual associations among the elements which

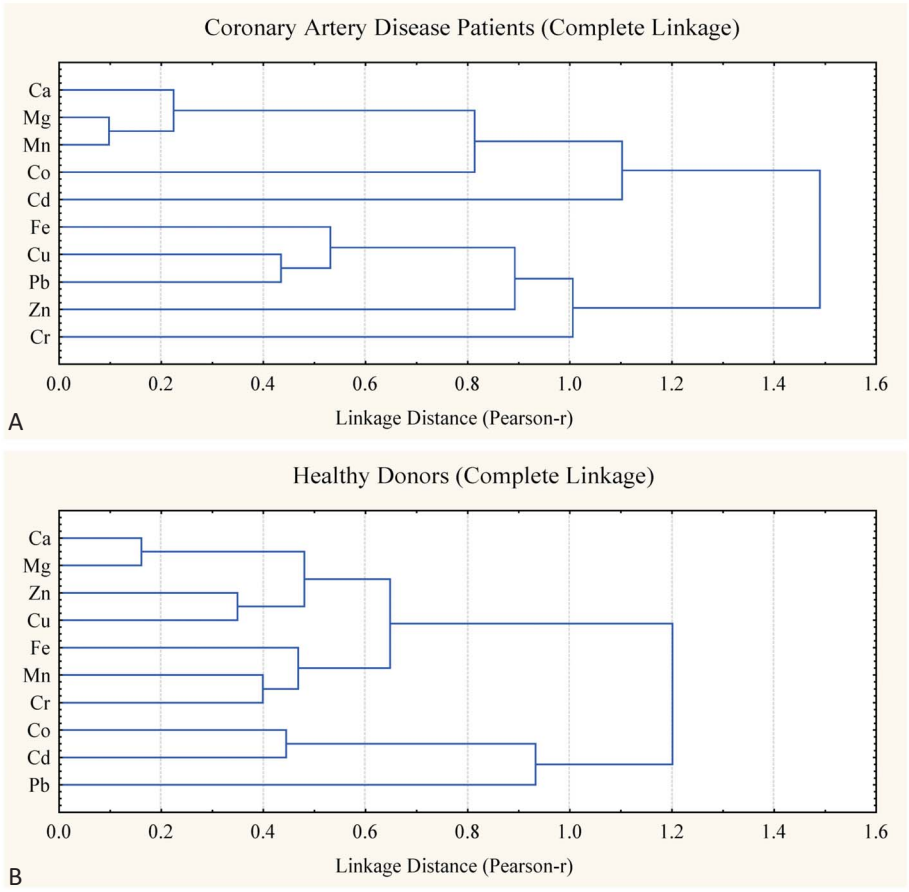

Figure 1. *Cluster analyses of selected trace elements in blood of coronary artery disease patients and healthy donors.*

are not so apparent in univariate methods such as correlation analysis. In the case of the first cluster, Cd and Co showed significant associations with Ca, Mg and Mn, thus indicating their mutual variations in the patients. Moreover, CA revealed that redox-active elements (Cu and Fe) and elements with accumulative property (Ca and Cd) shared common clusters with the essential minerals, thus indicating their critical role in the development of coronary artery disease. Numerous epidemiological studies established the role of these elements in the development of cardiovascular diseases (CVD); LaMarca et al., de Valk and Marx, Kazi et al., and Hemelrijck et al. in their separate studies all discussed the proposed mechanisms of these elements towards the progression of CVD.[7,24,25,29] In case of the healthy subjects, cluster analysis in the form of dendrogram is shown in Figure 1(B), which revealed very strong clusters of Ca-Mg-Cu-Zn, Cr-Mn-Fe and Co-Cd-Pb. Interestingly, most of the toxic elements shared common clusters, while most of the essential elements exhibited mutual associations and evidenced the true picture of normal elemental metabolism in the human body, which was significantly diverse compared to the patients. Consequently, multivariate cluster analysis of the elements in blood has the potential application to be used as an additional tool for the diagnosis/prognosis of the disease.

CONCLUSIONS

In conclusion, the present study provided evidence of marked disparities in the distribution of essential and toxic elements in blood of coronary artery disease patients compared with healthy subjects. Significantly higher concentrations of Pb, Cr, Cu, Fe, Ca and Mn were observed in the blood of CAD patients than in controls. Some noteworthy variations were also observed in the blood elemental levels based on gender, habitat, food habits and smoking habits of the subjects in both groups. The correlation study revealed appreciably different mutual variations of trace elements in blood of the patients as well as the controls. Multivariate CA exhibited diverse apportionment of the essential and toxic elements in the blood of coronary artery disease patients and healthy donors which evidenced the role of the selected elements imbalance in the development of the disease. The statistical mode of elemental analysis in the present study may be used as an additional tool for the prediction and progression of the disease.

CONFLICTS OF INTEREST

None.

ACKNOWLEDGMENTS

Funding that was provided by the Higher Education Commission, Government of Pakistan, to carry out this project is thankfully acknowledged. We are also grateful to the administration of the Punjab Institute of Cardiology, Lahore, Pakistan for their invaluable help during sample collection. Technical and financial help by Quaid-i-Azam University, Islamabad, Pakistan to execute this project is also acknowledged.

REFERENCES

1. Alissa EM, Bahjri SM, Ahmed WH, et al. Trace element status in Saudi patients with established atherosclerosis. *J Trace Elem Med Bio* 2006;20:105-14.
2. Tellez-Plaza M, Jones MR, Dominguez-Lucas A, et al. Cadmium exposure and clinical cardiovascular disease: a systematic review. *Curr Atheroscler Rep* 2013;15:356.
3. Jomova K, Valko M. Review: advances in metal-induced oxidative stress and human disease. *Toxicology* 2011;283:65-87.
4. Genuis SJ, Birkholz D, Rodushkin I, Beesoon S. Blood, urine, and sweat (bus) study: monitoring and elimination of bioaccumulated toxic elements. *Arch Environ Con Tox* 2011;61:344-57.
5. Eby GA, Halcomb WW. High-dose zinc to terminate angina pectoris: a review and hypothesis for action by ICAM inhibition. *Med Hypotheses* 2006;66:169-72.
6. Rubbo H, O'Donnell V. Nitric oxide, peroxynitrite and lipoxygenase in atherogenesis: mechanism insights. *Toxicology* 2005; 208:305-17.
7. Kazi TG, Afridi HI, Kazi N, et al. Distribution of zinc, copper and iron in biological samples of Pakistani myocardial infarction (1st, 2nd and 3rd heart attack) patients and controls. *Clin Chim Acta* 2008;389:114-9.
8. Tellez-Plaza M, Navas-Acien A, Crainiceanu CM, Guallar E. Cadmium exposure and hypertension in 1999-2004 national health and nutrition examination survey (NHANES). *Environ Health*

Perspect 2008;116:51-6.
9. Standstead HH, Klevay LM. History of nutrition symposium: trace element nutrition and human health. *J Nutr* 2000;130:483-4.
10. Agarwal S, Zaman T, Tuzcu EM, Kapadia SR. Heavy metals and cardiovascular disease: results from the national health and nutrition examination survey (NHANES). *Angiology* 2011;65:422-9.
11. Saghir M, Shaheen N, Shah MH. Comparative evaluation of trace metals in the blood of hepatitis-C patients and healthy donors. *Biol Trace Elem Res* 2011;143:751-63.
12. Pasha Q, Malik SA, Shaheen N, Shah MH. Investigation of trace metals in the blood plasma and scalp hair of gastrointestinal cancer patients in comparison with controls. *Clin Chim Acta* 2010; 411:531-9.
13. Zhao TT, Chen B, Wang HP, et al. Evaluation of toxic and essential elements in whole blood from 0- to 6-year-old children from Jinan, China. *Clin Biochem* 2013;46:612-6.
14. Brettell TA, Butler JM, Saferstein R. Forensic science. *Anal Chem* 2005;77:3839-60.
15. Pamphlett R, McQuilty R, Zarkos K. Blood levels of toxic and essential metals in motor neuron disease. *Neurotoxicology* 2001; 22:401-10.
16. Marroquin OC, Kip KE, Kelley DE, et al. Metabolic syndrome modifies the cardiovascular risk associated with angiographic coronary artery disease in women a report from the women's ischemia syndrome evaluation. *Circulation* 2004;109:714-21.
17. Afridi HI, Kazi TG, Kazi N, et al. Evaluation of toxic elements in scalp hair samples of myocardial infarction patients at different stages as related to controls. *Biol Trace Elem Res* 2010;134:1-12.
18. Aitio A, Jarvisalo J. Sampling and sample storage. In: Heber RFM, Stoeppler M, editors. *Trace element analysis in biological samples*. Amsterdam: Elsevier Science, 1994:21-52.
19. Bowen RAR, Hortin GL, Csako G, et al. Impact of blood collection devices on clinical chemistry assays. *Clin Biochem* 2010;43:4-25.
20. Sansoni B, Panday VK. Sample treatment of human biological materials. In: Herber RFM, Stoeppler M, editors. *Trace element analysis in biological samples*. Amsterdam; Elsevier Science, 1994.
21. StatSoft Inc. STATISTICA for Windows. Computer program manual. Tulsa, OK, 1999.
22. Jobson JD. Applied multivariate data analysis. New York: Springer, 1991.
23. Jomova K, Baros S, Valko M. Redox active metal-induced oxidative stress in biological systems. *Transit Met Chem* 2012;37: 127-34.
24. LaMarca BD, Gilbert J, Granger JP. Recent progress toward the understanding of the pathophysiology of hypertension during preeclampsia. *Hypertension* 2008;51:982-8.
25. de Valk B, Marx JJ. Iron, atherosclerosis and ischemic heart disease. *Arch Intern Med* 1999;159:1542-8.
26. Waalkes MP. Cadmium carcinogenesis in review. *J Inorg Biochem* 2000;79:241-4.
27. Messner B, Knoflach M, Seubert A, et al. Cadmium is a novel and independent risk factor for early atherosclerosis mechanisms and in vivo relevance. *Arterioscler Thromb Vasc Biol* 2009;29: 1392-8.
28. Gallagher CM, Meliker JR. Blood and urine cadmium, blood pressure, and hypertension: a systematic review and meta-analysis. *Environ Health Perspect* 2010;118:1676-84.
29. Hemelrijck MV, Michaelsson K, Linseisen J, Rohrmann S. Calcium intake and serum concentration in relation to risk of cardiovascular death in NHANES III. *PLOS ONE* 2013:8:e61037.
30. Rumberger JA, Schwartz RS, Simons DB, et al. Relations of coronary calcium determined by electron beam computed tomography and lumen narrowing determined at autopsy. *Am J Cardiol* 1994;73:1169-73.
31. Tulenko TN, Kleintop LL, Walter MF, Mason RP. Cholesterol, calcium and atherosclerosis: is there a role for calcium channel blockers in atheroprotection? *Int J Cardiol* 1997;62:55-66.
32. McLean RM. Magnesium and its therapeutic uses: a review. *Am J Med* 1994;96:63-76.
33. Gobbo LC, Imamura F, Wu JH, et al. Circulating and dietary magnesium and risk of cardiovascular disease: a systematic review and meta-analysis of prospective studies. *Am J Clin Nutr* 2013; 98:160-73.
34. Poreba R, Gac P, Porebab M, Andrzejak R. Environmental and occupational exposure to lead as a potential risk factor for cardiovascular disease. *Environ Toxicol Phar* 2011;3:267-77.
35. Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. *Toxicology* 2002;180:33-44.
36. Patrick L. Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. *Altern Med Rev* 2006;11:114-27.
37. Skoczyn´ ska A, Gruber K, Belowska-Bien K, Mlynek V. Risk of cardiovascular diseases in lead-exposed workers of crystal glassworks, part I. Effect of lead on blood pressure and lipid metabolism. *Med Pr* 2007;58:475-83.
38. Pepersack T, Rotsaert P, Benoit F, et al. Prevalence of zinc deficiency and its clinical relevance among hospitalised elderly. *Arch Gerontol Geriat* 2001;33:243-53.
39. Little PJ, Bhattacharya R, Moreyra AE, Korichneva IL. Zinc and cardiovascular disease. *Nutrition* 2010;26:1050-7.
40. Simonsen LO, Harbak H, Bennekou P. Cobalt metabolism and toxicology — a brief update. *Sci Total Environ* 2012;432:210-5.