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Variation of Serum Trace Element Levels in Patients with Hypertension, Acute Coronary Syndrome and Heart Failure: A Pilot Study

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Abstract

Cardiovascular diseases (CVD) contribute to significant mortality and morbidity where trace elements can affect the pathogenesis of CVD. We did a case-control study at the Teaching Hospital, Peradeniya, from January 2017 to August 2017. A trained interviewer was used to collect the patient information and the blood samples; analysis was carried out at the Geochemical Laboratory, Faculty of Science, the University of Peradeniya. Seventy-six individuals were included in the study consisting of 21 hypertension (HTN), 11 acute coronary syndromes (ACS), 20 heart failure (HF), and 24 controls. In HTN patients, Cu, Cd, and Pb showed a high serum level, and V, Cr, Mn, Fe, Ni, Zn, Sr, Ba, and Tl showed a low serum level compared to the control group ($p<0.050$). In ACS patients Al, As, Cd, and Tl showed a high serum level, and V, Mn, Fe, Zn, Rb, Sr, and Ba showed a low serum level compared to the control group ($p<0.050$). In HF patients Al, As, Cd, and Tl showed a significantly high serum level, and V, Mn, Zn, Sr, Ba, and Pb, showed a low serum level compared to the control group ($p<0.050$). Multivariate apportionment of the elements using cluster analyses showed mutual solid clusters among different elements considering patient groups separately. This study concludes that elements in the human body show a heterogeneous variation throughout CVD. Further large-scale studies are vital to establishing the cause-and-effect relationship of these elements to CVD.

Keywords: Cardiovascular diseases, Serum elements, Hypertension, Acute coronary syndrome, Heart failure

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of death globally [1]. Hypertension (HTN), acute coronary syndrome (ACS), and heart failure (HF) are three significant CVD contributing to significant mortality and morbidity [2,3]. HTN is characterized by persistently high blood pressure in the systemic arteries, commonly expressed as systolic and diastolic blood pressure [4]. It is a silent killer as rarely can any symptom be seen in its early stages until a severe medical crisis like heart attack, stroke, or chronic kidney disease[5]. ACS refers to a spectrum of diseases in which blood flow to the heart is decreased, which include ST-elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI), and unstable angina (UA) [6]. HF is a clinical syndrome caused by structural and functional defects in the

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myocardium resulting in impairment of ventricular filling or the ejection of blood [7].

According to Frieden's categorical classification of elements, twenty-nine elements present in the human body have been classified into five major groups[8]. Group I includes essential components of macromolecules such as carbohydrates, proteins, and lipids. The daily requirement of these macroelements for an adult person is above 100 mg/day. Examples include carbon (C), hydrogen (H), oxygen (O), and nitrogen (N). Group II includes nutritionally essential minerals, also referred to as macroelements, namely sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphorous (P), magnesium (Mg), and sulfur (S). Group III includes essential trace elements, and Group IV includes additional trace elements. An element is considered a trace element when its daily requirement is below 100 mg. Group V includes metals that are not essential, and their functions are unknown [8,9]. Essential trace elements of the human body include zinc (Zn), copper (Cu), selenium (Se), chromium (Cr), cobalt (Co), iodine (I), manganese (Mn), and molybdenum (Mo). Although these elements account for only 0.02% of the total body weight, they play significant roles, e.g., as active centers of enzymes or as trace bioactive substances [2]. The role of additional trace elements is unclear, and they may be essential. Examples include cadmium (Cd), nickel (Ni), silicon (Si), tin (Sn), vanadium (V), and aluminum (Al) [9].

A great majority of the trace elements, represented by metallic ions, serve chiefly as crucial components of essential enzyme systems or proteins with vital functions [10,11]. The functions of trace elements have a dual role. They are essential for stabilizing the cellular structures at normal levels, but they may stimulate alternate pathways and cause diseases in deficiency states [11]. Previous studies have demonstrated high or low serum levels of different trace elements in patients with CVD depending on the type of CVD and the trace element [2,12,13]. The relation of trace elements to CVD has also been studied using various clinical, epidemiological, and experimental data, where some studies have shown a significant correlation [2,12–16]. Cluster analysis of some studies has demonstrated mutual solid clusters among some serum trace elements sharing common sources such as dietary sources and anthropogenic activities regulated by internal body metabolism.

The main objective of this study was to identify the effect and variation of trace elements on CVD, including HF and ACS, and to identify common or specific trace elements for these diseases. Moreover, redox-active elements (e.g., Cu and Fe) and elements with accumulative property (e.g., Ca and Cd) shared common clusters with the essential minerals, thus indicating their critical role in the development of coronary artery disease [17]. Therefore, the detection and the interpretation of changes in tissue trace element concentrations that occur in association with CVD undoubtedly adds to our knowledge of the etiology and pathogenesis of these diseases, may open new avenues to prevention and therapy, and may supply new tools for diagnostic and prognostic evaluations [14].

MATERIALS AND METHODS

Study Design and the Participants

This case-control study was carried out to identify the variations of elements in human blood serum using patients with CVD and a healthy control group at the Teaching Hospital, Peradeniya, from January 2017 to August 2017. Sample processing and analysis were carried out in the Geochemical Laboratory, Faculty of Science, University of Peradeniya.

Ethical Clearance

Ethical clearance was obtained from the Ethical Review Committee, Faculty of Medicine, University of Peradeniya (Ethical clearance number. 2016/EC/19).

Patient Characteristics

The personal characteristics, including the name, age, contact details, and the clinical history to confirm HTN, ACS, or HF, were obtained by a trained interviewer from the participants according to a prevalidated structured datasheet.

Sample Collection and Sample Preparation

Separated serum was used to analyze trace elements using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The blood samples were collected into plain gel tubes and were allowed to clot. After completely clotted, they were centrifuged at 3000 rpm for 7 minutes.

Sample Processing

5ml of volume plain tubes were measured weight without blood serum sample before sampling. Then, the stored blood serum samples were collected randomly from a freezer (-80°C) at the Clinical Research Laboratory, Department of Medicine, Faculty of Medicine, University of Peradeniya. After randomly selected samples were allowed to thaw under ambient temperature, a sample volume of 200 µL blood serum samples was collected to a plain tube using a micropipette. Finally, the weight of the plain tube was measured with a serum sample.

Samples were digested, adding 1ml of (35%) hydrogen peroxide, and kept 24 hours at room temperature. Then 1ml of (≥69%) nitric acid was added to each serum sample. Finally, three (3ml) of deionized water was added to the sample and kept for 24 hours for digestion. Then the samples were filtered using 0.45µm (Whatman) filter papers. The filtrate was then mixed thoroughly using a vortex mixture before analyzing trace metals using ICP-MS.

Chemicals and Reagents

Analytical reagent grade chemicals and deionized water (< 0.055 Scm−1) prepared all solutions. Nitric acid (≥69.0% TraceSelect; Fluka, Switzerland) and hydrogen peroxide (35 wt. %; Sigma-Aldrich, Germany) were used to digest blood serum samples. Working standard solutions were prepared from intermediate standard solutions of each metal. The 10 mg/L intermediate standard solutions concentration was prepared by diluting the stock standard solution (ICP-MS-CAL2-1, ICP-MS Calibration Standard 2, ACCUStandard®, USA) (1000 mg/L) in 2% nitric acid. All the working standard solutions were stored in polypropylene bottles.

ICP-MS Analysis

Elemental analysis was performed using Inductively Coupled Plasma Mass spectrometry (ICP-MS, ICapQ, Thermo Fisher, Bremen, Germany) using standard operational conditions (Table 1). The instrument was optimized with a solution containing Ba, Bi, Ce, Co, In, Li, and U for sensitivity, resolution, and mass calibration. Polyatomic isobaric interferences were mitigated with the He collision/reaction cell. Elemental levels were quantified concerning mixed standard stock solutions, while ^{75}Re and ^{103}Rh were used as the internal calibration standards (10 g L^{-1}). Dissolved concentrations of Al, As, Ba, Be, Bi, Cd, Cs, Cr, Co, Cu, Ga, In, Fe, Pb, Li, Mn, Ni, Rb, Se, Ag, Sr, Tl, U, V, and Zn of human blood were analyzed.

Table 1: ICP-MS Instrumental Parameters used for Elemental Analysis

Parameter	Value
Plasma Power	1550W
Cool gas flow	14 L min^{-1}
Auxiliary gas flow	0.65 L min^{-1}
Nebulizer gas flow	1.03 L min^{-1}
Sample/Skimmer	Material: Nickel
Spray Chamber Temperature	27 °C
Dwell time	10 ms (40ms for Se, As)
Result Standard Deviation (RSD) (3 replicates) typical	<1%
Number of Sweeps	70
Sample flow	0.4 mL min^{-1}
Limit of Detection (LOD) typical	<0.1 ppb

All experiments were done at least in triplicates, and mean values were used for calculations. For the statistical interpretations, concentrations less than the limit of detection (LOD) are allocated a value of half LOD, and Arithmetic Mean (AM) and Arithmetic Standard Deviation (ASD) were taken as representative parameters of the distribution.

Statistical Analysis

The statistical analysis was carried out using the IBM SPSS Statistics 26.0 version for Windows. Descriptive analysis and mean comparison by independent samples t-test were used to compare the data from the control group with the cases group. ANOVA test was used to compare means between different categories of the cases group. All the tests were carried out at a 5% significant level. Multivariate analysis in cluster analysis (CA) was carried out to apportion the elements in the blood samples of the patients and controls.CA involves grouping the variables into clusters, each of which is a combination of objects having similar characteristics, thus resulting in internal homogeneity and external heterogeneity. CA aims to discover a system of organizing observations where variables share properties in common. We used the hierarchical clustering method, which is done at several stages. Initially, every single element is considered and paired as clusters of two. In the subsequent stages, the method joined the two clusters that are closest together. We used the complete linkage (furthest member) method for subsequent clustering, where the distance between the cluster was defined by the furthest related members of the two groups. Dendrograms demonstrated the clusters of elements where the reduced rescaled distance illustrated a stronger association between the elements/groups. Therefore, it was cognitively easier to predict mutual properties based on overall group membership.

RESULTS

Baseline characteristics

A total of 76 individuals were included in the study consisting of 52 cases and 24 controls. The cases included 21 patients with HTN (8 male, 13 female), 11 patients with ACS (5 male, 6 female), and 20 patients with HF (14 male, 6 female). The control group had 24 healthy individuals (11 male, 13 female). The mean age of the patients with HTN, ACS, and HF were 64.86±7.89, 63.09±11.91, and 67.15±9.78, respectively. The mean age of the control group was 49.79±11.71.

Serum levels of elements in patients with HTN

Table 2 compares the serum levels of elements between the hypertensive patients and the control group. The elements; V, Cr, Mn, Fe, Ni, Zn, strontium (Sr), barium (Ba), and thallium (Tl) showed a significantly low serum level compared to the control group ($p<0.050$). Cu, Cd, and Pb showed a significantly high serum level in patients with HTN compared to the control group ($p<0.050$).

Table 2: Mean serum levels of elements in patients with HTN compared to the control group

Elements (mean, SD)	Control (n=24)	HTN (n=21)	p-value
Na (mg/L)	2327.18 (60.03)	5197.69 (1208.36)	**≤0.001**
Mg (mg/L)	25.23 (12.66)	41.35 (10.59)	**≤0.001**
K (mg/L)	141.94 (47.14)	279.02 (61.91)	**≤0.001**
Ca (mg/L)	529.86 (616.17)	288.12 (133.50)	0.086
Al (µg/L)	2027.26 (2024.98)	2691.49 (1201.82)	0.196
V (µg/L)	15.06 (12.84)	8.46 (2.21)	**0.025**
Cr (µg/L)	270.98 (287.59)	130.00 (46.44)	**0.032**
Mn (µg/L)	200.14 (197.68)	98.06 (34.36)	**0.024**
Fe (µg/L)	4757.65 (2346.37)	2095.31 (965.99)	**≤0.001**
Co (µg/L)	4.78 (3.92)	3.58 (1.58)	0.197
Ni (µg/L)	157.53 (137.58)	86.78 (34.13)	**0.027**
Cu (µg/L)	1022.38 (562.49)	1461.38 (662.94)	**0.022**

Zn (µg/L)	21885.00 (28882.89)	6548.86 (5265.04)	**0.021**
As (µg/L)	5.06 (4.22)	4.56 (2.29)	0.634
Se (µg/L)	63.64 (30.73)	71.22 (23.05)	0.351
Rb (µg/L)	511.59 (301.80)	536.69 (133.80)	0.727
Sr (µg/L)	477.65 (505.13)	223.87 (74.39)	**0.028**
Cd (µg/L)	2.09 (2.05)	9.71 (7.83)	**≤0.001**
Ba (µg/L)	10200.44 (14676.91)	292.80 (78.80)	**0.004**
Tl (µg/L)	0.360 (0.234)	0.077 (0.067)	**≤0.001**
Pb (µg/L)	77.08 (68.03)	828.18 (597.37)	**≤0.001**

Serum levels of elements in patients with ACS

Table 3 compares the serum levels of elements between ACS patients and the control group. The elements; Al, arsenic (As), Cd, and Tl showed a significantly high serum level in patients with ACS compared to the control group (p<0.050). The elements; V, Mn, Fe, Zn, rubidium (Rb), Sr, and Ba, showed a significantly low serum level compared to the control group (p<0.050).

Table 3: Mean serum levels of elements in patients with ACS compared to the control group

Elements (mean, SD)	Control (n=24)	ACS (n=11)	p-value
Al (µg/L)	2027.2 (2024.9)	6423.8 (430.7)	**≤0.001**
V (µg/L)	15.0 (12.8)	3.9 (0.8)	**0.008**
Cr (µg/L)	270.9 (287.5)	146.3 (26.9)	0.164
Mn (µg/L)	200.1 (197.6)	54.6 (52.0)	**0.002**
Fe (µg/L)	4757.6 (2346.3)	2074.5 (799.2)	**≤0.001**
Co (µg/L)	4.7 (3.9)	6.8 (1.7)	0.112
Ni (µg/L)	157.5 (137.5)	129.7 (50.4)	0.523
Cu (µg/L)	1022.3 (562.4)	998.9 (162.1)	0.894
Zn (µg/L)	21885.0 (28882.8)	713.1 (789.1)	**0.022**
As (µg/L)	5.0 (4.2)	8.8 (2.1)	**0.009**
Se (µg/L)	63.6 (30.7)	51.7 (13.3)	0.230
Rb (µg/L)	511.5 (301.8)	281.6 (90.8)	**0.019**
Sr (µg/L)	477.6 (505.1)	58.8 (20.7)	**0.010**
Cd (µg/L)	2.0 (2.0)	4.9 (2.4)	**0.003**
Ba (µg/L)	10200.4 (14676.9)	78.1 (37.9)	**0.030**
Tl (µg/L)	0.3 (0.2)	2.4 (0.3)	**≤0.001**
Pb (µg/L)	77.0 (68.0)	66.7 (63.0)	0.665

Serum levels of elements in patients with HF

Table 4 compares the serum levels of elements between the HF patients and the control group. The elements; Al, As, Cd, and Tl showed a significantly high serum level in patients with HF compared to the control group (p<0.050). The elements; V, Mn, Zn, Sr, Ba, and Pb, showed a significantly low serum level compared to the control group (p<0.050).

Table 4: Mean serum levels of elements in a patient with HF compared to the control group

Elements (mean, SD)	Control (n=24)	HF (n=20)	p-value
Al (µg/L)	2027.2 (2024.9)	7712.0 (553.0)	**≤0.001**
V (µg/L)	15.0 (12.8)	5.9 (0.5)	**0.003**
Cr (µg/L)	270.9 (287.5)	182.7 (10.5)	0.178
Mn (µg/L)	200.1 (197.6)	51.3 (5.7)	**0.002**
Fe (µg/L)	4757.6 (2346.3)	4407.2 (1552.4)	0.571

Co (µg/L)	4.7 (3.9)	5.7 (1.5)	0.317
Ni (µg/L)	157.5 (137.5)	151.5 (8.1)	0.847
Cu (µg/L)	1022.3 (562.4)	1142.5 (151.2)	0.360
Zn (µg/L)	21885.0 (28882.8)	614.5 (232.6)	**0.002**
As (µg/L)	5.0 (4.2)	23.7 (1.8)	**≤0.001**
Se (µg/L)	63.6 (30.7)	61.4 (23.2)	0.790
Rb (µg/L)	511.5 (301.8)	266.0 (159.3)	0.059
Sr (µg/L)	477.6 (505.1)	60.6 (16.7)	**≤0.001**
Cd (µg/L)	2.0 (2.0)	3.4 (1.2)	**0.014**
Ba (µg/L)	10200.4 (14676.9)	94.8 (30.6)	**0.004**
Tl (µg/L)	0.3 (0.2)	5.2 (0.5)	**≤0.001**
Pb (µg/L)	77.0 (68.0)	40.9 (41.9)	**0.037**

Serum levels of elements in patients with CVD

Table 5 compares the serum levels of elements between patients with CVD taking HTN, ACS, and HF patients as one group. Serum Al, As, Cd, Tl, and Pb levels were significantly higher in patients with CVD than in the control group ($p<0.050$). Serum V, Cr, Mn, Fe, Zn, Sr, and Ba levels were significantly lower in patients with CVD than in the control group ($p<0.050$).

Table 5: Mean serum levels of elements in a patient with CVD compared to the control group

Elements (mean, SD)	Control (n=24)	CVD (n=53)	p-value
Al (µg/L)	2027.2 (2024.9)	5412.0 (2461.6)	**≤0.001**
V (µg/L)	15.0 (12.8)	6.5 (2.3)	**≤0.001**
Cr (µg/L)	270.9 (287.5)	153.7 (40.0)	**0.005**
Mn (µg/L)	200.1 (197.6)	70.9 (38.9)	**≤0.001**
Fe (µg/L)	4757.6 (2346.3)	2980.1 (1639.2)	**0.002**
Co (µg/L)	4.7 (3.9)	5.0 (2.0)	0.654
Ni (µg/L)	157.5 (137.5)	120.7 (42.9)	0.082
Cu (µg/L)	1022.3 (562.4)	1240.9 (472.0)	0.082
Zn (µg/L)	21885.0 (28882.8)	3031.9 (4422.3)	**≤0.001**
As (µg/L)	5.0 (4.2)	12.8 (9.0)	**≤0.001**
Se (µg/L)	63.6 (30.7)	63.3 (22.3)	0.963
Rb (µg/L)	511.5 (301.8)	417.1 (170.1)	0.086
Sr (µg/L)	477.6 (505.1)	126.1 (94.6)	**≤0.001**
Cd (µg/L)	2.0 (2.0)	6.2 (5.8)	**≤0.001**
Ba (µg/L)	10200.4 (14676.9)	171.2 (115.3)	**≤0.001**
Tl (µg/L)	0.3 (0.2)	2.5 (2.3)	**≤0.001**
Pb (µg/L)	77.0 (68.0)	364.3 (538.6)	**0.011**

Multivariate apportionment

Another critical aspect of the present study was the multivariate apportionment of the elements using cluster analyses. The dendrogram of the elements in blood samples of HTN patients is shown in Figure 1, which showed mutual solid clusters among Ca-Sr-Zn-Tl, Cd-Pb, Mn-Ni-Ba, V-Cr-Al-Fe, and K-Rb-Na-Mg. The dendrogram of the elements in blood samples of ACS patients is shown in Figure 2, which showed mutual solid clusters among Ca-Zn-Pb-Cr-Ni-Cd-V-Sr, Mg-Co, Ba-Tl-Se, Al-As, Mn-Cu, and Fe-Rb-K-Na. The dendrogram of the elements in blood samples of HF patients is shown in Figure 3, which showed mutual solid clusters among K-Rb-Na-Al-Tl, Mn-Pb-Co, Cr-Ni-As-V-Ba, Cu-Se-Fe, and Ca-Zn-Mg-Sr-Cd. The dendrogram of the elements in blood samples of normal healthy individuals is shown in Figure 4, which showed mutual solid clusters among Sr-Ba-Ca-Zn-Fe-Cu-Al-V-Co-Ni-Se-Tl, Na-Mg-K-Rb, and Cr-As.

DISCUSSION

Several animal studies have demonstrated that the pathogenesis of HTN is associated with serum levels of the elements; Cd, Pb, Hg, and Na, and Cu, Mg, K, and Se being depressors [2,14,15,18][2,14,15,17][2,14,15,17]. Mg deficiency, especially in patients with alcoholism, may contribute to HTN [19,20][18,19][18,19]. Cu, Zn, and Se deficiency have also been predisposed to HTN [21,22][20,21][20,21]. Asaolu et al. have shown higher values of Na, Fe, the toxic elements such as Pb, Co, Al, As, Cd, and Cr, and lower levels of K, Mg, Mn, Cu, and Ca for hypertensive patients in comparison with normal healthy controls[23][22][22]. Another study has found a higher serum level of Se in HTN patients [24][23][23]. According to our findings, Cu, Cd, and Pb serum levels were significantly higher in patients with HTN than in the control group ($p<0.050$), while V, Cr, Mn, Fe, Ni, Zn, Sr, Ba, and Tl serum levels showing a significantly low serum level compared to the control group ($p<0.050$). Therefore this study confirms the association of higher concentrations of Cd and Pb and lower concentrations of Zn and Mn in HTN patients. However, our findings show contradictory results for Cu, where they showed higher serum concentrations, and Fe showed a lower serum concentration in HTN patients giving contradictory results from the previous studies. At the same time, our results did not show any statistically significant difference in the serum concentrations of Al, Co, As, Se, and Rb in patients with HTN and the control group. Additionally, we have demonstrated low serum levels of V, Cr, Ni, Sr, Ba, and Tl in HTN, which have not been studied before. Several studies show marked disparities in the distribution of essential and toxic elements in the blood of ACS patients compared with healthy subjects [2,17,25,26][2,24–26][2,24–26]. Significantly higher Pb, Cr, Cu, Fe, Ca, and Mn concentrations have been observed in ACS patients' blood than in control groups [17][24][24]. Altekin et al. has shown increased serum levels of Cu and Fe and the decrease in serum levels of Zn and Se in patients with higher levels of cardiac biomarkers, implying the relationship of trace element levels with the degree of myocardial damage and thus may play a role in the pathogenesis of ischemic heart disease[25]. These findings on Se have been confirmed by Lubos et al., where a low Se concentration had been associated with future cardiovascular death in patients with ACS[27]. Similarly, recent studies have indicated that Se exerts a beneficial effect on coronary disease mortality and that selenium plus garlic produces significant anticancer activity[2]. At the same time, low serum Zn concentration is strongly associated with the etiopathogenesis of ACS[26,28]. Using a stunned heart model, Powell et al. demonstrated the zinc's protective effects in the isolated ischemic rat heart [29]. Anonna et al. demonstrated a contradictory finding regarding Cu from the previous studies where reduced concentration Cu has been found to have a role in the gradual development of ischaemic heart diseases. They have also suggested that depleted serum levels of Zn and Cu and elevated Fe and Mn levels may provide a prognostic indicator for managing the disease[28]. Our study demonstrated a high serum level of Al, As, Cd, and Tl in patients with ACS compared to the control group ($p<0.050$), and a low serum level of V, Mn, Fe, Zn, Rb, Sr, and Ba showed a significantly low serum level compared to the control group ($p<0.050$). Our findings confirmed the results of previous studies that low serum concentration of Zn in patients with ACS. However, we found a low serum concentration of Fe in ACS patients, contradicting the previous studies' findings. At the same time, we did not find any statistically significant difference in the serum concentrations of Pb, Cr, Cu, and Se in ACS patients compared to the control group ($p>0.050$), nullifying some findings from the previous studies.

The accumulation of trace elements has been found in idiopathic dilated cardiomyopathy (IDCM) but not in specific cardiomyopathies. This abnormality is limited to the myocardium and correlates with the severity of both HF and electrical instability[30]. Kosar et al. have shown that heart failure is associated with lower Se and Zn concentrations and higher Cu concentration, and serum Se, Zn, and Cu element profiles were similar in IDCM and ischaemic cardiomyopathy [31]. At the same time, a significant relationship has been demonstrated between N-terminal pro-B type natriuretic peptide (NT-proBNP) levels, whose elevated levels are considered to be associated with poor prognosis in patients with an ischemic

origin, increased serum Cu level and Cu / Zn ratio, and low serum Zn level[32]. Similarly, a meta-analysis also has concluded a significant association between low serum Zn levels and HF[33]. Serum Cu concentrations are higher in patients with severe heart failure than those with mild to moderate cardiac insufficiency and correlate with acute-phase protein concentrations [25]. An association of HF with serum Se concentration can be found concerning the Keshan disease, which manifests as fatigue after even mild exercise, cardiac arrhythmia and palpitations, loss of appetite, cardiac insufficiency, cardiomegaly, and congestive heart failure. The disease was prevalent among people under a selenium-deficient diet, who showed a rapid improvement after fortification[9,11]. Our study confirmed the previous findings on Zn, where we found a significantly low serum level of Zn in HF patients compared to the control group. However, the serum Cu and Se levels did not show any statistically significant difference in patients with HF with the control group ($p>0.050$). The Manchester syndrome was ascribed to beer contamination by arsenic, or, at least, to the enhancement of the toxic effect of arsenic by the alcoholic vehicle as it appears that arsenic may cause myocardial necrosis both in animals in men[14]. Our study also found an increased concentration of serum As in HF patients supporting the above findings ($p<0.050$). Serum Mg concentration is associated with HF, where hypomagnesemia is present in <10% of patients with mild to moderate heart failure but is more common in severe congestive heart failure. Hypomagnesaemia has been shown to increase the frequency of arrhythmias in heart failure but is not associated with increased mortality[19][18][18]. The association between serum Fe can be suggested concerning iron deficiency anemia, leading to heart failure[11]. However, our results did not show any significant difference in serum Fe concentrations in patients with HF compared to the control group ($p>0.050$). A study on eight patients who died of uremic heart failure has found significantly increased concentrations of 10 elements; Co, As, Br, Ce, La, Sb, Sc, Fe, and P compared to a control group of non-uremic individuals. As some of these elements are cardiotoxic, supporting the opinion that an excess of certain trace elements, Co, in particular, may be etiological agents of importance in uremic heart failure[34]. In total HF patients, our study found an increased serum level of Al, As, Cd, and Tl and a decreased serum level of V, Mn, Zn, Sr, Ba, and Pb compared to the control group ($p<0.050$).

Aluminum: Granadillo et al. have demonstrated that plasma A1 is elevated in hypertensive individuals, suggesting the possible influence of this metal in the appearance of arterial HTN [35]. However, our patients with HTN did not show an elevated Al level compared to the control group, but ACS and HF patients did. Nevertheless, studies on serum Al levels in patients with ACS and HF are limited in the medical literature.

Vanadium: Vanadium is a probable essential trace element known to have an anti-atherosclerotic action, as demonstrated by reducing the cholesterol level in plasma and the aorta in rabbits and healthy human subjects. This anti-atherosclerotic action appears to be due to interference with cholesterol synthesis by inhibiting utilization of mevalonic acid and acceleration of cholesterol catabolism. It may be interesting that hard water, whose consumption is statistically associated with a lower incidence of cardiovascular diseases in many world areas, contains vanadium, whereas soft waters do not [11,14,36,37]. Also, Schroeder (1966) found a significant negative correlation between the vanadium content of municipal waters and atherosclerotic-heart death rates [14]. A high dose of vanadium blocks cholesterol synthesis and reduces phospholipid and cholesterol in the blood [36]. At the same time, some studies have assumed that several elements are suspected of being involved in the genesis of arteriosclerotic heart disease, including vanadium [16]. According to our findings, all three groups of patients, HTN, ACS, and HF, showed a low serum level of V compared to the control group. This result supports the previous finding that vanadium has an anti-atherosclerotic action, thereby a cardiovascular protective effect.

Chromium: Epidemiological data on chromium intake and CVD risk are limited. However, studies suggest that increased chromium intake may be necessary for diabetes and CVD prevention [38]. The European Community Multicenter Study on

Antioxidants, Myocardial Infarction, and Breast Cancer (EURAMIC) have found that the toenail chromium concentration is inversely associated with the risk of a first myocardial infarction in men. Nevertheless, the mechanism by which chromium may decrease CVD incidence is not known [38]. Nevertheless, in our study, the serum Cr concentration of ACS and HF patients compared to the control group did not show any significant difference. However, HTN patients did show a significantly low level of Cr compared to the control group. Epidemiologic studies suggest that tissue levels of chromium are reduced among diabetic individuals, especially in those with existing CVD, compared with healthy control subjects [39]. Anyhow, A meta-analysis of randomized control trials did not show the beneficial effects of chromium supplementation on blood pressure in adults [40]. Another study on rats has concluded that administering amlodipine may increase chromium accumulation in the internal organs, increase the antioxidant status, and suppress cells' inflammatory response [41].

Manganese: Mn is essential for health in trace amounts but toxic at higher levels. A few reports in the literature examine the effects of excess oral exposure of humans to Mn [42]. Although it remains inadequate, animal and human evidence support the view that Mn exposure significantly alters cardiovascular function despite the lack of epidemiological data on cardiovascular morbidity and mortality. Mn exposure produces abnormal electrocardiogram and inhibits myocardial contraction. Concerning vascular function, Mn exposure dilates the blood vessel and induces hypotension [43]. The daily manganese intake of normotensive adults has shown a significant negative correlation with systolic blood pressure, indicating a positive effect of manganese on blood pressure [44]. Also, urinary manganese may play some role in blood pressure and protecting against HTN [45]. In this study, we found a significantly low level of Mn in HTN, ACS, and HF patients compared to the control group.

Interestingly, previous studies have also found a negative correlation between manganese concentrations and cardiovascular diseases in humans and experimental animals. The theory has explained its effect that manganese as superoxide dismutase present in the mitochondrial matrix of cardiac endothelial cells could have a protecting role against cardiovascular diseases [37]. Anonna et al. have reported a strong association of the pathogenesis of ischaemic heart diseases with depleted serum levels of Zn and Cu and elevated Fe and Mn levels, which may provide a prognostic tool for treating this concerning the disease [28].

Iron: Iron is an essential element in human nutrition, but it can be toxic. Iron deficiency, probably the most common nutritional deficiency, is believed by some authorities to affect 80% of the world's population. Low socioeconomic status, high dietary phytate, other inhibitors of iron bioavailability and low consumption of flesh foods, and chronic blood loss increase the risk of deficiency. Some studies have found lower serum Cr, Co, and Fe in hypertensive subjects compared with normotensive subjects. However, some studies disagreed with this and found higher Fe, Co, and Cr values for hypertensive patients than normal healthy controls [2,22]. This study confirmed the former where the serum Fe concentration of HTN patients was significantly lower than the control group. Iron and copper belong to the category of redox-active elements. They are vital for life and can be toxic when present in excess. The body's free redox iron and copper generate damaging reactive free radicals via Fenton chemistry. These harmful free radicals stimulate lipid peroxidation, especially low-density lipoprotein, and ultimately tissue damage. LaMarca et al. have shown that elevated iron levels produce reactive oxygen species (ROS), predisposing them to coronary disease and myocardial infarction [24,46]. Our finding contradicts this theory where ACS patients showed a reduced serum Fe concentration compared to the healthy individuals. Alterations in iron metabolism are associated with adverse outcomes in patients with coronary artery diseases, and it is a well-established fact that there is an association between chronic iron deficiency and heart failure [47,48]. However, our results showed no statistically significant difference in serum Fe concentration in HF patients compared to healthy individuals.

Cobalt: The effect of cobalt on the cardiovascular system is one of many aspects of cobalt

metabolism in humans. A study on children with essential HTN showed significantly higher systolic and diastolic blood pressure and significantly lower serum cobalt concentrations than controls [49]. Oxidative stress, inflammation, and apoptosis are pathologic mechanisms in rats' $CoCl_2$ induced HTN and cardiovascular complications [50]. However, our study did not show any significant difference in serum Co concentration in HTN, ACS, and HF patients compared to the control group. Anyhow, cobalt-related cardiomyopathy is a well-known entity reported in patients with metal implants where a delay or missed diagnosis may lead to significant morbidity and mortality, and avoidance of metal articulations in revision for fractured ceramic implants may help in management [51,52].

Nickel: Serum Ni concentration in HTN patients was significantly low compared to the control group; however, there was no significant difference in ACS and HF patients. Nevertheless, a study on hypertensive patients has found significantly higher levels of As, Cd, Ni, and Pb in the biological samples (scalp hair, blood, and urine) of hypertensive patients when related to controls of both genders [53,54]. In ACS also, it is found that the plasma level of Ni increases sharply following myocardial infarction (Masironi, 1969). Our study contradicts these findings. However, some studies have also suggested an association between nickel and carotid atherosclerosis [55].

Copper: Humans are exposed to Cu via food and drinking water in the general environment. Cu deficiency can cause heart diseases. A study using data from 6683 National Health and Nutrition Examination Survey (NHANES) participants from 2011 to 2016 has concluded no association between the serum Cu level and HTN [23]. In our study, the serum Cu concentration of HTN patients was significantly higher than healthy individuals, although ACS and HF patients did not show a significant difference. Lower serum copper was found in hypertensive with Heart Disease subjects compared with normotensive subjects. The abnormal deficiency of copper in hypertensives probably contributes to decreased lysyl oxidase and superoxide dismutase, which fail collagen and elastin cross-linking and impair defense against free radicals [2,22].

Zinc: is an essential trace element found in all food and potable water like salt or an organic complex. The principal source of zinc is usually the diet. More than 300 enzymes are dependent on zinc for catalytic, structural, regulatory, and noncatalytic functions. Zn deficiency occurs in human beings when intake is low. Lower serum of Selenium and Zn was also found in hypertensive subjects with heart disease compared with normotensive subjects [2]. Poor zinc is associated with increased coronary heart disease risk [56]. Zinc is an essential component of copper-zinc superoxide dismutase, which plays an emergent role in coronary heart disease. Zn deficiency can cause an increase in tissue oxidation damage. The antagonistic relationship between these metals is also associated with increases in Cu and Fe. Zinc may be an essential element in the cause of ischaemic heart disease due to imbalances between Cu and Zn [28]. Heart failure is associated with lower Zn concentration, and serum Se, Zn, and Cu element profiles were similar in idiopathic dilated cardiomyopathy and ischaemic cardiomyopathy [31]. Zn possesses cardiac cytoprotective qualities and supports the concept that this metal can decrease hydroxyl free-radical formation by affecting copper reactivity [29]. The results of this study support the hypothesis that Zn has a cardiovascular protective effect, that the serum Zn concentrations of HTN, ACS, and HF patients were significantly low compared to the healthy individuals.

Arsenic: Inorganic As is a naturally occurring toxic metalloid found primarily in drinking water and food, with an estimated 100 million people worldwide exposed to arsenic at levels exceeding 50 µg/L. In epidemiologic studies, high-chronic arsenic exposure has been linked to CVD, including coronary heart disease, stroke, and peripheral arterial disease [57]. A study done in Zrenjanin municipality, Serbia, has demonstrated that consumption of As above national standards were associated with a higher risk for the occurrence of the ACS and an insignificantly higher probability of a fatal outcome [58]. Thus, in our ACS and HF patients sample, the serum As concentration was significantly higher than the control group, although HTN patients did not show any significant difference. However, a systematic review has identified an association between As and the

prevalence of HTN [59]. Interpreting a causal effect of environmental arsenic on HTN is limited by the small number of studies, the presence of influential studies, and the absence of prospective evidence. Additional evidence is needed to evaluate the dose-response relationship between environmental arsenic exposure and HTN [59].

Selenium: Bastola et al. have found a positive association between serum selenium and HTN, irrespective of age or antihypertensive medications intake [23]. However, a low selenium concentration is associated with future cardiovascular death in patients with ACS [27]. Se can lead to a form of cardiomyopathy, especially in children and pregnant women, observed in the Keshan province of China due to selenium deficiencies in soil and food and the presence of some unknown factor in food [37,60]. Heart failure is associated with lower Se concentration [31]. Se deficiency is often associated with reduced activity of the enzyme glutathione peroxidase, which prevents lipid peroxidation and aggregation of blood platelets. Low platelet glutathione peroxidase activity and serum selenium concentration always seem to occur in patients with chronic renal failure, but a further reduction of both is found in those patients with additional cardiovascular complications. On the other hand, the influence of Se on cardiovascular disease is sometimes explained by the binding of cardiovascular disease stimulating cadmium through the formation of a non-toxic high-molecular cadmium-selenium-protein complex [37]. However, our study did not find any discrepancy of Se concentrations between patients with HTN, ACS, and HF compared to healthy individuals.

Rubidium: Studies on Rb related to CVD are sparse; however, the Rb level is high on heart tissues in patients who died due to CVD compared to healthy hearts [61]. In our study, the Rb concentration in ACS patients was low compared to the control group, although HTN and HF patients did not show any significant difference.

Strontium: The increased risk for cardiac events, including ACS with strontium ranelate, has been detected in randomized control trials but not in real life. Interestingly, serum Sr level was significantly low in HTN, ACS, and HF patients compared to the control group. There are no previous studies measuring serum Sr levels in CVD patients to compare these results. Strontium ranelate remains a valuable therapeutic alternative in patients with severe osteoporosis without cardiovascular contraindications who cannot take another osteoporosis treatment [62,63].

Cadmium: Soluble cadmium salts accumulate and result in toxicity to the liver, kidneys, brain, lungs, heart, testes, and the central nervous system [64]. In animal studies, lead and cadmium-induced aortic atherosclerosis have been demonstrated. In humans, the accumulated evidence strongly suggests a potential role of metals such as Cd and Pb in cardiovascular risk [55]. Our findings support this evidence where the serum Cd level in HTN, ACS, and HF patients was significantly higher than in the control group. Cd concentration has been significantly higher in scalp hair, blood, and urine samples of smokers and nonsmokers HTN patients than in referents [54]. Toxic elements high exposure may synergize with risk factors associated with hypertension [53]. Chronic exposure to lower doses of Cd gives rise to alterations in cell membranes and cytoplasm due to interactions with various enzymes. Destruction of mitochondria affects the functioning of muscle cells, for instance, in the heart leading to a reduction in coronary flow rate. Blood vessels of different types undergo morphological changes in the endothelial cells, changes in the elastic lamellae and the muscular layers of the media, and increased frequency of plaques formation. In the heart, the most important effect of low cadmium dose seems to be enlargement of a part of the fissure of the intercalated disks, the sites of connections between the heart muscle cells [37].

Barium: Chronic effects of barium on the cardiovascular system are unclear. An occupational study on barium workers exposed to other chemicals found a higher incidence of elevated blood pressure in the barium-exposed group. However, studies in populations exposed to drinking water found no significant differences in blood pressure, heart disease, stroke, kidney disease, or lung disease [55]. The high barium communities have significantly higher death rates for ‘all cardiovascular diseases’ and ‘heart disease’

than the low barium communities [65]. A study on rats revealed that rats exposed to 100 ppm Ba for 16 months exhibited depressed rates of cardiac contraction and depressed electrical excitability in the heart. Hearts from these maximally exposed rats also had significantly lower ATP content and phosphorylation potential. Although the barium-induced increase in the blood pressure of rats was modest, comparable mild HTN in humans would have significant health implications [66]. However, in our sample, patients with HTN, ACS, and HF showed a significantly low serum Ba concentration compared to the control group.

Thallium: The toxicological action of several heavy metal ions, including Cd, Pb, Hg, and Tl, can cause HTN by affecting hormone metabolism, vasoconstriction, and renal tubular function [2]. Serum Tl level has not been a popular focus for studied previously. In this study, the Tl concentration in HTN and ACS patients was significantly low, while it was significantly high in HF patients.

Lead: Pb occurs in ore and soil as both inorganic and organic compounds with different types of toxicity to humans. Pb has been used in paint, ceramics, and in-home utensils. The primary exposure route is via food and drinking water [2]. The high serum concentration of both toxic elements Pb and Cd was observed in hypertensive Heart Disease patients [2]. There has been enough evidence to infer a causal relationship of Pb exposure with HTN [67–69]. Specifically, low-level Pb exposure increases blood pressure and may increase the risk of HTN, though some studies do not show any significant association between the serum Pb level and HTN [70,71]. Our study has reproduced this result where HTN patients had a significantly high serum concentration of Pb compared to healthy subjects, although ACS patients did not show any statistically significant difference. Some studies have demonstrated a higher Pb level in ACS patients than normal ones [2]. However, some studies have found that Pb is not associated with coronary heart disease incidence in univariate and multivariate analyses [72]. Serum Pb level has been associated with left ventricular hypertrophy [73]. Interestingly in our sample, HF patients showed a significantly low level of Pb compared to the control group.

Multivariate apportionment

Multivariate apportionment using cluster analyses was for the HTN, ACS, HF, and the control group separately, and the dendrograms for each group are shown in Figure 1-4. The dendrogram for HTN patients showed mutual solid clusters among Ca-Sr-Zn-Tl, Cd-Pb, Mn-Ni-Ba, V-Cr-Al-Fe, and K-Rb-Na-Mg. The dendrogram for ACS patients showed mutual solid clusters among Ca-Zn-Pb-Cr-Ni-Cd-V-Sr, Mg-Co, Ba-Tl-Se, Al-As, Mn-Cu, and Fe-Rb-K-Na. The dendrogram for HF patients showed mutual solid clusters among K-Rb-Na-Al-Tl, Mn-Pb-Co, Cr-Ni-As-V-Ba, Cu-Se-Fe, and Ca-Zn-Mg-Sr-Cd. The dendrogram for ordinarily healthy individuals showed mutual solid clusters among Sr-Ba-Ca-Zn-Fe-Cu-Al-V-Co-Ni-Se-Tl, Na-Mg-K-Rb, and Cr-As. Na and K have been in the same cluster, being the two most common cations in the blood [74]. In normal healthy individuals, many trace elements of dietary origin have been clustered into one, including Sr, Ba, Fe, Cu, Al, V, Co, Ni, Se, and Tl. However, Cr and As are separated from other trace elements in the same cluster. The source of Cr to the human body is mainly via foods and As, found in soil, sediments, and groundwater [75,76]. This indicates the possibility of food contamination of As. This is further supported by As, Cr, Ni, V, and Ba being in the same cluster in HF patients. A cluster of Cd and Pb is found in HTN patients, wherein ACS patients are also in the same cluster. Both Cd and Pb accumulate in the human body by contaminated foods with industrial materials and occupational exposure [77,78]. This supports the hypothesis that occupational exposure of Pb and Cd may have a causal effect on HTN.

One drawback of this study is that we analyzed the serum samples of these patients for serum element levels after storing them for a few months in the refrigerator under – 80 ˚C, unlike in clinical practice, the samples are analyzed within a short period. Therefore, the resulting element levels are not comparable to the reference ranges of the particular elements. However, since all the samples are treated with the same conditions, it is possible to compare the serum element levels between the given groups.

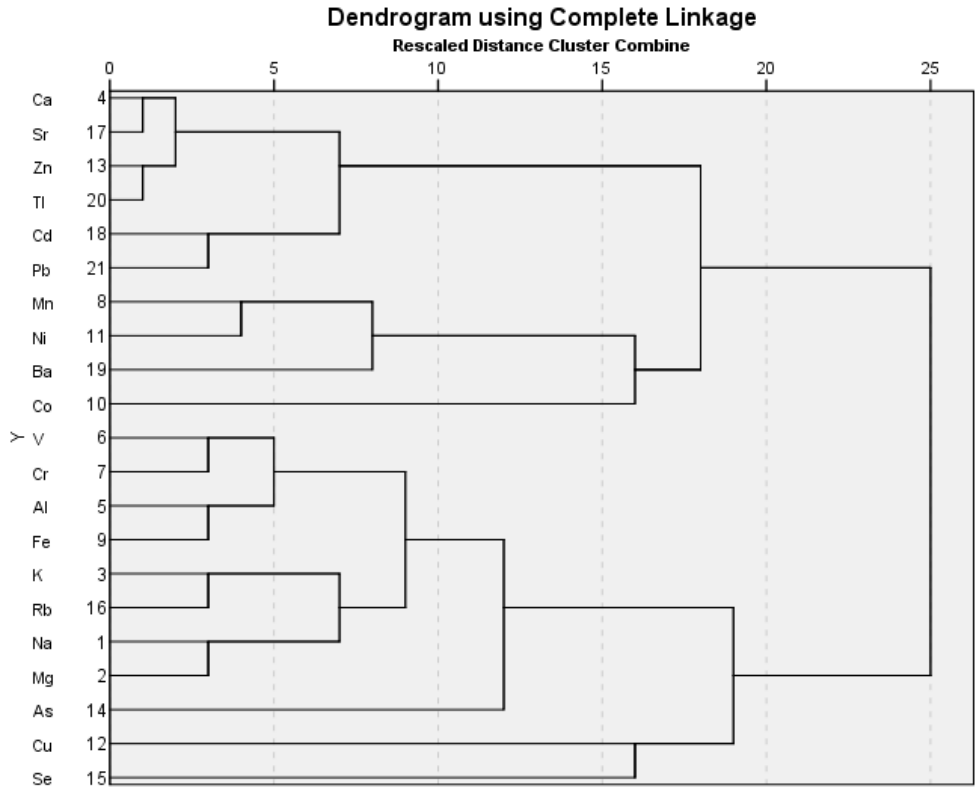

Figure 1. Cluster analyses of selected elements in blood of HTN patients

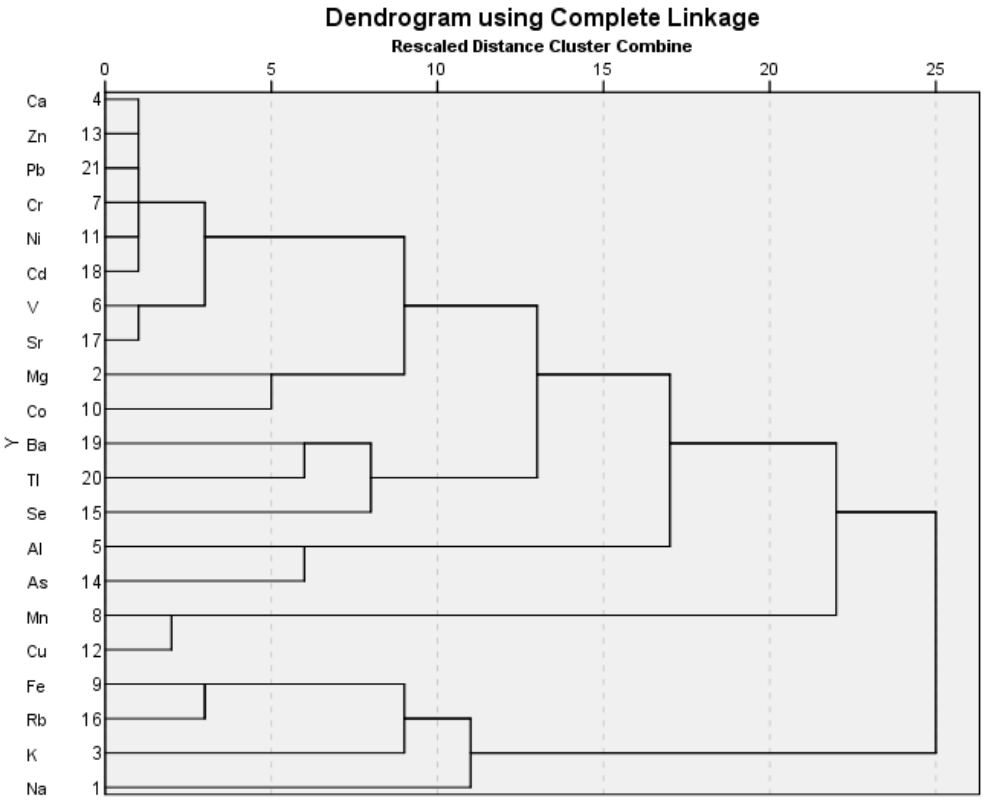

Figure 2. Cluster analyses of selected elements in blood of ACS patients

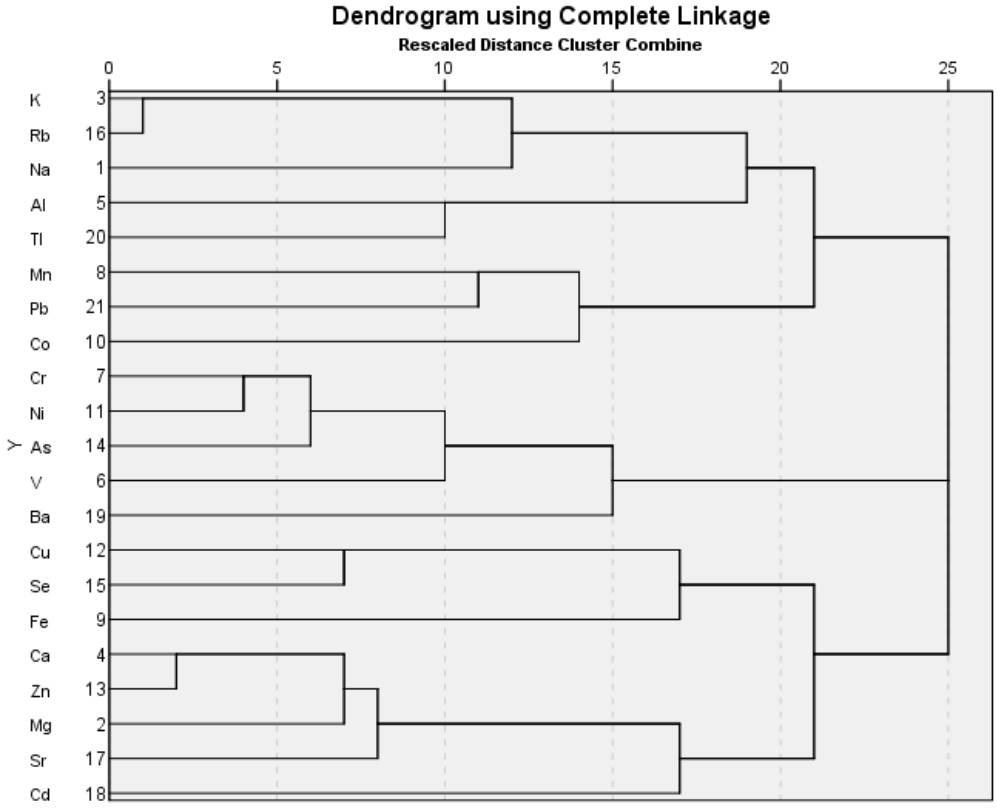

Figure 3. Cluster analyses of selected elements in blood of HF patients

CONCLUSION

Elements in the human body, either macroelements or trace elements, show a heterogeneous variation throughout CVD, including HTN, ACS, and HF. This is evident by our results, where the serum element concentrations were varied in CVD compared to the healthy individuals. HTN patients showed higher Cu, Cd, and Pb serum levels and lowered V, Cr, Mn, Fe, Ni, Zn, Sr, Ba, and Tl serum levels compared to the control group. ACS patients showed a high serum level of Al, As, Cd, and Tl and a low serum level of V, Mn, Fe, Zn, Rb, Sr, and Ba compared to the control group. HF patients showed a high serum level of Al, As, Cd, and Tl and a low serum level of V, Mn, Zn, Sr, Ba, and Pb compared to the control group. Cluster analysis of these elements supports the hypothesis that some of the elements' exposure to food contamination and occupational exposure may contribute to their presence in the human body. Our evidence suggests that these elements may affect the pathogenesis of HTN, ACS, and HF. However, further large-scale studies are vital in this area to establish these elements' cause and effect relationship to CVD, which may contribute to disease prevention strategies.

Author declaration

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Conflicts of Interest:
The authors declare that there is no conflict of interest regarding the publication of this paper.

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Ethics Approval and Consent to Participate:
Ethics approval was granted from the Institutional Ethical Review Committee (IERC) of the Faculty of Medicine, University of Peradeniya. Informed signed consent was obtained from all participants before the collection of data.

Consent for Publication:
All participants gave written consent for publication before data collection.

Data Availability:
All the data are stored in a password-protected computer, and hard copies are also under the corresponding author's custody. The data used to support the findings of this study are available from the corresponding author upon request.

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