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Journal of Hazardous Materials 153 (2008) 1215–1221
Journal of Hazardous Materials
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Statistical analysis of trace metals in the plasma of cancer patients versus controls

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Received 24 February 2007; received in revised form 22 September 2007; accepted 24 September 2007
Available online 3 October 2007

Abstract

The plasma of cancer patients ($n = 112$) and controls ($n = 118$) were analysed for selected trace metals (Al, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Sr and Zn) by flame atomic absorption spectroscopy. In the plasma of cancer patients, mean concentrations of macronutrients/essential metals, Na, K, Ca, Mg, Fe and Zn were 3971, 178, 44.1, 7.59, 4.38 and 3.90 ppm, respectively, while the mean metal levels in the plasma of controls were 3844, 151, 74.2, 18.0, 6.60 and 2.50 ppm, respectively. Average concentrations of Cd, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sr and Zn were noted to be significantly higher in the plasma of cancer patients compared with controls. Very strong mutual correlations ($r > 0.70$) in the plasma of cancer patients were observed between Fe–Mn, Ca–Mn, Ca–Ni, Ca–Co, Cd–Pb, Co–Ni, Mn–Ni, Mn–Zn, Cr–Li, Ca–Zn and Fe–Ni, whereas, Ca–Mn, Ca–Mg, Fe–Zn, Ca–Zn, Mg–Mn, Mg–Zn, Cd–Sb, Cd–Co, Cd–Zn, Co–Sb and Sb–Zn exhibited strong relationships ($r > 0.50$) in the plasma of controls, all were significant at $p < 0.01$. Principal component analysis (PCA) of the data extracted five PCs, both for cancer patients and controls, but with considerably different loadings. The average metals levels in male and female donors of the two groups were also evaluated and in addition, the general role of trace metals in the carcinogenesis was discussed. The study indicated appreciably different pattern of metal distribution and mutual relationships in the plasma of cancer patients in comparison with controls.

Keywords: Cancer; Plasma; Trace metals; Multivariate analysis; Correlation; AAS; Pakistan

1. Introduction

Blood is one of the widely used specimens for biological trace metal research because of its natural significance and ease of sampling [1]. It is the medium of transport of trace metals and provides direct evidence of metabolism about the trace metal concentrations [2,3]. Therefore, whole blood, plasma and serum are convenient samples for the determination of trace metal status of an individual [1,4]. A number of investigations have been carried out over the past years to assess values of trace metals in the body liquids [5–7]. Plasma can be separated more rapidly than serum from cells and procedure is more gentle towards the cells. Therefore, plasma is preferred over serum when trace metals with an unequal distribution in the blood are analyzed, although the addition of anticoagulants adds a risk of contamination [8,9].

The role of different trace metals in the normal vital activities and initiation of some diseased has long been known [10,11]. Nevertheless, until recently clinical recognition was limited to very few of the trace metals, but now it can be assumed that all chemical elements are involved in physiological processes in varying degrees. Moreover, any changes in the environment as well as in the human body itself can trigger changes in trace metal composition of any organ or tissue and as a consequence, some disease can be produced [12]. Biological and medical trace metal research has progressed constantly in recent years, yielding an enormous abundance of data [13–15].

Cancer was primarily considered to be genetically linked, however, it is now well recognized that diet has a significant effect on cancer incidences [16,17]. In fact, food consumption patterns could provide major insights into cancer risk

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doi:10.1016/j.jhazmat.2007.09.115

and prevention despite the fact that their significance is not fully appreciated [18]. Epidemiological studies showed that the majority of the factors leading to the development of tumour in humans have arisen from environmental factors and 65–70% of all cancers in humans are associated with the environment, including the work environment, 30–40% with nutritional habits and only 2% with consequences of genetic predispositions [19,20]. Cancer is a multi-etiological and multi-factorial complex disease. The role of trace metals in the development and inhibition of cancer has a complex character and raises many questions [21]. Several studies have focused on the relationship between trace metals and cancer in humans [13,22–24]. A relatively wide range of minor and trace metals are known to play important roles in biological processes including the activation or inhibition of enzymatic reaction, competition amongst elements and metalloproteinase for binding sites and modification of the permeability of cell membranes [15,25,26]. It would seem reasonable, therefore, to assume that the trace metals might influence the carcinogenic process [27–29].

The elevated levels of carcinogenic metals may be associated with a number of physiological disorders in humans. It has been reported that Cd is a mutagen in mammalian [20,30] and enhanced concentration of Cd may result in prostate, renal and lung cancers [31–33]. Similarly, higher Pb contents in dietary intake were linked with the cancers of stomach, small intestine, large intestine, ovary, kidney, lung, myeloma, all lymphorms and all leukemia [3,30,34,35]. Some studies on experimental animals, such as, mice, related higher Cr and Zn concentrations with accelerated tumor growth [36–38]. Significant correlations have been reported between blood Cr and Zn levels and mortalities from cancers of breast, colon, rectum, ovary, lung, pancreas, leukemia and bladder [30,32,39,40]. Association of Cr intake with respiratory, lung and nasal cancers has also been reported [41–43]. Moreover, Cr and Zn had been reported to be significantly higher in the blood of breast cancer patients as compared with healthy donors [39], and had been significant experimental evidence that Cr stimulate oxygen radical production which induces DNA damage [44]. Breast cancer mortality was found to be strongly contributed by Cr, followed by Cd and Zn, while for prostate cancer, Cd revealed the strongest contribution followed by Zn and Cr [30,31,36]. Ni is another mutagen [25,45,46], which is also associated with lung and nasal cancer [30,47]. Among the other metals, Sb and Co have been linked to lung cancer [48,49], and Fe may be a carcinogen but needs further experimental evidences [50]. Although Cu is an essential element for humans and animals, its significantly higher levels were found in malignant plasma than the plasma of healthy donors which could induce growth proliferation and cancer by damaging DNA with free hydroxyl radicals [51–53].

The present study is carried out to estimate the comparative trace metal distribution and correlation in the plasma of cancer patients and controls. Multivariate method of principal component analysis has been used for apportionment of the trace metals in the two groups of donors. The selected metal contents in the plasma are also evaluated for relative gender-based variations in both categories.

2. Materials and methods

A total of 112 metastasize cancer patients, 58 women and 54 men, ages between 15 and 94 years, were included in this study. Subjects were selected from the patients admitted in hospitals of Rawalpindi district, Pakistan, namely POF Hospital Wah Cantt ($n = 67$) and Christian Hospital, Taxila ($n = 45$). The blood samples from the cancer patients were collected prior to any chemo- or radiotherapy and diagnosis of primary carcinoma was confirmed histologically. The controls ($n = 118$) were selected from the same localities with matched age groups, including 69 women and 49 men, ages between 13 and 80 years. The blood sampling was carried out during January 2001 to July 2003. The blood samples were collected from an antecubital vein by using appropriate precautions to prevent contamination with exogenous trace elements [8]. Venous blood was obtained in heparinized evacuated tubes (venoject, 10 mL). Each sample was gently shaken by hand and centrifuged at 2000 rpm for 15 min. The plasma was separated carefully by using Finn pipette into another clean polyethylene vial duly labelled with relevant codes related to the donor's name, age, eating and drinking habits, social and general health status, all recorded and compiled on regular proforma at the time of sampling. Samples were stored at −70 °C until analyses were performed [54,55]. The plasma samples were digested with nitric acid–perchloric acid (10:1, v/v) mixture with subsequent heating to a soft boil until white dense fumes evolved. Samples were then cooled to room temperature and diluted to proper volume with doubly distilled water [56]. The blank was prepared the same way but without plasma sample.

All reagents used were of ultrahigh purity (certified > 99.99%) procured from E-Merck. Working solutions were prepared by serial dilution of 1000 ppm standard solutions. Quantitative analysis was carried out on Flame Atomic Absorption Spectrophotometer, Varian 240-FS, with automatic background compensation and under optimum analytical conditions. The plasma samples were analyzed for 18 metals; Al, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Sr and Zn.

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 SRM (OL-96). The samples were also analyzed at an independent laboratory for comparison of the results. A maximum of 5% difference was observed in the results of two laboratories. STATISTICA software was used for statistical analyses of plasma metal data [57].

3. Results and discussion

The mean metal concentrations in the plasma of cancer patients and controls, along with basic statistical distribution parameters, are presented in Tables 1 and 2, respectively. Highest mean metal levels are observed for Na (3971 ppm), followed by K (178 ppm), Ca (44.1 ppm), Mg (7.59 ppm), Sr (5.44 ppm), Sb (5.35 ppm), Fe (4.38 ppm), Zn (3.90 ppm), Pb (3.45 ppm), Cu (3.43 ppm) and Al (3.17 ppm) in the plasma of cancer patients. In

Table 1
Statistical distribution parameters for selected metal concentrations (ppm) in the plasma of cancer patients ($n = 112$)

	Range	Mean	Median	S.D.	S.E.	Skewness
Al	0.01–9.93	3.17	2.73	2.25	0.21	1.08
Ca	3.02–455	44.1	33.4	66.0	6.24	5.27
Cd	0.13–7.51	0.76	0.57	0.92	0.09	5.02
Co	0.13–3.05	0.69	0.57	0.46	0.04	2.84
Cr	0.05–4.03	0.78	0.76	0.50	0.05	3.02
Cu	0.88–17.7	3.43	3.11	2.00	0.19	4.27
Fe	0.05–56.0	4.38	1.49	9.74	0.92	4.10
K	8.26–456	178	178	66.3	6.26	0.96
Li	0.06–1.48	0.33	0.31	0.21	0.02	2.54
Mg	1.73–29.5	7.59	6.96	4.42	0.42	2.71
Mn	0.03–5.95	0.37	0.15	0.84	0.08	4.75
Mo	0.002–7.86	1.98	1.76	1.55	0.15	1.02
Na	43.2–9634	3971	3710	1379	130	1.45
Ni	0.01–18.4	2.06	1.50	2.94	0.28	3.46
Pb	0.29–29.7	3.45	2.32	4.38	0.41	3.48
Sb	0.30–11.2	5.35	5.61	2.74	0.26	0.15
Sr	0.10–31.5	5.44	4.83	4.33	0.41	2.50
Zn	0.03–57.3	3.90	0.96	9.53	0.90	3.89

comparison, the dominant metal levels in the plasma of controls are Na (3844 ppm), K (151 ppm), Ca (74.2 ppm), Mg (18.0 ppm), Fe (6.60 ppm), Zn (2.50 ppm) and Al (2.69 ppm). The dispersion and asymmetry of the metals data are significantly high in the plasma of cancer patients compared with the controls, manifested by standard deviation (S.D.), standard error (S.E.) and skewness.

The average metal level in the plasma of cancer patients reveals the following decreasing order in their concentrations: Na > K > Ca > Mg > Sr > Sb > Fe > Zn > Pb > Cu > Al > Ni > Mo > Cr > Cd > Co > Mn > Li. The corresponding order of mean metal levels in the plasma of controls is: Na > K > Ca > Mg > Fe > Al > Zn > Pb > Cu > Sr > Sb > Mo > Co > Ni > Li > Cd > Cr > Mn. The two orders are the same for both categories (patients and controls) in terms of Na, K, Ca, Mg, Zn, Pb, Cu and Mo. Two tailed Student's t-test ($p < 0.01$) of the data show that there is no significant difference between the levels of Al, Co, K, Li and Na in the plasma of cancer patients and healthy donors. However, there is a significant difference in the levels of all other selected metals.

Table 2
Statistical distribution parameters for selected metal concentrations (ppm) in the plasma of controls ($n = 118$)

	Range	Mean	Median	S.D.	S.E.	Skewness
Al	0.32–9.44	2.69	2.06	1.84	0.17	1.34
Ca	54.8–237	74.2	72.8	20.8	1.92	5.86
Cd	0.07–0.57	0.23	0.22	0.09	0.01	0.73
Co	0.01–2.50	0.71	0.67	0.38	0.04	1.18
Cr	0.001–1.29	0.11	0.07	0.15	0.01	4.98
Cu	0.08–5.37	1.26	1.05	0.84	0.08	1.64
Fe	3.02–17.4	6.60	6.09	2.79	0.26	1.51
K	50.4–256	151	149	20.9	1.92	0.33
Li	0.01–1.09	0.39	0.26	0.33	0.03	0.52
Mg	1.04–33.3	18.0	17.8	3.16	0.29	−0.27
Mn	0.001–0.28	0.10	0.10	0.03	0.003	0.69
Mo	0.002–2.84	0.83	0.64	0.77	0.07	0.78
Na	2303–6311	3844	3779	662	60.9	0.99
Ni	0.01–2.91	0.51	0.39	0.49	0.05	1.99
Pb	0.04–6.74	1.58	1.30	1.24	0.11	1.51
Sb	0.07–2.48	0.86	0.78	0.39	0.04	1.32
Sr	0.001–1.98	0.97	0.99	0.48	0.04	−0.08
Zn	1.17–6.21	2.50	2.29	0.87	0.08	1.89

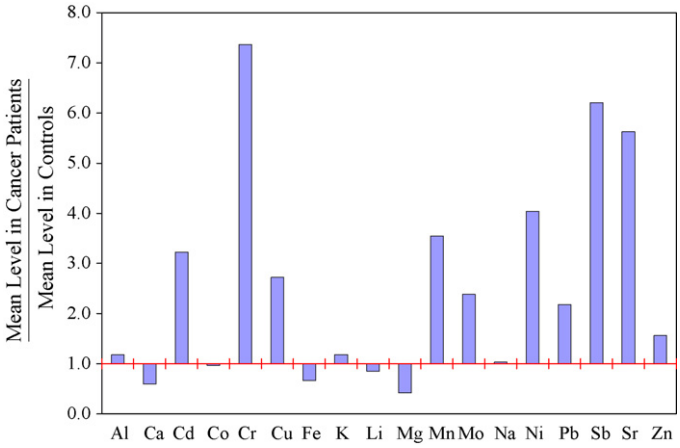

Fig. 1. Comparative mean trace metal ratios in the plasma of cancer patients and controls.

An examination of the tabulated data reveal that among the selected metals, Cd, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sr and Zn exhibit significantly higher mean metal levels in the plasma of cancer patients than controls, while, average concentrations of Ca, Fe and Mg are noticeably higher in controls and Al, Co, K, Li and Na show almost comparable levels in the plasma of both groups. This is also shown in Fig. 1 as ratios of mean metal levels in the plasma of two groups. Markedly elevated concentrations of some toxic trace metals in cancer patients than healthy donors indicate a build-up in the metal levels in cancer patients at the expense of macronutrients, which exhibit lower mean concentrations in the plasma of cancer patients. A decreased plasma Fe in cancer subjects indicates that the utilization of heme molecule was impaired, because the cancer itself might have been affecting the bone marrow function adversely [58]. The statistical distribution parameters evidenced that the metal data in cancer patients are spread over large concentrations as compared with the controls, as reflected by range, S.D., S.E. and skewness values. For cancer patients the average concentrations of Cd, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sr and Zn are 1.6–7.4 times higher as compared with controls, as shown in Fig. 1.

The comparison of average metal levels as revealed by their ratios in the plasma of male and female cancer patients is shown in Fig. 2. Average concentrations of Al, Cd, Co, Fe, Mo, Sb, Sr and Zn in the plasma of male and female cancer patients are not significantly different from each other. However, appreciably higher average levels of Ca, Mg, Mn, Ni and Pb are observed in the plasma of male cancer patients while, in the case of female cancer patients, the mean concentrations of Cr, Cu, K, Li and Na are higher than male patients. Similarly, comparison of average metal levels in terms of their ratios in the plasma of male and female healthy donors is shown in Fig. 3. Half of the selected trace metals (Al, Co, K, Mg, Mo, Na, Ni, Pb and Sb) exhibit

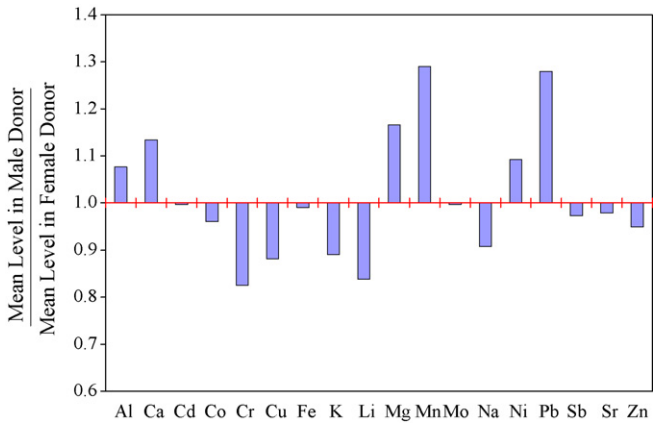

Fig. 2. Comparative gender-based ratios of mean metal levels in the plasma of cancer patients.

almost comparable levels in the plasma of male and female donors of control group. The average levels of Cr, Cu and Li are found to be slightly higher in the plasma of female donors while in the cases of Ca, Cd, Fe, Mn, Sr and Zn, the average levels are estimated to be noticeably higher in the plasma of male donors. In comparison with the controls, relative gender-based variations of trace metals in the plasma of cancer patients are significantly different, which may be due to the different gender-related ways of detoxifying, thus evidencing imbalances of the trace metals in the plasma of cancer patients.

The data on metal-to-metal correlations in the plasma of cancer patients and controls are given in Tables 3 and 4, respectively. For cancer patients (Table 3) very strong positive correlations are found between: Cr–Li ($r = 0.93$), Fe–Mn ($r = 0.81$), Ca–Mn ($r = 0.76$) Ca–Ni ($r = 0.76$), Ca–Co ($r = 0.76$), Cd–Pb ($r = 0.75$), Co–Ni ($r = 0.75$), Mn–Ni ($r = 0.75$), Mn–Zn ($r = 0.75$), Ca–Zn ($r = 0.74$) and Fe–Ni ($r = 0.70$). Other notably strong correlation are observed for Ni–Zn, Fe–Zn, Ca–Fe, Cd–Li, Ni–Pb, Co–Mn, Co–Fe, Co–Li, Co–Zn, Ca–Pb, Co–Pb, Mo–Ni and Co–Cr. In addition, some significant correlations are also noted between Cr–Cu, Cu–Li, Mn–Pb, Cd–Co, Ca–Mg, Cr–Cu, Cr–Pb, Li–Pb, Mo–Sb, Cr–Na, Co–Mg and Pb–Zn. This simple correlation study reveals some common origin of these metals in the plasma

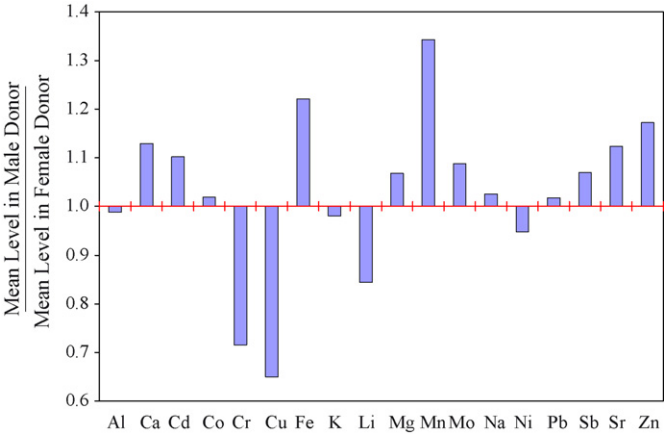

Fig. 3. Comparative gender-based ratios of mean metal levels in the plasma of controls.

of cancer patients. On the basis of above observation it can be concluded that essential metals such as Ca, Mn, Zn and Fe are directly related with the toxic trace metals such as Cr, Co, Ni, Cd, Pb, Sb, Li and Mo thus evidencing the uptake of these toxic metals in cancer patients.

As given in Table 4, the selected trace metals in the plasma of controls reveal strong positive correlations between, Ca–Mn ($r = 0.89$), Cd–Sb ($r = 0.84$), Fe–Zn ($r = 0.65$), Cd–Co ($r = 0.65$), Ca–Zn ($r = 0.63$), Ca–Mg ($r = 0.63$), Cd–Zn ($r = 0.58$), Co–Sb ($r = 0.54$), Mg–Zn ($r = 0.52$), Sb–Zn ($r = 0.52$) and Mg–Mn ($r = 50$). In addition, some significant correlations are also noted between Al–Co, Al–Li, Al–Mo, Cd–Fe, Cd–Mg, Co–Li, Co–Mo, Co–Zn, Fe–Mg, Fe–Pb, Fe–Sb, Li–Mg and Mg–Zn. Among the selected metals, Na, K, Cr, Ni and Sr are not significantly correlated with any other metal, evidencing their independent role in the plasma of healthy donors. The case of Cu is unique because it is significantly negatively correlated with Cd ($r = -0.49$), Sb ($r = -0.43$) and Fe ($r = -0.39$). The correlation study suggests that the essential metals, Ca, Mg, Fe, Mn and Zn are contributed by some common sources, while the inter-relationship of toxic metals such as Cd, Sb, Co, Al, Li and Mo indicate another common source in the plasma of controls.

The inter-relationship between pairs of selected metals in the plasma of two groups brings out a marked difference in metal-to-metal correlations (Tables 3 and 4). In case of cancer subjects, very strong positive correlations among essential metals and toxic metals indicate build up of the toxic/carcinogenic metals in the plasma. Also Ca is strongly correlated with Mg in controls but in cancer patients only a weak correlation is observed. The absence of strong correlation between Ca and Mg in cancer patients is noteworthy because the metals are well known for their positive correlation [39,59]. The range and mean levels of Ca and Mg are also found lower in cancer patients than in healthy donors, most likely due to some metabolic process whereby these metals are depleted and replaced by carcinogenic metals in the plasma of cancer patients as compared with the controls. It may be inferred from this behaviour that Ca and Mg are less bio-available in cancer patients, which may lead to a number of physiological disorders. Another significant difference is observed in the cases of Na and K, which show significant correlations with some toxic metals in the plasma of cancer patients but no such relationship is found in healthy donors. Similarly, Al exhibits a non-significant correlation pattern with all other metals in case of cancer patients while, for controls, Na, K, Cr, Ni and Sr are insignificantly correlated with other metals. Although Cu is negatively correlated with Cd, Fe and Sb in controls but exhibit significantly positive relationship with Cr and Li in cancer patients. Likewise, Mn and Zn exhibit almost comparable correlations with Ca and Mg in both categories. All selected metals reveal divergent mutual correlations in the plasma of two groups of donors.

The present study takes up metal source identification and apportionment for the two groups using standard statistical model reported in the literature [12,56,60–62]. Accordingly, multivariate technique of principal component analysis (PCA) is employed to identify possible sources and grouping of selected metals in the plasma of the two groups of donors. The principal

Table 3
Pearson correlation coefficient matrix of selected metals in the plasma of cancer patients ($n = 112$)

	Al	Ca	Cd	Co	Cr	Cu	Fe	K	Li	Mg	Mn	Mo	Na	Ni	Pb	Sb	Sr
Ca	0.02																
Cd	0.02	0.21															
Co	0.19	**0.76**	**0.35**														
Cr	0.18	0.12	**0.43**	**0.58**													
Cu	0.13	0.09	0.15	0.27	**0.35**												
Fe	0.01	**0.64**	0.09	**0.56**	0.05	0.04											
K	0.23	0.01	**0.38**	0.23	**0.44**	0.20	0.10										
Li	0.20	0.15	**0.55**	**0.58**	**0.93**	**0.35**	0.06	**0.49**									
Mg	0.01	**0.47**	0.08	**0.39**	0.05	0.19	0.25	0.19	0.06								
Mn	0.09	**0.76**	0.13	**0.66**	0.06	0.04	**0.81**	0.05	0.10	**0.34**							
Mo	0.12	0.27	0.12	0.29	**0.34**	0.09	0.15	0.20	**0.40**	−0.05	0.24						
Na	0.15	0.03	0.10	0.16	**0.35**	0.26	−0.09	0.11	0.28	0.21	−0.06	0.01					
Ni	0.04	**0.76**	0.14	**0.75**	0.24	0.23	**0.70**	0.11	0.27	**0.33**	**0.75**	**0.51**	−0.07				
Pb	0.07	**0.53**	**0.75**	**0.60**	**0.37**	0.15	**0.38**	0.24	**0.47**	0.20	**0.48**	**0.35**	0.01	**0.56**			
Sb	0.08	0.06	0.08	0.09	0.16	0.04	−0.01	0.19	0.14	−0.08	−0.02	**0.46**	0.01	0.23	0.05		
Sr	−0.07	0.07	0.12	0.23	**0.32**	0.05	0.06	0.14	**0.31**	−0.06	0.11	0.09	0.02	0.23	0.20	0.06	
Zn	0.10	**0.74**	0.10	**0.55**	0.02	0.11	**0.65**	0.02	0.04	**0.37**	**0.75**	0.24	−0.06	**0.64**	**0.41**	0.02	0.05

Bold values are significant at $p < 0.01$.

component (PC) loadings, extracted by using varimax normalized rotation on the metal data-set, are shown in Tables 5 and 6, respectively, for cancer patients and controls. Five principal components (PCs) are extracted with eigen values > 1 for each group of donors, commutatively explaining more than 70% of total variance for cancer patients (Table 5) and more than 64% of total variance for controls (Table 6).

In the case of cancer patients, PC 1 shows higher loadings for Ca, Co, Fe, Mg, Mn, Ni, Pb and Zn. For controls, PC 1 shows higher loadings for Ca, Mg, Mn and Zn. An important difference emerging here is that in the plasma of cancer patients, macronutrients and essential metals (Ca, Mg, Fe and Zn) share a common PC with toxic metals (Co, Ni and Pb), while such association is absent in controls. PC 2 shows higher loadings for Cd, Cr, K, Li and Pb in cancer patients while for controls, Cu, K, Li and Na contribute maximum loadings to PC 2. The maximum loadings of Na and Cu, along with Cr and Li are shown in PC 4 for cancer patients. These findings suggest that Na in the plasma of cancer patients has different source as compared with the healthy donors, probably due to the difference in the metabolism of the body. PC 3, for cancer patients, shows elevated loadings of Mo and Sb, whereas, for controls, third PC exhibits maximum loadings in favour of Cd, Co, Fe, Pb, Sb and Zn. Similarly, PC 4 shows higher loadings for Cr, Ni and Sr and PC 5 evidenced significant loadings of Al, Mo and Co in controls. Likewise, PC 5 shows dominant loadings of Al and Sr for cancer patients. The PCA demonstrates significantly different grouping/origin of the metals in the plasma of cancer patients and controls. In healthy donors, macronutrients share common PCs with essential metals and therefore, show mutual dependence while toxic metals reveal independent sources compared to essential metals. In cancer patients, the toxic and carcinogenic metals reveal

Table 4
Pearson correlation coefficient matrix of selected metals in the plasma of controls ($n = 118$)

	Al	Ca	Cd	Co	Cr	Cu	Fe	K	Li	Mg	Mn	Mo	Na	Ni	Pb	Sb	Sr
Ca	−0.04																
Cd	0.26	0.28															
Co	**0.42**	0.11	**0.65**														
Cr	0.21	−0.03	0.02	0.10													
Cu	−0.20	0.01	**−0.49**	−0.23	−0.08												
Fe	0.14	0.28	**0.47**	0.20	0.12	**−0.39**											
K	0.01	0.17	0.08	0.08	0.06	−0.07	0.08										
Li	**0.33**	0.05	0.26	**0.48**	0.07	−0.06	−0.08	0.22									
Mg	0.14	**0.63**	**0.34**	0.23	0.19	−0.19	**0.30**	0.16	**0.32**								
Mn	0.01	**0.89**	0.19	0.14	−0.05	0.07	0.27	0.12	0.09	**0.50**							
Mo	**0.36**	0.03	0.23	**0.41**	0.08	−0.10	−0.09	−0.08	0.09	0.08	0.01						
Na	−0.14	−0.05	−0.23	−0.29	−0.07	0.06	0.11	−0.11	−0.28	−0.09	0.05	−0.17					
Ni	0.19	0.11	0.28	0.19	0.28	−0.24	0.25	0.07	0.13	0.25	0.03	0.07	0.04				
Pb	−0.08	0.04	0.14	−0.11	0.01	−0.13	**0.33**	−0.02	−0.27	−0.10	0.02	−0.11	0.10	−0.05			
Sb	0.20	0.15	**0.84**	**0.54**	0.09	**−0.43**	**0.46**	0.11	0.16	0.20	0.08	0.27	−0.20	0.21	0.28		
Sr	−0.01	0.12	0.29	0.19	−0.18	−0.16	0.15	0.03	−0.16	−0.10	0.11	0.16	0.05	−0.08	0.22	0.29	
Zn	0.11	**0.63**	**0.58**	**0.34**	0.01	−0.28	**0.65**	0.15	0.03	**0.46**	**0.52**	0.11	−0.01	0.22	0.22	**0.52**	0.15

Bold values are significant at $p < 0.01$.

Table 5
Principal Component loadings[a] of selected metals in the plasma of cancer patients

	PC 1	PC 2	PC 3	PC 4	PC 5
Al	−0.03	−0.11	−0.28	−0.33	**0.52**
Ca	**0.90**	0.11	0.01	0.06	0.02
Cd	0.09	**0.92**	−0.08	−0.02	0.01
Co	**0.73**	0.36	0.12	0.37	0.16
Cr	0.02	**0.56**	0.29	**0.60**	0.31
Cu	0.10	0.09	0.04	**0.64**	0.00
Fe	**0.83**	0.04	0.02	−0.07	0.02
K	−0.02	**0.56**	0.23	0.28	−0.19
Li	0.05	**0.68**	0.29	**0.52**	0.26
Mg	**0.49**	0.03	−0.30	0.38	−0.27
Mn	**0.91**	0.07	0.03	−0.04	0.01
Mo	0.25	0.18	**0.79**	0.02	0.06
Na	−0.07	0.01	−0.10	**0.74**	−0.05
Ni	**0.85**	0.10	0.33	0.08	0.18
Pb	**0.51**	**0.75**	0.04	−0.07	0.09
Sb	−0.01	0.02	**0.79**	−0.01	−0.06
Sr	0.08	0.12	0.10	0.15	**0.79**
Zn	**0.85**	0.01	0.04	−0.01	−0.09
Eigen values	5.84	2.98	1.52	1.32	1.07
% Total variance	32.5	16.6	8.47	7.32	5.92
% Cumul. variance	32.5	49.1	57.5	64.8	70.8

[a] Higher loadings are shown in bold.

Table 6
Principal Component loadings[a] of selected metals in the plasma of controls

	PC 1	PC 2	PC 3	PC 4	PC 5
Al	−0.02	0.22	0.14	0.35	**0.58**
Ca	**0.94**	0.02	0.07	−0.08	−0.03
Cd	0.23	0.32	**0.78**	0.01	0.27
Co	0.14	0.41	**0.52**	0.03	**0.53**
Cr	−0.04	−0.01	0.05	**0.70**	0.08
Cu	−0.09	**0.66**	0.08	0.22	0.04
Fe	0.34	−0.20	**0.69**	0.25	−0.12
K	0.15	**0.52**	0.15	0.03	−0.47
Li	0.10	**0.77**	−0.04	0.20	0.14
Mg	**0.67**	0.25	0.11	0.36	0.03
Mn	**0.93**	−0.01	−0.02	−0.09	0.03
Mo	0.03	0.09	0.07	−0.05	**0.80**
Na	−0.06	**0.64**	0.06	−0.10	0.10
Ni	0.11	0.00	0.26	**0.65**	0.08
Pb	−0.04	−0.38	**0.52**	−0.16	−0.23
Sb	0.09	0.23	**0.82**	−0.03	0.22
Sr	−0.05	0.14	−0.43	**0.54**	−0.21
Zn	**0.66**	−0.02	**0.57**	0.06	0.03
Eigen values	4.56	2.36	2.03	1.49	1.16
% Total variance	25.4	13.1	11.3	8.30	6.43
% Cumul. variance	25.4	38.5	49.8	58.1	64.5

[a] Higher loadings are shown in bold.

common PCs with the macronutrients and essential metals and hence the similar variations and sources. This situation calls for a comprehensive investigation on the role of macronutrients and toxic metals in cancer patients in comparison with controls.

4. Conclusions

In conclusion, the present study brings out marked differences in the distribution and correlations of selected trace metals in the plasma of cancer patients compared with the healthy donor. On the average basis, Cd, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sr and Zn are significantly higher in the plasma of cancer patients than controls. Similarly, the gender-based variations of most of the selected metals are also considerably different in the two groups of donors. PCA and CA also support the different apportionment mechanism of trace metals in the plasma of cancer patients and controls which evidences that the body metabolism in the cancer patients is being significantly affected by the particular trace metal concentrations.

Acknowledgments

The funding by Higher Education Commission, Government of Pakistan, to carry out this project is gratefully acknowledged. Thanks are due to the administration of POF Hospital, Wah Cantt and Christian Hospital, Taxilla, for their invaluable help during the sampling.

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