



# Study of Trace Metal Imbalances in the Scalp Hair of Stomach Cancer Patients with Different Types and Stages

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## Abstract

Stomach cancer is among the most common forms of cancers, and diet and environmental factors play important roles in its malignancy. This study was conducted to evaluate the trace metal contents in the scalp hair of stomach cancer patients and healthy donors to investigate probable relationship between metal imbalances and cancer. The samples were digested in HNO<sub>3</sub>-HClO<sub>4</sub> mixture and the metals were quantified by flame atomic absorption spectrophotometry. Median level of Cr was found to be significantly higher in the patients than in the controls, while median levels of Fe, Mn and Cd were considerably reduced. The correlation pattern of metals in the patients manifested significantly divergent mutual relationships compared with the controls. Multivariate analyses showed appreciably diverse apportionment of the metals in the patients and healthy donors. Variations in the metal levels were also observed for various types (adenocarcinoma and gastrointestinal stromal tumour) as well as stages (I, II, III and IV) of stomach cancer patients. Most of the metals revealed noticeable disparities in their levels based on gender, habitat, dietary habit and smoking habit of patients and controls. Accordingly, the essential/toxic metals exhibited significant imbalance due to pathogenesis of stomach among the patients.

**Keywords** AAS · Metal · Pakistan · Scalp hair · Statistical analysis · Stomach cancer

## Introduction

During recent years, considerable increase in the trace metal emanations into the environment has been observed mainly due to excessive industrialization, urbanization and globalization [54]. Accordingly, chronic exposure to toxic metals can cause adverse effects including varieties of diseases, such as cancer even at relatively low quantities [12, 32]. Cancer of the stomach, also known as gastric cancer, is a disease in which the epithelial tissues of stomach are affected thereby producing malignant cells [43]. This cancer leads to inflammation, ulceration and ultimately tumour formation because of abnormal hyperplasia that starts in the inner lining of cells [35]. It is the fifth

leading cause of cancer and the third leading cause of death from cancer, making up 7% of cases and 9% of deaths worldwide [20, 42]. The prevalence of stomach cancer increases with age and the highest rate is found in ages 80 years or older [5].

Stomach cancer is a heterogeneous disease with many histopathological types: adenocarcinoma, lymphoma, gastrointestinal stromal tumour and carcinoid tumour, etc. [43]. The stage of stomach cancer depicts the extent of the cancer and helps determine how serious the cancer is and how best to treat it [53]. The aetiology of stomach cancer is multicausal/multifactorial and the infection with *Helicobacter pylori* is the strongest established risk factor for this malignancy [9]. Other well-established risk factors include tobacco smoking, alcohol consumption, Epstein-Barr virus, obesity, salt and salted preserved food, exposure to metals, nitrate and nitrite ingestion and occupation exposure to various types of dust [12, 22, 35, 42]. Among the stomach cancer risk factors, heavy metals are classified as certain/probable carcinogens by the International Agency for Research on Cancer (IARC) [50]. Although some trace metals serve as co-enzymes that are essential for intracellular processes, but most of the toxic trace metals induce various ailments. Trace metals present in small levels have been evaluated as a potential key constituent in various cancers. Some trace metals do play a preventive role

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against malignant growth by involving in protection against oxidative stress that contribute to cancer development [17]. Because of its anatomic position, the stomach is in direct contact with ingested trace metals through water and food. Some previous studies have also advocated the existence of a relationship among trace metal exposure and stomach cancer risk [18, 52, 54].

In humans, blood, milk, plasma, urine, saliva, sweat, nails and tissue samples are commonly employed to monitor and quantify the trace metal levels as indices for evaluating nutritional status, identifying systemic intoxication and diagnosis diseases [6, 18, 20, 34]. In the present work, scalp hair sample is selected for metal analyses due to many advantages; for instance, collection of hair is non-invasive/painless, low cost, easy transport, it is stable, robust and its composition does not fluctuate over short time. Moreover, sampling of hair is easy to collect and does not need special storage/handling and specific professional skills. Finally, it poses no risk of contagious diseases [34], although some restrictions such as scarcity of well-defined reference values, highly variable intra-hair growth rates, probability of external contamination and a need for improved understanding of hair biology/pharmacokinetics are also associated with hair samples [18, 34].

The role of trace metals in the initiation and progression of stomach cancer has been investigated in some recent investigations, but the results are inconsistent and random [12, 18, 20, 34, 52]. There has been an apparent lack of investigation regarding the association of metal exposure and the risk of stomach cancer with respect to cancer types and stages. The aim of the present study was to explore the relation between exposure of metals and the risk of stomach cancer especially the cancer types and stages in Pakistan. The results of the present work may be useful for the treatment and prevention of stomach cancer and will be used as a database for the researchers and oncologist.

## Experimental Methodology

### Study Design and Subjects

Newly diagnosed stomach cancer patients ( $n = 95$ ) admitted in (i) Nuclear Oncology and Radiotherapy Institute (NORI), Islamabad, Pakistan, and (ii) Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan, and matching controls ( $n = 91$ ) were recruited in the present study on a volunteer basis. The ethical review committees of the institutes reviewed and approved the protocol of the study. Clinicopathological features of the patients were obtained from medical records. The inclusion criteria were (a) pathologically confirmed new diagnosis of stomach cancer patients, (b) no transfusion of blood and no use of any kind of mineral/supplement for the last 6 months, (c) no prior radiotherapy/chemotherapy or anti-

neoplastic treatment and no clinical or biochemical evidence of other complications, and (d) the ability to understand and answer questions. The exclusion criteria were (a) alcohol consumption, (b) unaware of suffering from any malignancy and chemotherapy, (c) any type of gastric surgery and (d) other organ dysfunctions. Healthy donors were also selected on a volunteer basis from similar places with matched gender, age groups and comparable socioeconomic status. In most of the cases, healthy donors were either a family member or a closely related person to the cancer patient. The information regarding the donor's age, health, ailment, lifestyle, nutritional habits, smoking habits, work history, socio-demography, abode, income and education was recorded on a pro-forma at the time of sample collection. Prior to the sample collection, the participants were given a brief description on the goals and the procedure of the study and a written informed consent was acquired from all the subjects or their next of kin/parents in the case of young participants (for under 18 years).

### Sample Collection and Preparation

In the present study, scalp hair specimens (1.0–3.0 g) were collected from the sub-occipital region of the head with a pair of stainless-steel scissors. The collected hair samples were put into small polyethylene bags, labelled with relevant codes and stored at room temperature until digestion and analysis of metals were performed. The hair samples were thoroughly washed to offer an accurate assessment of endogenous metal contents. Before washing, the samples were cut into small pieces (approximately 0.5 cm) and mixed to make a representative sample. Afterwards, each hair sample was washed in series with 5% detergent solution, 0.5% Triton X-100 solution and deionized water. First of all, the scalp hair sample was taken in a conical flask containing 50 mL of 5% detergent solution and mixed well. The flask contents were then shaken on an auto-shaker at 320 vibrations per minute for about 30 min. After leaving it at room temperature for at least 2 h, it was washed with plentiful water. Then, 30 mL of non-ionic detergent Triton X-100 (0.5% v/v) solution was added to each flask and again placed on the auto-shaker for 30 min. The samples were then washed with deionized water followed by drying in an electric oven overnight at 70 °C [34].

For digestion purpose, 10 mL of  $\text{HNO}_3$  was added to each flask which contained accurately weighed quantity of dried and cleaned hair sample and heated for about 30 min on a hot plate at 70–80 °C. The sample contents were then cooled to room temperature, followed by the addition of 5.0 mL of perchloric acid (70%) in each flask and again placed on the hot plate. The flasks were heated again until white dense fumes evolved which indicated the completion of the digestion process. Samples were cooled to room temperature and diluted

appropriately with 0.1 M HNO<sub>3</sub> [18, 34]. Blank digests were carried out in the same way but without scalp hair sample.

### Quantification of Selected Metals

Selected essential/toxic metals (Zn, Pb, Fe, Cu, Cr, Mn and Cd) were quantified in the digested samples using flame atomic absorption spectrophotometer (Shimadzu AA-670, Japan). The instrument was operated with automatic background compensation under optimum analytical conditions which are given in Table S1. The average level of metal in each sample was computed based on the measurement of three sub-samples of each sample that were run separately onto the spectrophotometer. Standard Reference Material (Human Hair, GBW 07601) was used for quality assurance and accuracy. These results showed very good agreement/recoveries (Table S1). For comparison of the data, samples were also analysed at an independent laboratory and the two results showed almost comparable levels ( $\pm 2\%$  difference). All the reagents used were obtained from E-Merck or BDH and they were of ultrahigh purity (certified > 99.99%). Working standards were prepared by serial dilution of the stock standard solutions (1000 mg/L) just before the analysis of the metal on the instrument [34].

### Statistical Analysis

Statistical analyses were applied on the results of metal data using STATISTICA (6.0) software [45]. Descriptive analysis was conducted to obtain basic statistical parameters including range, mean, median, 25th percentile and 75th percentile levels in the scalp hair samples. The distribution of selected metal levels in the scalp hair of the patients and controls was evaluated by Anderson-Darling test. The data were further analysed by Wilcoxon rank-sum test to compare the median levels of the metals between various sub-groups in each category. The level of  $p < 0.05$  was noted as statistically significant. Pearson correlation analysis was used to investigate the mutual associations among the metals in the scalp hair. Apportionment of the metals in the scalp hair of the patients and controls was done by multivariate methods including cluster analysis (CA) and principal component analysis (PCA) [34]. PCA has been proven to be a useful and efficient chemometric approach frequently employed for pattern recognition in a large data set. It is an excellent tool used to acquire hidden information that is not apparent from a conventional data analysis [27]. CA involves grouping of the variables having similar characteristics into clusters which results in internal homogeneity and external heterogeneity. It is informative to examine the CA in conjunction with PCA as they give similar information in different forms [34].

## Results and Discussion

### Characteristics of the Study Subjects

The demographic parameters related to the stomach cancer patients and healthy donors are displayed in Table 1. Stomach cancer malignancy was confirmed histopathologically along with clinical examination. The age of the stomach cancer patients ranged from 17 to 63 years with a mean value of 45 years while for healthy donors, it ranged from 15 to 65 years with a mean value of 42 years. Majority of the participants (> 50%) in both groups were vegetarians and 63% of the patients and 64% of the healthy donors resided in the rural areas. More than 50% of the patients and healthy subjects were not addicted to tobacco (smoking). Most of the patients (68%) were suffering from adenocarcinoma. Based on the division of histopathological stage, 32% of the patients were diagnosed at stage II, 25% at stage I, and 23% at stage III while 20% at stage IV of stomach cancer in the present study.

### Distribution of Metals

Basic statistical parameters were computed to find out the relative distribution of selected essential/toxic metal levels

**Table 1** Characteristics of the subjects

Parameters	Patients	Controls
<i>n</i>	95	91
Age (years)		
Range (Mean)	17–63 (45)	15–65 (42)
Gender		
Female	43 (45%)	31 (34%)
Male	52 (55%)	60 (66%)
Diet		
Vegetarian	59 (62%)	49 (54%)
Non-vegetarian	36 (38%)	42 (46%)
Habitat		
Urban	35 (37%)	33 (36%)
Rural	60 (63%)	58 (64%)
Tobacco use (smoking)		
No use	55 (58%)	58 (64%)
Use	40 (42%)	33 (36%)
Types of stomach cancer		
Adenocarcinoma	65 (68%)	--
Gastrointestinal stromal tumour	30 (32%)	--
Pathological stage		
Stage-I	24 (25%)	--
Stage-II	30 (32%)	--
Stage-III	22 (23%)	--
Stage-IV	19 (20%)	--

( $\mu\text{g/g}$ , dry weight) in the scalp hair of both donor groups as shown in Table 2. Most of the metals exhibited large spread and diverse dispersion in both study groups as shown by their minimum/maximum levels, mean/median levels and interquartile ranges (25th percentile and 75th percentile). An examination of the data revealed that major contributions in the scalp hair were noted for Zn, followed by Fe, Pb, Cu and Cr while Mn and Cd showed lowest contributions. Most of the metals demonstrated random distribution pattern in their levels as revealed by Anderson-Darling probability plots which are shown in Fig. S1 (supplementary material) as well as interquartile range (IQR). However, relatively normal distribution was found for Cd levels in the scalp hair of controls. Likewise, Cu levels in the scalp hair of controls also exhibited moderately normal distribution (Fig. S1). Majority of the metals exhibited large dispersion and more randomness in their levels in the scalp hair of the patients compared with the counterpart controls as shown in Fig. S1. Nonetheless, some of the metals, including Zn, Mn and Cr, showed predominantly random distribution in the scalp hair of both donor groups.

Comparison of the metal data among the patients and controls in terms of Wilcoxon rank-sum test ( $p < 0.05$ ) showed that the median levels of Cd, Cr, Fe and Mn were significantly different in the patients and controls; maximum difference was observed for Cd which showed that the median level was 53% higher in the scalp hair of controls than in the patients. Similarly, the median levels of Fe and Mn in the scalp hair of controls were 37% and 31% higher than those of the patients, respectively. Nevertheless, the median level of Cr in the scalp hair of the patients was found to be 30% higher compared with the controls. Among the rest of the metals, median levels of Cu and Zn were almost comparable in the scalp hair of both donor groups while the median level of Pb was slightly

higher in the case of controls than in the patients, but the difference was not statistically significant. Overall, relative distribution of most of the metals was considerably divergent in both study groups; such variances can be ascribed to the imbalances of the toxic and essential metals in the patients.

## Correlation Study

The correlation coefficient matrix ( $r$ ) relating to the metal levels in the scalp hair of stomach cancer patients and controls is shown in Table 3, wherein the italicized  $r$  values are significant at  $p < 0.05$ . In the case of the patients, significant positive correlations were noted between Mn-Cu ( $r = 0.669$ ), Cr-Cd ( $r = 0.596$ ), Pb-Cu ( $r = 0.528$ ), Pb-Cd ( $r = 0.455$ ), Fe-Cr ( $r = 0.411$ ), Fe-Cu ( $r = 0.404$ ), Fe-Cd ( $r = 0.373$ ) and Pb-Fe ( $r = 0.358$ ), thus demonstrating their probable common variations/origin in the patients. The rest of the metals exhibited statistically insignificant positive/negative relationships. However, among the metals, only Zn was not significantly associated with any other metal; thus, it revealed almost independent dispersion in the scalp hair of the patients. Toxic metals showed positive association with the essential metals which indicated build-up of the toxic metals in the patients.

In the case of healthy donors, the correlation study revealed significantly different mutual associations among the metals (Table 3). Statistically, strong/significant correlation ( $p < 0.05$ ) was found between Fe-Cr ( $r = 0.674$ ), while fairly significant relationships were displayed by Fe-Cd ( $r = 0.424$ ), Mn-Cr ( $r = 0.401$ ) and Cr-Cd ( $r = 0.367$ ). The correlation study indicated more or less independent distribution of Cu, Zn and Pb; they showed insignificant relationships with other metals in the case of healthy subjects. Overall, the results of the correlation study of the metals were considerably divergent in the healthy donors than in the patients, which may be attributed to the disproportions/

**Table 2** Statistical distribution parameters for the levels ( $\mu\text{g/g}$ , dry weight) of selected metals in the scalp hair of stomach cancer patients and controls

		Cd	Cr	Cu	Fe	Mn	Pb	Zn
Patients	Minimum	0.073	0.103	3.300	2.790	0.299	0.085	64.32
	25th percentile	0.344	3.500	8.045	20.50	1.263	6.514	117.4
	Mean	0.961	6.294	10.65	40.39	3.709	15.80	186.1
	Median	0.681	4.910	9.300	34.60	2.991	10.89	160.6
	75th percentile	1.384	7.155	12.02	46.36	4.118	20.26	251.3
	Maximum	6.554	33.78	39.95	170.7	16.01	84.67	665.7
Controls	Minimum	0.036	0.107	0.456	12.08	0.379	0.219	15.63
	25th percentile	0.840	1.728	7.400	29.24	2.622	6.806	137.6
	Mean	1.720	4.349	10.50	57.16	5.606	15.51	184.2
	Median	1.460	3.460	9.578	55.29	4.304	12.04	159.6
	75th percentile	2.626	5.862	13.04	79.93	7.509	23.72	208.7
	Maximum	5.825	14.89	37.77	139.4	17.38	46.39	649.0
	*Z value	-3.882	-3.431	-1.483	-4.073	-3.614	-1.908	-1.323
	*p value	0.0001	0.0006	0.115	0.00001	0.0003	0.0816	0.187

\*Wilcoxon rank-sum test

**Table 3** Correlation coefficient ( $r$ ) matrix for selected metal levels in the scalp hair of stomach cancer patients (below the diagonal) and controls (above the diagonal)

	Cd	Cr	Cu	Fe	Mn	Pb	Zn
Cd	1	<i>0.367</i>	0.099	<i>0.424</i>	0.15	0.204	0.118
Cr	<i>0.596</i>	1	0.029	<i>0.674</i>	<i>0.401</i>	0.084	0.072
Cu	0.316	0.331	1	0.194	0.132	0.1	0.348
Fe	<i>0.373</i>	<i>0.411</i>	<i>0.404</i>	1	0.342	0.207	-0.029
Mn	0.138	0.057	<i>0.669</i>	0.144	1	0.081	0.022
Pb	<i>0.455</i>	0.232	<i>0.528</i>	<i>0.358</i>	0.069	1	0.004
Zn	-0.107	-0.038	0.155	0.041	0.024	-0.192	1

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

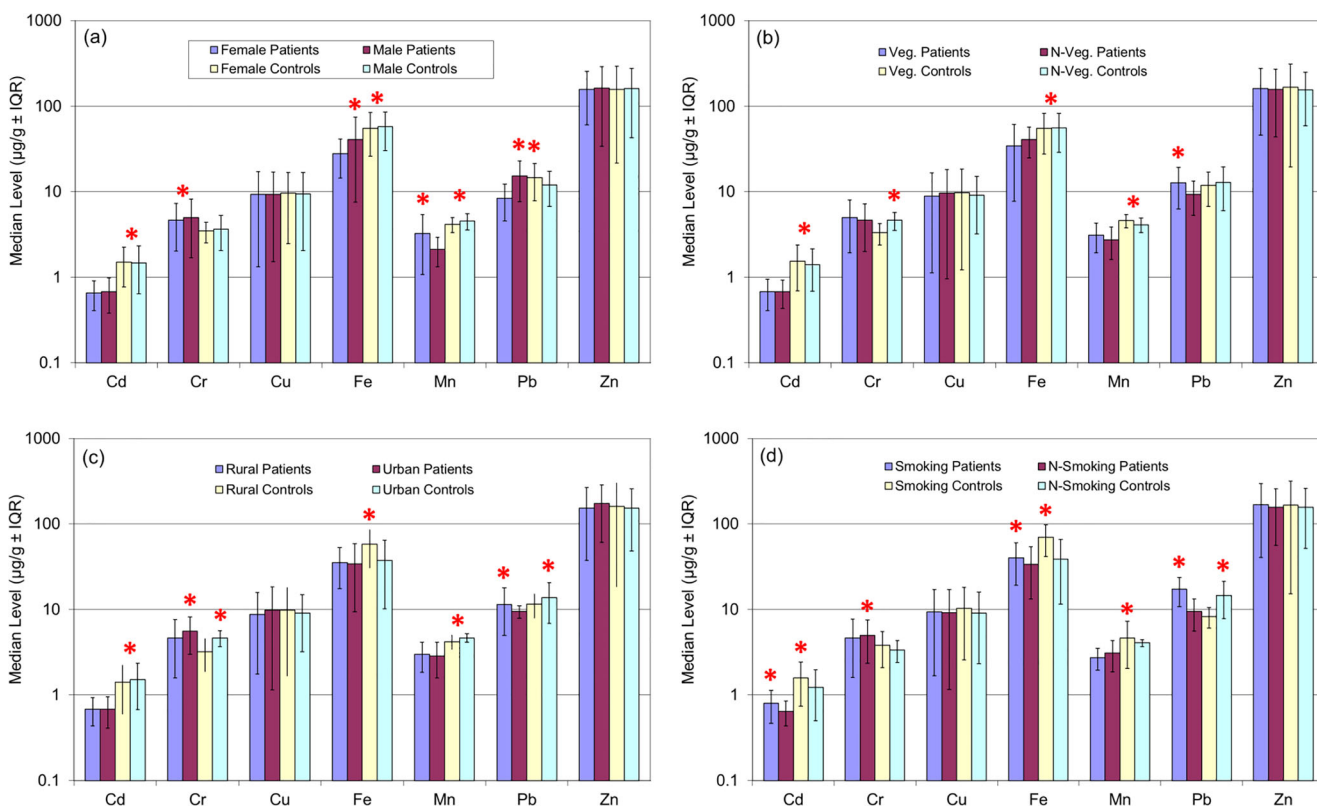
imbalances of metals in the stomach cancer patients. In the present study, Pb and Fe exhibited significant positive correlation in the case of the cancer patients; these two metals can play a critical role in the initiation and progression of oxidative stress which may lead towards the progression of various malignancies.

### Comparison of the Metal Levels Based on Gender, Abode, Diet and Smoking Habits

Comparison of median levels of the metals ( $\pm$  IQR) in the scalp hair of patients and healthy donors based on gender, habitat, dietary and smoking habits is given in Fig. 1a–d, wherein the statistically significant differences are highlighted

as asterisk (\*). In the case of gender-based comparison, median levels of Cd were found to be 54–56% higher in the scalp hair of male/female controls than in the counterpart patients. However, median levels of Cr in the case of female and male patients were 25–26% higher than in the female and male controls ( $p < 0.05$ ). In the case of Fe, median levels found in the scalp hair of male and female controls were 29–49% higher than in the corresponding patients among which median level of Fe was 32% higher in male donors than in female donors. Likewise, median levels of Mn in the scalp hair of female/male controls were 22–53% higher than in the counterpart patients; however, female patients showed considerable increase (34%) in Mn level compared with the male patients. An opposing variation was noted for Pb; median levels found in the male patients and female controls were 45% and 18% higher than in the female patients and male controls, respectively. Among the selected metals, median levels of Cu and Zn showed almost comparable contributions and insignificant gender-based differences for both patient and control groups (Fig. 1a).

Diet is the main pathway by which exposure of metals occurs in the human body. Diet has been postulated as an important aspect in the aetiology of stomach cancer [9], and many studies have been carried out on the associations between various dietary constituents and this malignancy [49]. It is obvious therefore that food habits and intake of nutrients



**Fig. 1** Comparison of the median levels of metals ( $\pm$  IQR) in the scalp hair of patients and controls based on **a** gender, **b** dietary habits, **c** habitat and **d** smoking habits



play a key role in the prevention and causation of stomach cancer [1]. Figure 1b shows comparative median levels of the metals with respect to nutritional habits which include vegetarian and non-vegetarian. Based on the median levels, Cd and Fe exhibited considerably elevated levels (52–56% and 26–38%, respectively) in the scalp hair of non-vegetarian and vegetarian controls than in the counterpart patient groups. However, Cr showed a significant increase (29%) in the case of non-vegetarian controls than in the vegetarian controls. The median level of Mn was found to be 33% higher in the scalp hair of vegetarian controls than in the vegetarian patients. Nonetheless, vegetarian patients showed 27% higher median Pb level than in non-vegetarian patients. Median levels of Cu and Zn showed insignificant dietary-based differences in both patients and controls (Fig. 1b).

Habitat-based comparison as shown in Fig. 1c revealed that rural/urban controls showed considerably higher contributions of Cd and Mn (52–55% and 29–39%, respectively) than the counterpart patient groups. However, median levels of Cr in the scalp hair of urban patients and urban controls were 18% and 31% higher than the rural patients and controls, respectively. The median level of Fe was found to be 35% higher in the scalp hair of rural controls than the urban controls. Pb showed conflicting results; relatively higher median levels were found in the rural patients (17%) than the urban patients while urban controls showed comparatively higher level (15%) than the rural controls. Zn and Cu once again showed insignificant differences in their median levels among the rural and urban subjects of both donor groups.

Epidemiological studies revealed that cigarette smoking plays a critical role in the development of stomach cancer [35]. Smoking is considered to reduce prostaglandins which establish gastric mucosal integrity [29]. Tobacco smoke is known to influence the growth/development of precursor gastric lesions such as ulceration, gastritis, and intestinal metaplasia [29]. It was found that the aetiology of stomach tumour in tobacco smokers is nearly twofold higher than in non-smokers [35]. Almost 18% of stomach cancer risk is comprised of smoking as shown in the European Prospective Study. A few other reports revealed the similarly high rates in their findings [11]. So, it was requisite to evaluate the effects of the metal exposure through cigarette smoking. The smoking-based comparison shown in Fig. 1d pointed out that the median levels of Cd were considerably higher in the scalp hair of smoking patients (20%) and smoking controls (22%) than the counterpart non-smoking subjects. Similarly, median levels of Fe were also found to be higher for smoking patients (15%) and smoking controls (44%) than the non-smoking patients/controls. However, the median level of Pb was found to be 45% higher in the scalp hair of smoking patients than the non-smoking patients while non-smoking controls exhibited 43% rise in the median level of Pb than the smoking controls. In the case of Mn, smoking controls showed 41% increase in the

median level than the smoking patients while in the case of Cr, non-smoking patients showed 32% higher median level than the non-smoking controls. Median levels of Zn and Cu were almost comparable in all groups, thus manifesting insignificant variations in the study population.

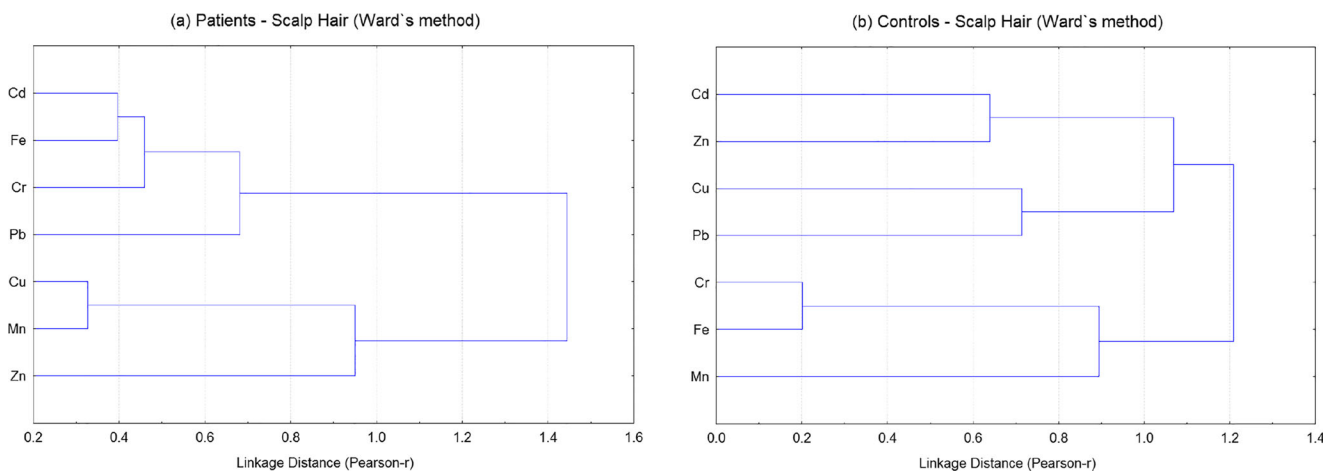
## Multivariate Analysis

Multivariate statistical methods such as PCA and CA were implemented for the source apportionment of metals in the scalp hair of the patients and controls [27]. The PCA of metals in the scalp hair of patients extracted by using varimax normalized rotation on the data set is depicted in Table 4, while their corresponding CA based on Ward's method is displayed in Fig. 2a. Two principal components (PCs) (eigenvalues > 1) were extracted which accounted for more than 63% of the total variance of data. The first principal component accounted for 42.70% of the total variance, showing higher loadings for Cd, Fe, Cr and Pb which shared a common cluster of Cd, Fe, Cr and Pb in CA. This PC pointed out that these metals can be controlled by the lifestyle of the donors and originated mostly from the mixed sources comprising of the anthropogenic contaminations/environmental pollutants. It is advocated that Cd, Cr and Pb are known to augment the oxidative stress of the body which is believed to be a reason for the initiation of cancer [24, 26]. Similarly, the second principal component with 20.42% loading of the total variance exhibited dominant loadings for Cu, Mn and Zn with a joint cluster of these metals in CA. This group of metals was mainly contributed by the nutritional habits of the patients. Thus, CA revealed that toxic trace metals share communal clusters with the essential metals, demonstrating the imbalances of metals in the patients.

In the case of healthy donors, three major components with eigenvalues > 1 were extracted which demonstrated more than

**Table 4** Principal component loadings of selected metals in the scalp hair of the stomach cancer patients and controls

	Patients		Controls		
	PC 1	PC 2	PC 1	PC 2	PC 3
Eigenvalue	2.989	1.429	2.429	1.308	1.078
Cumulative eigenvalue	2.989	4.418	2.429	3.738	4.815
Total variance (%)	42.70	20.42	34.71	18.69	15.40
Cumulative variance (%)	42.70	63.11	34.71	53.40	68.79
Cd	0.883	-	0.395	-	0.738
Cr	0.754	-	0.883	-	-
Cu	0.497	0.787	-	0.766	-
Fe	0.795	-	0.913	-	-
Mn	-	0.833	0.511	-	-
Pb	0.677	-	-	0.806	-
Zn	-	0.589	-	-	0.844



**Fig. 2** a, b Cluster analysis of selected metals in the scalp hair of patients and controls

68% of the cumulative variance of the metal data (Table 4). The corresponding CA displayed in the form of dendrogram is shown in Fig. 2b. The first principal component which accounts for 34.71% of the total variance indicated the highest build-up of Fe, Cr and Mn, with a similar strong cluster in CA. The major contributors for the second principal component with 18.69% of the total variance were Cu and Pb, duly supported by a strong cluster in CA. The highest loadings of Cd and Zn were noted in the third principal component which contributed a loading of 15.40% of total variance. These three PCs were chiefly contributed by the dietary habits and environmental contamination by anthropogenic sources. Overall, PCA and CA showed communal associations of essential and toxic metals in the patients. Such relationships may be attributed to the onset/progression of the disease including cancer.

### Comparison of the Metal Levels Based on Types/Stages of Cancer

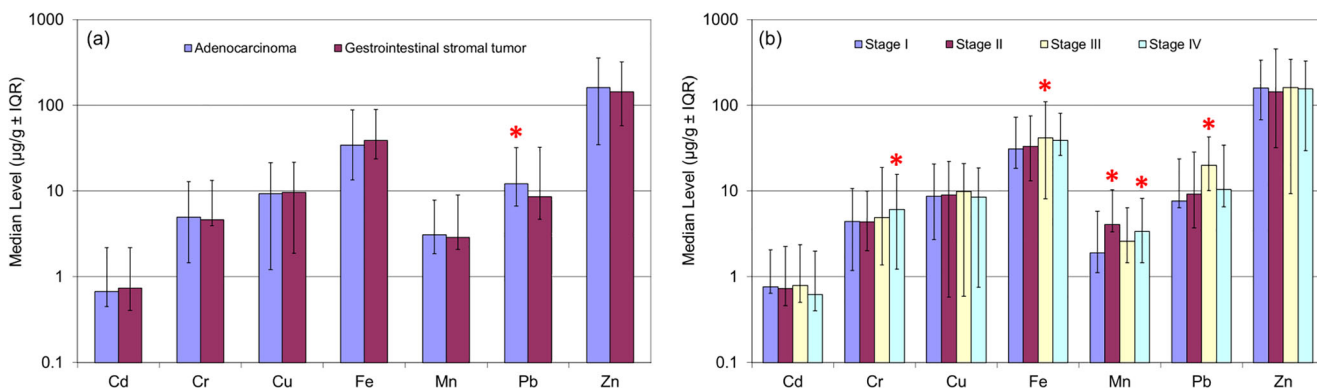
Comparative evaluation of median metal levels in the scalp hair of various types (i.e., adenocarcinoma and gastrointestinal stromal tumour) and stages (I, II, III and IV) of stomach cancer patients is displayed in Fig. 3, wherein the statistically significant differences are highlighted as asterisk (\*). The

median level of Pb was found to be significantly higher (30%) in the scalp hair of gastrointestinal stromal tumour patients compared with the adenocarcinoma patients. However, the median levels of the rest of the metals (Cd, Cr, Cu, Fe, Mn and Zn) were almost comparable and exhibited statistically insignificant variations in the scalp hair of both types of stomach cancer patients (Fig. 3a).

Based on the stage, Cd, Cu and Zn showed insignificant differences among their median levels at all four cancer stages. However, Cr exhibited 28% increase in the median level at stage IV compared with stage I. Likewise, Fe showed 27% increase in the median level moving from stage I to stage III. In the case of Mn, the median level at stage II was found to be 53% higher than at stage I, while the median level at stage IV was 23% higher than at stage III. Pb also showed a gradual build-up in its level with cancer stages; the median level of Pb at stage III was found to be 62% higher compared with stage I (Fig. 3b).

### Role of Trace Metals in Stomach Cancer

Chromium in trivalent form is considered as essential playing a significant role in carbohydrate metabolism while in hexavalent form it is classified as carcinogen to humans by



**Fig. 3** Comparison of median metals levels ( $\pm$  IQR) in the scalp hair of patients based on the a types and b stages of stomach cancer

IARC (Group 1). Cellular metabolism of Cr can cause both oxidative and non-oxidative forms of DNA damage and it also revealed genotoxic effects [12, 31]. Chromium-induced mutations can be generated through different types of DNA damage such as DNA protein cross-links, DNA-DNA cross-links, Cr-DNA adducts and oxidative damage [13]. Many studies suggested that Cr may have carcinogenic effects in the stomach of humans, which is consistent with the results reported in rodent studies [52]. It has been reported that Cr can induce DNA lesions in peripheral blood lymphocytes and human gastric mucosa cells partly due to direct contact between Cr and the stomach mucosa. The epidemiological evidence for increased risk of stomach cancer with ingestion of Cr is limited [59]. The p53 tumour suppressor protein is decreased in the stomach of rats treated with Cr [54]. In China, high incidence in stomach cancer mortality was noted in an ecologic study of villagers exposed to Cr in drinking water [2]. In the present study, Cr level was found to be considerably higher in the scalp hair of patients compared to controls clearly showing the adverse effect of Cr overload in the patients.

Cadmium is a toxic pollutant which can affect multiple cellular processes including cell cycle progression, proliferation, differentiation, DNA replication and apoptosis [5, 32]. It can interfere via substitution with Fe, Cu and Zn in various cytoplasmic and membrane proteins, and enhancing the cellular amount of free redox active metals could induce oxidative stress via Fenton reaction [5]. Chronic exposure to Cd could inhibit the activity of superoxide dismutase as one of the strongest antioxidant enzymes [32]. Lipid peroxidation by Cd impairs the gastric mucosa, disrupting the mucosal barrier and increasing the vulnerability of the gastric mucosa and ulceration [51]. Chronic Cd exposure may result in the formation of dysplastic lesions in gastric glandular epithelium [30]. Cadmium-induced disruption of E-cadherin affects cell-cell junctions and may describe a key step in the cancer-initiating and tumour-promoting properties of Cd [36, 54]. It can accelerate cancer development by activating protooncogenes and genes involved in cell proliferation and by inhibiting DNA methylation, which increases clonal expansion of damaged and mutated cells, as well as mitigates p53 function, thus accelerating cancer development [3].

Zinc is a constituent of superoxide dismutase, an enzyme that eliminates free radicals [56]. It is a cofactor of many proteins which regulate critical cellular functions such as, immunity, cell cycle progression, response to oxidative stress, DNA replication and damage/repair [25]. Zinc plays a key role in antiangiogenic activity of endostatin cell proliferations and intracellular signalling pathways [14]. Some case-control studies investigated the association between Zn intake and risk of stomach cancer [25, 33]. It has been reported that higher Zn intake was linked with reduced stomach cancer risk [33]. A study revealed that serum Zn level was significantly lower in stomach cancer patients than controls, and decreased serum Zn

level may be associated with an increased risk of stomach cancer [56]. An inverse association between Zn level in toenail and stomach cancer risk was reported by Campos et al. [6].

Copper is considered essential but due to its redox activity, it is well suited to facilitate the formation of ROS which can attack DNA and cause mutation, and/or promote epigenetic changes [19, 28]. In humans, a strong link has been established between oxidative damage to cells and cancer [15]. It is an essential component of angiogenesis, therefore, utilized by tumours for growth. In addition, Cu can be involved in the activation of several organic peroxides and making them more carcinogenic. The level of Cu in stomach cancer patients was found to be significantly increased in comparison with healthy individuals [4] but scalp hair Cu level was significantly higher in the stomach cancer patients as compared to controls [18].

The IARC classified inorganic Pb compounds as probably carcinogenic to humans (Group 2A) and its exposure is recognised as augments risk of stomach cancer [16]. Lead is involved in the components of cellular damage by inducing oxidative stress via formation of reactive oxidants [24]. It can inhibit DNA repair and acts synergistically with other mutagens [54, 58]. Epidemiologic studies suggested possible relation of Pb exposure and stomach cancer [21, 41]. An increased risk of stomach cancer among workers with high exposure to Pb was reported in some studies [26, 46]. The mechanisms involved can be DNA-repair inhibition, oxidative DNA damage, gene amplification, genomic instability, aneuploidy and epigenetic effects [48].

Manganese is a structural constituent of antioxidant enzyme, manganese peroxide dismutase that eases the toxic effects of ROS [40]. This enzyme was reported to be deficient in lung and breast cancers patients [7, 55]. Some studies showed a probable association of Mn with cancer, but the results were conflicting [44, 57]. According to Janbabai et al. [18], level of Mn in hair samples was significantly elevated in stomach cancer cases than in controls. However, the present study showed increased Mn level in the controls compared with the stomach cancer patients.

Iron is vital for oxygen transport and oxidative metabolism; both excess and deficiency of Fe is detrimental [37]. Stomach cancer has been linked with low level of Fe [23]. Its deficiency can boost DNA damage, reduce antioxidant defence, decrease enzymatic activity and increase genomic instability [47]. Its deficiency also augments and accelerates *H. pylori* which was reported to induce stomach inflammation and carcinogenesis [6]. It was found that an Fe level was lower in the cancerous tissues compared with normal tissues [38] and it may be due to lesser absorption of Fe in the cancerous tissue [39]. Cohort studies demonstrated inverse associations between lower level of serum Fe and gastric cancer [8]. The results of the present study manifesting lower Fe level in the scalp hair of stomach cancer patients compared to controls (Table 2) corroborate with the above findings.



Stomach cancer is a major health problem among Pakistani population [10]. Most of the patients at the time of diagnosis were found at late cancer stage; late diagnosis and late seeking of medical treatment are resulting in low survival rates. Mass screening programs may help to detect this malignancy at the early stage and will prove useful as preventive measures. Another important approach to control this lesion is the dietary modifications because food is the main source of metals. Local authorities should adopt more stringent measures to reduce the contaminant emissions and monitor them in food products. Further, effective measures should be adopted to combat the tobacco epidemic.

## Conclusions

In conclusion, the median level of Cr was significantly higher ( $p < 0.05$ ) in the scalp hair of stomach cancer patients, while median levels of Cd, Fe and Mn were significantly elevated in the scalp hair of healthy donors. The correlation study revealed divergent relationships among the toxic/essential metals in the patients and controls; it was further clarified by PCA and CA which revealed divergent sources/grouping of the metals in the patients and controls. Most of the metals exhibited considerable disparities in their median levels based on habitat, food habits, smoking habits and gender of the subjects. Among the types of cancer, gastrointestinal stromal tumour patients showed significantly elevated level of Pb in their scalp hair. Similarly, median levels of Fe and Pb were found to be considerably elevated at stage III, while Cr level was found to be higher at stage IV in the scalp hair of cancer patients. In addition, Mn showed the highest level at stage II. Hence, the disruption in the balance of trace/toxic metals in the scalp hair of the patients may possibly indicate the development and progression of the malignancy.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflicts of interest.

**Ethical Approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Ethical Review Committee, NORI and PIMS, Islamabad REF. NO. QAUC-2014-A631 and QAUC-2014-A633, respectively) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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