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Investigation of imbalances in essential/toxic metal levels in the blood of laryngeal cancer patients in comparison with controls

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Abstract Laryngeal carcinoma is one of the common types of head and neck cancer, with men being more likely than women to develop it. Diet, age, gender, smoking habits, and environmental factors play important roles in its development. The goal of this study was to ascertain if there were imbalances in essential and toxic trace metals owing to the initiation and progression of laryngeal cancer. Atomic absorption spectrometry was employed to quantify selected macroelements, and essential/toxic trace metals in blood of the cancerous patients and matching controls. Significantly higher concentrations of Pb, Cu, Fe, and Sr while substantially lower levels of Na, K, Ca, and Mg were observed in the cancer patients compared with the controls. Considerably disparate mutual relationships among the macroelements, and essential/toxic trace metals in the patients and controls were manifested by their correlation coefficients. Similarly, multivariate apportionment of the metal levels showed appreciably diverse associations and grouping in the patients and controls. The laryngeal cancer patients exhibited significant disparities in the metal levels among various sub-types (supraglottic, subglottic, transglottic, and glottic cancer) and stages (I, II, III, and IV) of the disease. Most of the metals revealed distinct differences based on the gender, habitat, age, eating preferences, and smoking habits in both donor groups. Overall, the study demonstrated significant imbalances among the macroelements, and essential/toxic trace metal levels in the blood of laryngeal cancer patients compared to the controls.

Keywords Laryngeal cancer · Blood · Toxic metal · FAAS · Pakistan

Introduction

Laryngeal cancer is characterized by the formation of malignant cells in the tissue of the larynx. It is the second most common respiratory cancer after lung cancer and eleventh most common form of cancer among men worldwide (Alessandrini et al. 2020). The larynx is present between the base of the tongue and trachea; it is further divided into three anatomical regions including glottis, supraglottis and subglottis. It is also known as a sound box as it contains the vocal cords, which vibrate and make sound (Zhang et al. 2020). Laryngeal cancer is usually characterized by sore throat or cough for a long period of time, trouble/pain when swallowing, ear pain, lump in the neck or throat and hoarseness in the voice. Use of tobacco products, smoking and too much consumption of alcoholic beverages can cause laryngeal cancer

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(Franz et al. 2020). Anatomically most common type of laryngeal cancer is one which originates in glottis known as galactic tumour, while supraglottic and subglottic tumours are less common. Laryngeal cancer can spread to nearby tissues, such as, trachea, thyroid or oesophagus (Elicin and Giger 2020; Games et al. 2020). In 2018, almost 177,000 laryngeal cancer cases and 94,800 deaths were reported worldwide, while in 2020 almost 12,370 adults were reported with laryngeal cancer (associated 3750 deaths) in the United States only (Dong et al. 2020; He et al. 2020).

Trace metals play several important roles in a variety of physiological processes in the human body, but an imbalance in the metal levels can lead to a variety of disorders, including cellular damage, inflammation, cardiovascular disease, and cancer (Crans and Kostenkova 2020). In general, the carcinogenic progressions are linked to an excess or enrichment of toxic/carcinogenic metals, while most of the essential metals are found to be depleted or deficient in the cancerous patients. The exposure to toxic metals may occur through variety of processes; they are primarily acquired through occupational exposure (including industries and chemical laboratories), combustion processes and contaminated food/water (Khan et al. 2020). The essential metals, on the other hand, are found to be protective, however the excessive intake of these essential minerals can also lead to harmful effects (Fu and Xi 2020). Evaluation of trace elements in blood is considered imperative as imbalances in the essential and toxic trace metals play vital role in the occurrence and diagnosis of various physiological disorders (Brrow and Bargiul 2021; Chen et al. 2020; Duan et al. 2020; Stojsavljević et al. 2020). Blood is the most important medium to transport the macronutrients and trace metals in the tissues/organs and thus it can provide dependable and prompt information about the body's metabolism (Ahmed et al. 2020; Bao et al. 2021). In addition, it can provide indication about the absorbed materials by the body in recent times, and it is relatively easy to collect the blood sample (Goyal et al. 2021; Qayyum and Shah 2019). The literature reported studies related to the associations among the essential and toxic trace metals in the blood of laryngeal cancer patients are scarce (Lubinski et al. 2021; Taysi et al. 2003). The present study was therefore designed to evaluate the levels of selected macroelements (Na, K, Ca, Mg, and Fe), and essential/toxic trace metals including Sr, Zn, Cu, Mn, Co, Li, Ag, Cr, Cd, Ni, and Pb in the blood samples collected from newly diagnosed laryngeal cancer patients and counterpart controls. The primary objective was to appraise the plausible imbalances in the macroelements, and essential/toxic trace metal contents in the cancer patients in comparison with the controls and their impact on the patients' health. The study also focuses on the assessment of a potential relationships between demographic characteristics of the patients and controls through appropriate statistical methods in order to examine if the metal contents are statistically divergent and dependant on the cancer type and/or stage.

Experimental methodology

Study design and subjects

The blood sample was collected from newly diagnosed laryngeal cancer patients ($n=80$) admitted in Nuclear Oncology and Radiotherapy Institute (NORI), Islamabad, Pakistan. Their counterpart controls ($n=80$) were selected on a volunteer basis, and they were chosen in such a way that their demographic parameters such as, age, gender, habitat, diet and socioeconomic status matched with the corresponding patient (Russano et al. 2020). The whole research programme and its purposes were explained to ethical review committee of the institute and the samples were collected after the approval from the ethical review committee of NORI. Only those patients were included in the current research study who were newly diagnosed and had not undergone chemotherapy or radiotherapy. Controls were mostly close family members and acquaintances of the patients. For each subject, pro-forma was filled out that included the donor's age, health, ailment, lifestyle, nutritional habits, smoking habits, employment history, socio-economic status, and education (Chen et al. 2020). Before the sample collection, all participants were briefed about the study's goals and procedures, and all subjects or their next of kin/parents in the case of young participants (under 18 years) signed a written informed consent form (Duan et al. 2020).

Collection and processing of the samples

Blood samples (approximately 3 mL) were collected from an antecubital vein through the vein puncture method and the samples were stored in metal free vacutainer tubes (BD Vacutainer Ref. 366,430) which were without any anticoagulant. Before further processing of the blood samples, they were stored in a refrigerator (Ahmed et al. 2020). In order to transform the blood sample into a form in which the metal analysis can be performed, about 3 mL of the blood sample was transferred from the collection tube to a digestion flask and the digestion process was carried with the combination of two mineral acids (HNO_3 and $HClO_4$). First 10 mL of HNO_3 was added to the digestion flask containing the sample and placed on a hot plate for 3 h. Then digestion flask was removed from the hot plate and 10 mL of $HClO_4$ was added into it after cooling. The digestion flask was again placed on the hot plate and the digestion process completed within 3–4 h until the evolution of white dense fumes. Blank was prepared in the same way with each batch of 5 samples. After the completion of digestion process, each sample was diluted with 0.1 N HNO_3 in a 50 mL volumetric flask (Stojsavljević et al. 2020; Brrow and Bargiul 2021).

Quantification of the metals

A flame atomic absorption spectrophotometer (Shimadzu AA-670, Japan) was used to analyse the selected macroelements (Na, K, Ca, Mg, and Fe), and essential/toxic trace metals including Sr, Zn, Cu, Mn, Co, Li, Ag, Cr, Cd, Ni, and Pb under optimal operational conditions which are mentioned in Table S1 (supplementary material). Both essential and toxic metal contents were evaluated in the present study in order to establish any plausible relationship between the imbalances of the elemental levels and the onset/progression of the carcinogenesis in the patients. Analytical grade reagents were employed to prepare the samples and standards in the present study. The working standards were prepared by serial dilution of 1000 mg/L standard stock solution right before the samples analysis on the instrument. All measurements were made in triplicate and run independently on the spectrophotometer to pool the average concentration. The samples were also examined at an independent laboratory for comparison purposes, and the results of the two laboratories differed by no more than 2.5% as shown in Table S2 (supplementary material). Standard reference material (NIST SRM 1598a) was also analysed to verify the accuracy of quantified results and it revealed excellent recoveries as shown in Table S2 (supplementary material). The blank contributed about $<5\%$ of the observed concentrations of the samples in general (Manousi et al. 2021; Bahadır and Mermer 2021).

Statistical analysis

Univariate and multivariate statistical methods were used to investigate the relative distribution and mutual relationships among the macroelements, and essential/toxic trace metal levels in the patients and controls. The basic statistical parameters such as, quartiles, range, median, mean, relative standard deviation, and skewness were computed to assess the distribution of individual metals while student t-test was used to validate the significant differences between the patients and controls ($p < 0.05$). Spearman correlation was calculated to establish the relationships among the macroelements, and essential/toxic trace metals in the blood of both donor groups. Multivariate principal component analysis (PCA) and cluster analysis (CA) were performed to evaluate the complex relationships among the metal levels in the patients and controls (Leventeli and Yalcin 2021). The PCA was performed on the dataset using a varimax normalised rotation, while the CA was performed on the standardised matrix and the results are displayed in the form of a dendrogram which demonstrates the levels of similarity between the variables using Ward's method (Liu et al. 2021).

Result and discussion

Characteristics of the study subjects

Table 1 shows the demographic characteristics of the laryngeal carcinoma patients and counterpart controls. Along with the clinical evaluation, the malignancy of laryngeal cancer was established by histopathological assessment. The patients ranged in age from 11 to 66 years, with a mean of 44 years, while the controls ranged in age from 13 to

Table 1 Demographic characteristics of the subjects

Characteristics	Laryngeal cancer patients	Controls/healthy donors
n	80	80
Age (years)		
Range	11–66	13–66
Mean	44	44
Gender		
Female	38 (48%)	38 (48%)
Male	42 (52%)	42 (52%)
Diet		
Vegetarian	41 (51%)	36 (45%)
Non-vegetarian	39 (49%)	44 (55%)
Habitat		
Urban	39 (49%)	38 (47%)
Rural	41 (51%)	42 (53%)
Tobacco use (Smoking)		
No use	39 (49%)	51 (64%)
Use	41 (51%)	29 (36%)
Stages of the cancer		
Stage I	30 (37%)	
Stage II	31 (39%)	
Stage III	15 (19%)	
Stage IV	04 (5%)	
Types of laryngeal cancer		
Supraglottic cancer	15 (19%)	
Subglottic cancer	25 (31%)	
Transglottic cancer	19 (24%)	
Glottic cancer	21 (26%)	

66 years, with a mean of 44 years. More than half of the participants (52%) were the male subjects in both groups. About 51% of the patients and 45% of the controls were vegetarians. Similarly, 49% of the patients and 47% of the controls were inhabiting in urban settings. Almost half of the patients (49%) were not addicted of smoking while among the controls, the non-smoker subject accounted for 64% of the cases. According to the type of laryngeal cancer, 19% were suffering from supraglottic cancer, 31% from subglottic cancer, 24% of transglottic cancer, and 26% from glottic cancer. In the current study, 37% of the patients were diagnosed at stage I, 39% at stage II, 19% at stage III, and 5% at stage IV of laryngeal cancer.

Distribution of the metals

Table 2 shows the descriptive statistics related to the concentrations of macroelements, and essential/toxic trace metals in the blood of the patients and controls. It was evident from the data that there were significant disparities among the measured concentrations in both donor groups; practically majority of the metals exhibited wide range of concentrations as well as broad and lopsided variations in the quartile levels as shown in Table 2 and Figure S1 (supplementary material). The average levels of K in the blood of the patients were predominantly higher, followed by Fe, Na, and Ca while on the other hand, appreciably lower levels were noted for Li, Ag, Cd, and Mn. The mean and median levels for most of the metals were considerably different hence demonstrating random distribution of the metals. Relatively lower RSD and skewness was shown by Zn and Fe compared with the other metals, thus indicating rather Gaussian/symmetrical distribution of these metals in the patients. However, Cu and Cd showed more randomness and asymmetry in the patients as manifested by their relatively higher RSD and skewness values (Table 2).

In the case of controls, K was found to be the dominant contributor, followed by Na, Fe, Ca, and Mg whereas Ag, Cd, and Li demonstrated the lowest mean concentrations. Relatively higher dispersion in the controls was shown by Fe, although Zn, Co, and Cd also revealed noticeably higher randomness and asymmetry. On the average basis, the macroelements, and essential/toxic trace metal contents in the blood of patients exhibited following order: K > Fe > Na > Ca > Mg > Pb > Ni > Zn > Co > Sr > Cu > Cr > Mn > Cd > Ag > Li, while in the case of controls, following was the decreasing order: K > Na > Fe > Ca > Mg > Zn > Ni > Co > Cr > Pb > Sr > Cu > Mn > Ag > Cd > Li. It is evident from this comparative evaluation that Na > Fe in controls while Fe > Na in the case with patients. Sodium is an important electrolyte, and it controls extracellular fluid and movement of the molecules across cell membranes and any fluctuation in the required amount of Na can lead to the disturbance in the blood pressure (Landi et al. 2019; Lee et al. 2018). Deficiency of Na is termed as hyponatremia and it is characterized by large number of physiological disorders ranging from the loss of consciousness to heart disorders (Allison and Fouladkhah 2018). Nevertheless, Fe was found to be significantly higher

Table 2 Statistical distribution parameters for the concentrations of selected macroelements, and essential/toxic trace metals (μg/g) in the blood of laryngeal cancer patients and controls

	Patients						Controls						*p*-value
	Min	Max	Mean	Median	RSD	Skewness	Min	Max	Mean	Median	RSD	Skewness	
Na	115.8	764.3	219.4	183.4	0.527	2.880	99.89	776.3	240.7	200.7	0.533	1.874	NS
K	179.8	968.4	414.2	378.1	0.333	1.803	223.3	967.0	450.0	389.6	0.384	1.104	NS
Ca	9.894	149.7	48.37	44.74	0.524	1.700	10.89	199.4	56.52	52.98	0.650	1.736	NS
Mg	12.99	46.37	27.92	27.92	0.197	0.293	12.41	66.49	31.42	30.36	0.238	1.227	NS
Sr	0.146	8.804	2.056	1.525	0.938	1.967	0.030	4.750	1.431	1.402	0.672	0.759	NS
Fe	76.91	622.6	396.9	400.2	0.300	– 0.380	46.39	387.0	95.83	82.49	0.614	3.775	< 0.05
Zn	0.282	6.964	3.562	3.578	0.430	– 0.003	1.484	70.69	9.864	5.058	1.176	3.059	< 0.05
Cu	0.239	10.66	1.679	1.348	0.883	3.748	0.018	2.644	1.066	0.951	0.585	0.508	NS
Mn	0.033	2.110	0.531	0.423	0.802	1.716	0.021	2.833	0.636	0.521	0.868	1.913	NS
Co	0.024	11.35	2.938	2.093	0.952	1.520	0.376	34.89	5.899	4.055	1.064	2.192	< 0.05
Li	0.020	1.103	0.172	0.100	1.207	2.885	0.027	0.701	0.187	0.131	0.886	1.403	NS
Ag	0.016	0.690	0.176	0.157	0.795	1.483	0.000	2.568	0.497	0.340	1.008	1.834	< 0.05
Cr	0.027	5.898	1.419	1.231	0.788	1.941	0.181	10.82	2.708	2.399	0.752	1.632	< 0.05
Cd	0.019	1.806	0.230	0.158	1.386	3.547	0.028	1.057	0.223	0.132	1.015	2.033	NS
Ni	0.130	19.17	3.862	3.324	0.861	2.202	0.459	25.59	8.548	7.463	0.756	0.788	< 0.05
Pb	0.118	19.72	4.271	2.592	1.043	1.727	0.052	4.212	1.704	1.848	0.647	0.352	< 0.05

NS nonsignificant

($p < 0.05$) in the blood of patients than controls; although it is an essential nutrient, but its excess can cause hemochromatosis, and cell injury (Muckenthaler et al. 2017; Musallam and Taher 2018). The hemochromatosis can induce cancer by damaging the hepatocytes which cause excessive absorption and retention of iron inside the body (Gozzelino and Arosio 2016; Zhou et al. 2018; Galaris et al. 2019). Excess of iron can also alter the T-lymphocytes subset and their distribution in different compartments of the immune system (Handa et al. 2016). An adequate amount of iron is obligatory for regulating the expression of T-lymphocytes surface markers, hence increased iron content in the body can disrupt the immune system, leading to excessive growth of some cells, which ultimately turns into a carcinoma (Terpilowska and Siwicki 2018).

The present study also revealed some significant imbalances among the trace metal contents; in case of the patients Pb > Ni > Zn > Co > Cr while in the case of controls Pb < Cr < Co < Ni < Zn (Table 2). The concentration of Pb was found to be significantly higher ($p < 0.05$) in the patients compared to the controls, and it is even higher than Zn which is an essential trace metal. Lead is one of the toxic trace metals for humans and classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC). It can directly damage DNA and participate in the formation of reactive oxygen species (ROS), thus causing oxidative damage to DNA. Higher concentration of Pb can act as a substitute for Zn in many proteins which function as transcriptional regulators. It also decreases the ability of these proteins to get bound to recognition elements in genomic DNA, which leads to an altered gene expression and ultimately cancer.

In the case of controls, it was observed that Zn > Ni > Pb, however, in the patients Pb > Ni > Zn, which clearly showed that the essential trace metal, Zn was significantly higher than the toxic trace metals (Pb and Ni) in the controls. Nonetheless, the patients showed significantly lower concentration of Zn ($p < 0.05$) which is one of the essential trace metals and act as an antioxidant and inflammatory agent. An earlier study also reported significantly lower levels of Zn in blood serum of the laryngeal cancer patients than controls (Taysi et al. 2003). Many cancers are instigated by the oxidative stress and inflammations, but Zn can provide an effective remedy in the prevention of cancer. It can inhibit NF-kappa B, which is involved in the activation of antiapoptotic gene.

Therefore, Zn increases apoptosis in the cancer cells, due to which it is commonly used in the chemoprevention of various cancers. It has been reported that risk of death in patients with lower Zn contents was much higher compared to the patients with elevated Zn levels (Lubinski et al. 2021). Consequently, the deficiency of Zn in cancer patients can be one of the contributing factors towards the onset and progression of carcinogenesis. In addition, some other trace metals including Co, Ag, Cr and Ni also exhibited significant differences ($p < 0.05$) among the patients and controls thus indicating their imbalance in the blood of cancer patients. However, the measured levels of Na, K, Ca, Mg, Sr, Cu, Mn, Cd, and Li revealed insignificant disparities among the patients and controls. One of the previous studies reported insignificant differences among the Cd, Co, and Ni levels in cancerous and non-cancerous tissues of laryngeal cancer patients (Klatka et al. 2011).

Correlation study

The correlation study was undertaken to evaluate the relationships between the metal pairs in the blood of both donor groups as shown in Table 3, wherein the significant *r*-values are highlighted ($p < 0.05$). The following pairs were found to exhibit strong positive correlations ($r > 0.500$) in the blood of patients: Cd-Na, Na-K, Cd-Cu, Cd-K, Cd-Sr, Ni-Na, Sr-Na, Sr-K, Zn-Fe, Mn-Sr, Pb-Cd, Cu-K, Cu-Sr, Mn-Na, Mn-K, Na-Pb, K-Pb, and Co-Na. In addition, some significant correlations were noted between Fe-Mg, Cu-Na, Cu-Ca, Li-K, Li-Na, Cr-Na, Cr-Mn, Cd-Li, Ni-Sr, Ni-Mn, Pb-Co, and Pb-Ni in the patients. The correlation analysis revealed mutual associations among Na, K, Sr, Cd, Cu, Ni, Mn, Pb, Cr, and Li in the blood of the patients; hence, the study demonstrated significant interferences of toxic trace metals in the physiological functions of the macroelements, and essential metals. In the case of controls, significantly strong correlations were observed among Na-K, Mn-Na, Mn-K, Co-Na, Co-Mn, and Cd-Co (Table 3). Consequently, unlike the patients, no significant interferences of toxic trace metals were found in the controls. The correlation study revealed that Pb, Cd, and Ni (which are the toxic trace metals) demonstrated strong positive correlations with K and Na (which are essential metals) in the patients but there were no such correlations in the controls. Lead can inhibit the maturation of brain by blocking the receptor known as N-methyl-D-aspartate, thus leading to the abnormal thinking process, abnormal social behaviour, aggression, and difficulties in coordinating fine movements (Grant 2020). The effect of Pb poisoning is more obvious in children than in adults in terms of damaged nervous system (Kumar et al. 2020). Lead poisoning can also result in the abdominal pain, constipation, tiredness, headache, irritability, loss of appetite, memory loss, pain or tingling in the hands or feet and weakness (O'Connor et al. 2020). High exposure of Pb can cause stillbirths, miscarriages and infertility. The cardiovascular system is also disturbed by Pb exposure leading to heart attack and eventually death (Mohammadyan et al. 2019).

Potassium showed strong positive correlations with the toxic trace metals in the patients. It as an essential electrolyte and its channels are unique in their function and can control membrane potential and nerve/cardiac actions. It is also important for neurotransmitter release, insulin release, differentiation, activation, proliferation, apoptosis, and several other physiological functions (Leanza et al. 2016; Peruzzo et al. 2016). Although no strong evidence between the cancer and K levels had been established, but several researchers reported that balanced K levels can help in the prevention of tumorigeneses (Huang and Jan 2014; Litan and Langhans 2015; Prevarskaya et al. 2018). In recent studies various anticancer drugs containing potassium proves its significance in cancer prevention (Hoffmann and Lambert 2014; Checchetto et al. 2016; Prevarskaya et al. 2018). Accordingly, the correlation study revealed significantly dissimilar mutual dependence of the macroelements, and essential/toxic trace metals in the patients and controls.

Multivariate analysis

Figure 1 shows the CA of selected macroelements, and essential/toxic trace metals in the blood of both donor groups based on Ward's method while PCA extracted by varimax normalised rotation on the dataset is shown in Table S3 (supplementary material). In the case of patients, four principal components (PCs) with eigenvalues greater than unity were obtained, accounting for more than 62% of the total variance of the data. PC 1 with a total variance of 33.78%, revealed higher loadings for K, Na, Mn, Co, Cd, Ni, and Pb (supported by strong mutual cluster) which

Table 3 Correlation coefficient (r)* matrix for selected macroelements, and essential/toxic trace metal levels in the blood of laryngeal cancer patients (below the diagonal) and controls (above the diagonal)

	Na	K	Ca	Mg	Sr	Fe	Zn	Cu	Mn	Co	Li	Ag	Cr	Cd	Ni	Pb
Na	1	**0.881**	− 0.063	0.050	**0.415**	− 0.067	0.180	0.178	**0.630**	**0.638**	**0.356**	0.297	**0.313**	**0.398**	0.266	0.282
K	**0.947**	1	− 0.160	0.034	**0.400**	− 0.146	0.244	0.243	**0.698**	**0.453**	**0.498**	0.153	**0.366**	**0.369**	0.221	0.283
Ca	0.124	0.160	1	**0.375**	0.183	− 0.191	0.243	0.063	0.184	− 0.169	− 0.036	− 0.094	0.275	− 0.121	0.111	0.001
Mg	0.073	0.121	0.275	1	0.202	**− 0.313**	0.079	− 0.221	0.030	0.043	0.179	− 0.092	0.114	− 0.122	− 0.036	− 0.094
Sr	**0.768**	**0.691**	0.180	0.074	1	**− 0.324**	− 0.036	0.218	**0.321**	**0.371**	0.244	0.159	0.227	0.122	0.038	0.017
Fe	− 0.213	− 0.109	− 0.092	**0.427**	− 0.183	1	− 0.102	0.100	-0.122	− 0.172	− 0.200	0.218	− 0.195	− 0.119	− 0.153	0.116
Zn	− 0.108	− 0.014	− 0.180	0.091	− 0.074	**0.608**	1	0.012	0.230	0.058	0.120	0.139	0.193	0.267	0.173	0.217
Cu	**0.491**	**0.501**	**0.460**	0.112	**0.529**	− 0.144	-0.101	1	**0.371**	− 0.028	0.106	0.205	0.228	− 0.101	0.097	0.040
Mn	**0.564**	**0.561**	0.075	0.184	**0.641**	− 0.198	− 0.100	**0.307**	1	**0.574**	**0.384**	0.233	**0.400**	**0.360**	0.284	0.103
Co	**0.517**	**0.521**	0.064	0.157	**0.359**	− 0.135	-0.152	**0.353**	**0.459**	1	0.214	**0.420**	0.106	**0.699**	0.229	0.022
Li	**0.402**	**0.435**	**0.367**	0.133	**0.441**	− 0.111	− 0.206	**0.505**	0.261	0.201	1	0.018	0.264	0.076	0.078	0.203
Ag	0.126	0.111	− 0.172	− 0.046	0.178	− 0.135	− 0.067	− 0.070	0.112	0.039	0.007	1	− 0.011	**0.445**	− 0.052	0.057
Cr	**0.499**	**0.375**	− 0.152	0.027	**0.581**	− 0.252	0.137	0.036	**0.487**	0.021	− 0.036	0.270	1	− 0.081	0.128	0.177
Cd	**0.780**	**0.662**	**0.354**	0.125	**0.680**	− 0.189	− 0.252	**0.707**	**0.545**	**0.375**	**0.428**	− 0.035	0.007	1	0.087	**0.305**
Ni	**0.610**	**0.528**	0.273	− 0.020	**0.402**	− 0.046	− 0.215	0.130	**0.412**	0.211	0.259	− 0.063	0.028	**0.537**	1	0.031
Pb	**0.533**	**0.611**	**0.307**	0.055	**0.516**	− 0.029	− 0.282	**0.502**	**0.414**	**0.463**	**0.533**	0.117	0.034	**0.650**	**0.433**	1

*r-values shown in bold are significant at $p < 0.05$

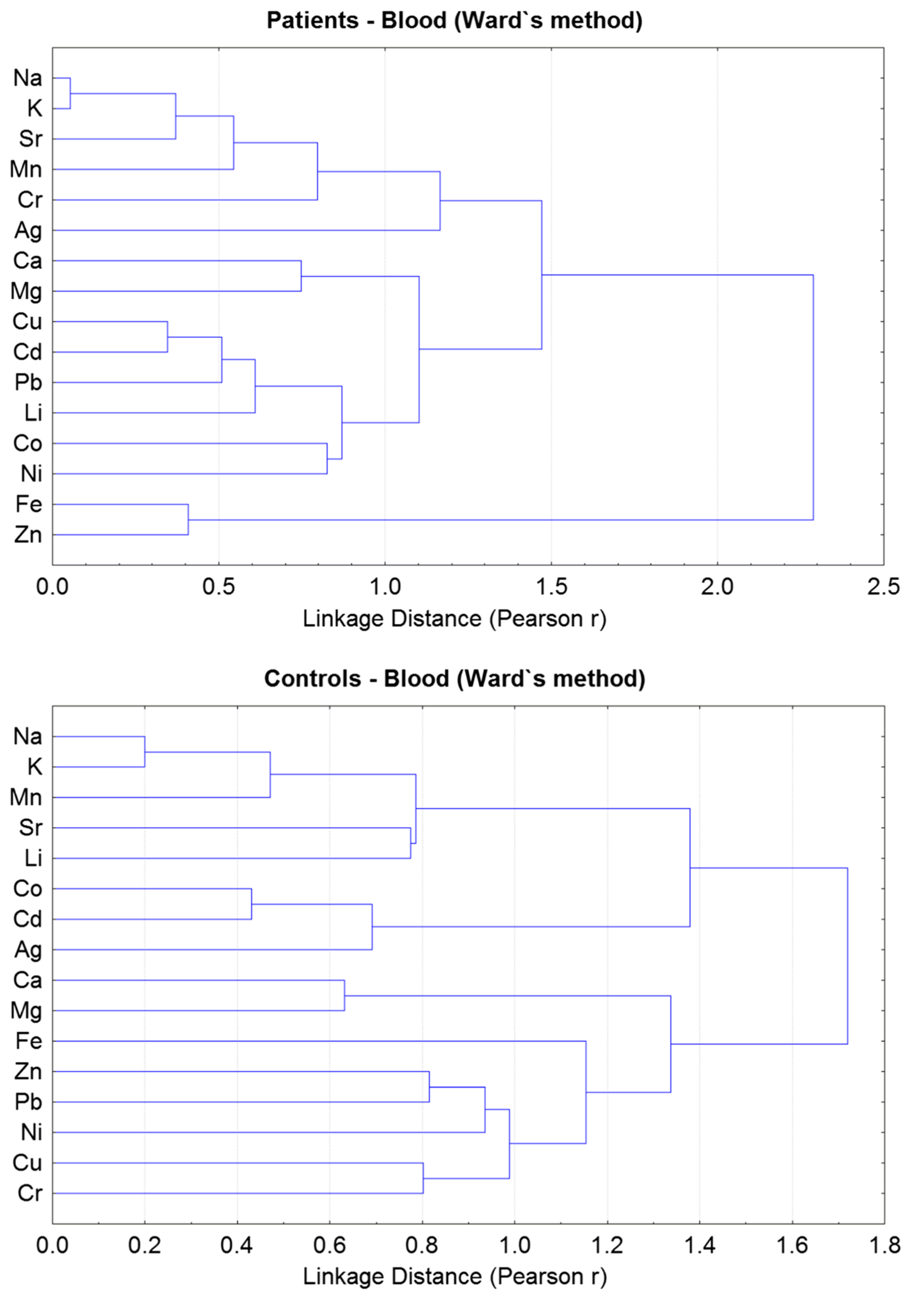


Fig. 1 Cluster analysis of selected macroelements, and essential/toxic trace metal levels in the blood of laryngeal cancer Patients and controls

were mostly associated with the anthropogenic contamination and nutritional habits of the subjects. This PC/cluster also indicated the interferences of toxic metals with the essential metals. PC 2 (11.53% of total variance) showed considerable loading for Cr, Ag, and Sr while PC 3 indicated elevated loadings for Fe, Zn, and Mg, accounting for 10.75% of the total variance. These metals also revealed strong associations/clusters in the CA (Fig. 1). Such grouping suggested considerable interferences of these metals in the cancer patients. PC 4, which showed higher loadings for Ca, Cu, and Cd and well supported by a shared cluster in CA, was primarily linked with the contamination of dietary sources. PCA and CA results of the cancer patients showed combined group of Ca with Cu and Cd. Cadmium and copper can replace Ca, which is one of the essential metals, deficiency of which can promote several types of cancers, such as, colon cancer, breast cancer and head and neck cancer (Plaschke et al. 2019; Wasson et al. 2020). Cadmium is an established carcinogen, and it can interfere with Ca-channels thereby interfering its functioning which may result in some malignancy. Elevated exposure to Cu can pose serious damages to human health and its cluster with calcium, which is an essential metal, showed serious complications. Although Cu is not directly involved in the formation of cancerous cells, but it can enhance the growth of tumour and its excess can cause leukaemia and lymphoma (Asrami et al. 2020; Maarman et al. 2020). The medical specialists have introduced an effective treatment of carcinogenesis using Ca which has no genotoxic and cytotoxic effects (Gibot et al. 2020). Calcium electroporation is most widely recommended nowadays because of reduced chances of secondary malignancies (Frandsen et al. 2020).

In the case of controls, six PCs (with eigenvalues > 1) accounting for more than 68% of the cumulative variance of the data were obtained as shown in Table S3 while Fig. 1 depicts the related CA in the form of a dendrogram. Highest loadings of K, Na, Li, and Mn were found in PC 1 (with a total variance of 24.01%); CA also revealed a communal cluster of these metals. Significant loadings of Mg, Ca, and Fe were found in PC 2 (with a total variance of 12.63%), which were strongly supported by a combined cluster in CA. The metals in these two PCs/clusters were mainly associated with the nutritional intake/dietary sources of the subjects. Higher loadings of Co, Ag, and Cd were found in PC 3, while Zn and Pb exhibited highest loadings in PC 4. These results were in good agreement with the mutual clusters observed in CA. PC 5 and 6 showed highest loadings for Cu and Ni, respectively. These metals were mostly contributed by anthropogenic activities and contamination of biological segments in the environment. Overall, the multivariate apportionment of the macroelements and essential/toxic trace metals in the patients indicated considerable interventions of the toxic metals with the macroelements; it was significantly divergent compared with the controls.

Comparison of the metal levels based on gender, abode, age, diet, and smoking habits

Figure 2 (a–d) shows comparison of the average levels of the macroelements and essential/toxic metals in the blood of patients and controls based on gender, habitat, dietary, and smoking habits. Comparative evaluation based on gender showed that male patients exhibited higher levels of Na, Ca, Sr, Cu, Mn, Li, Cd, Ni, and Pb than female patients, whereas mean levels Fe, Ag, and Cr were found to be relatively higher in female patients. However, average concentrations of Mg, Zn, and Co were comparable in male and female patients. In the case of controls, male donors showed comparatively higher levels of Ca, Mg, and Fe, whereas female controls revealed higher mean levels of Na, K, Cu, Mn, Co, Cr, Ni, and Pb in their blood. Nevertheless, the mean levels of Sr, Zn, and Cd in male and female controls were almost comparable (Fig. 2a). Some researchers reported that gender has a significant effect on human health and the inception of various diseases as the physiological difference in genders may demonstrate different metal profiles (de Martel et al. 2018; Goyal et al. 2021). It has been reported that Cd typically tends to be higher in the male subjects as it is evident in the present study, however, the effect of gender on metal's profile needs more focus in the futuristic studies (Satarug et al. 2021; Kowalska and Milnerowicz 2016).

The urban patients demonstrated comparatively higher level of Na, K, Zn, Mn, Co, and Cr than their rural counterparts, who exhibited fairly higher levels of Cu, Li, Ag, and Cd in their blood. Nonetheless, average levels Ca, Mg, Fe, Ni, and Pb in both patient groups were found to be comparable (Fig. 2b). In the case of controls, considerably elevated mean levels of Zn, Ag, Cr, and Pb were found in the rural than urban subjects, although mean levels of Na, K, Mg, Sr, and Li were practically comparable in both groups. Average levels of Mn, Co, Cd, and Ni were found to be relatively higher in the urban controls than the rural controls. Habitat may exhibit significant impacts on human health; people living in industrial areas are more prone to health implications than those living in green areas. Anthropogenic emissions associated with industrial wastes can cause serious implications for the exposed populaces (Elnabris et al. 2013; Mills et al. 2017; Klatka et al. 2011).

Figure 2c depicts the comparison of the mean metal levels as a function of dietary habits (vegetarian and non-vegetarian) of the subjects. The vegetarian patients showed relatively higher levels of Ca, Co, and Ni, whereas non-vegetarian patients exhibited rather higher levels of Sr, Cu, Mn, Cr, and Cd.

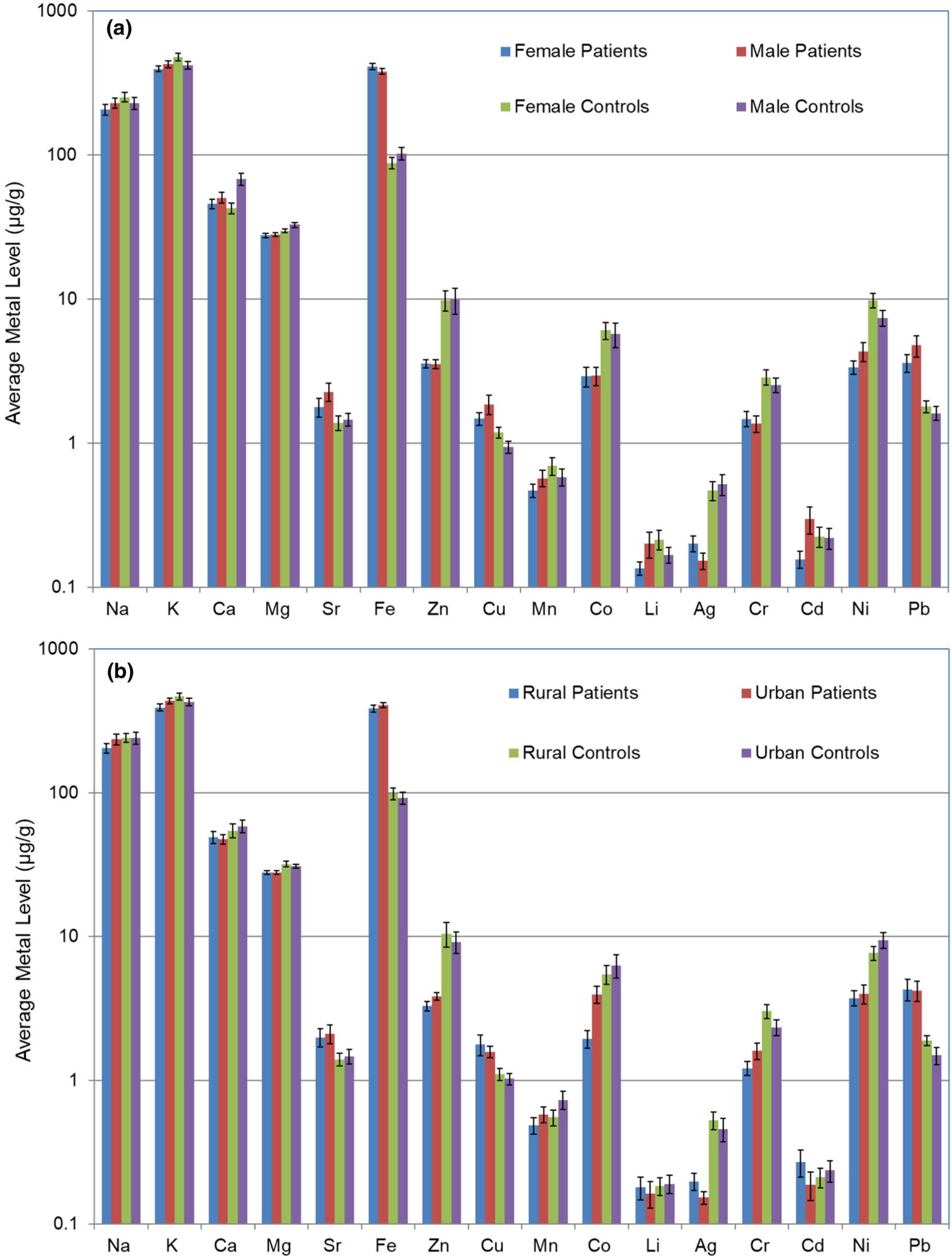


Fig. 2 Comparison of the average concentrations (µg/g, ± SE) of selected macroelements, and essential/toxic trace metals in the blood of laryngeal cancer patients and controls based on their **a** gender, **b** habitat, **c** food habits and **d** smoking habit

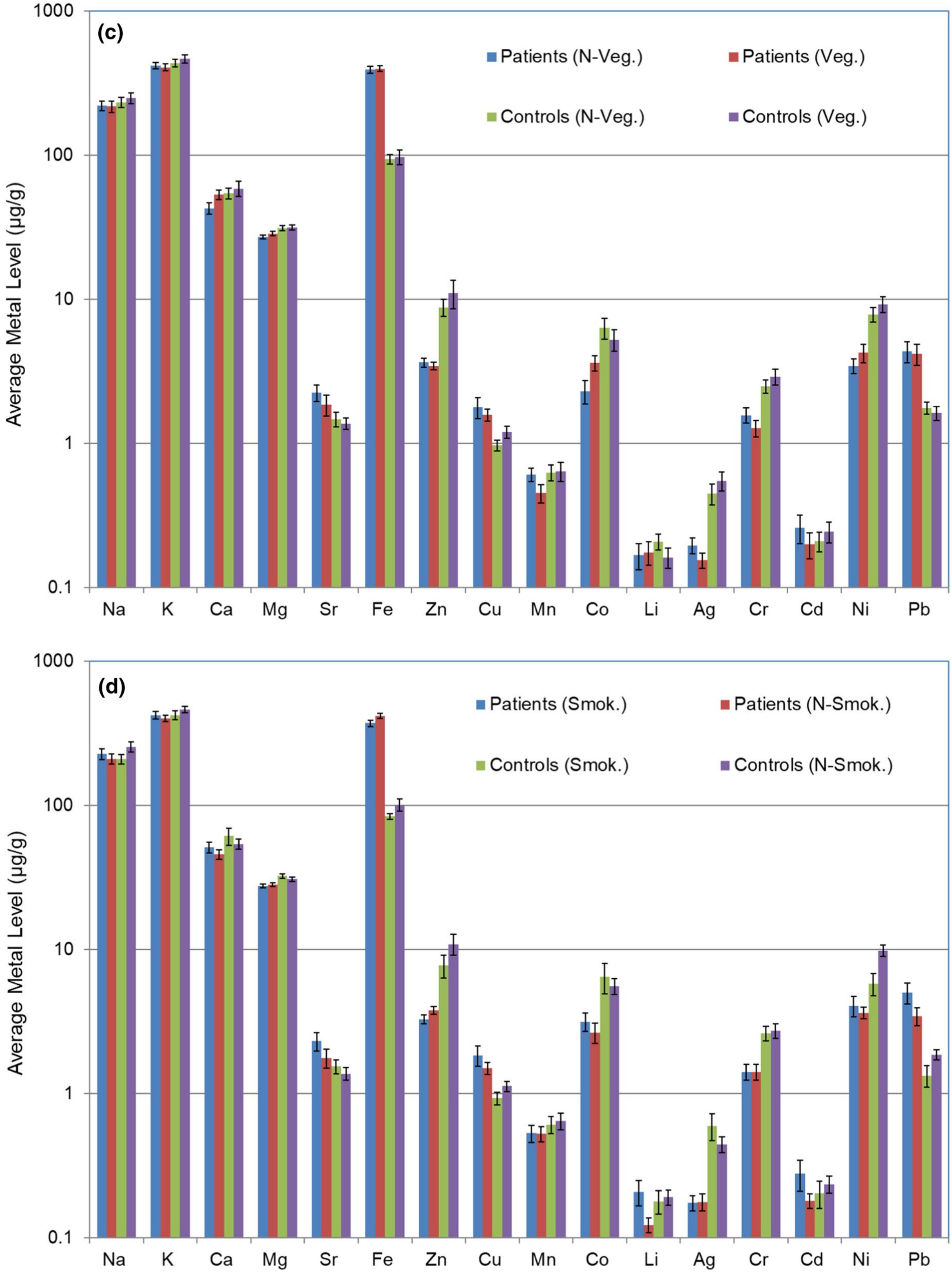
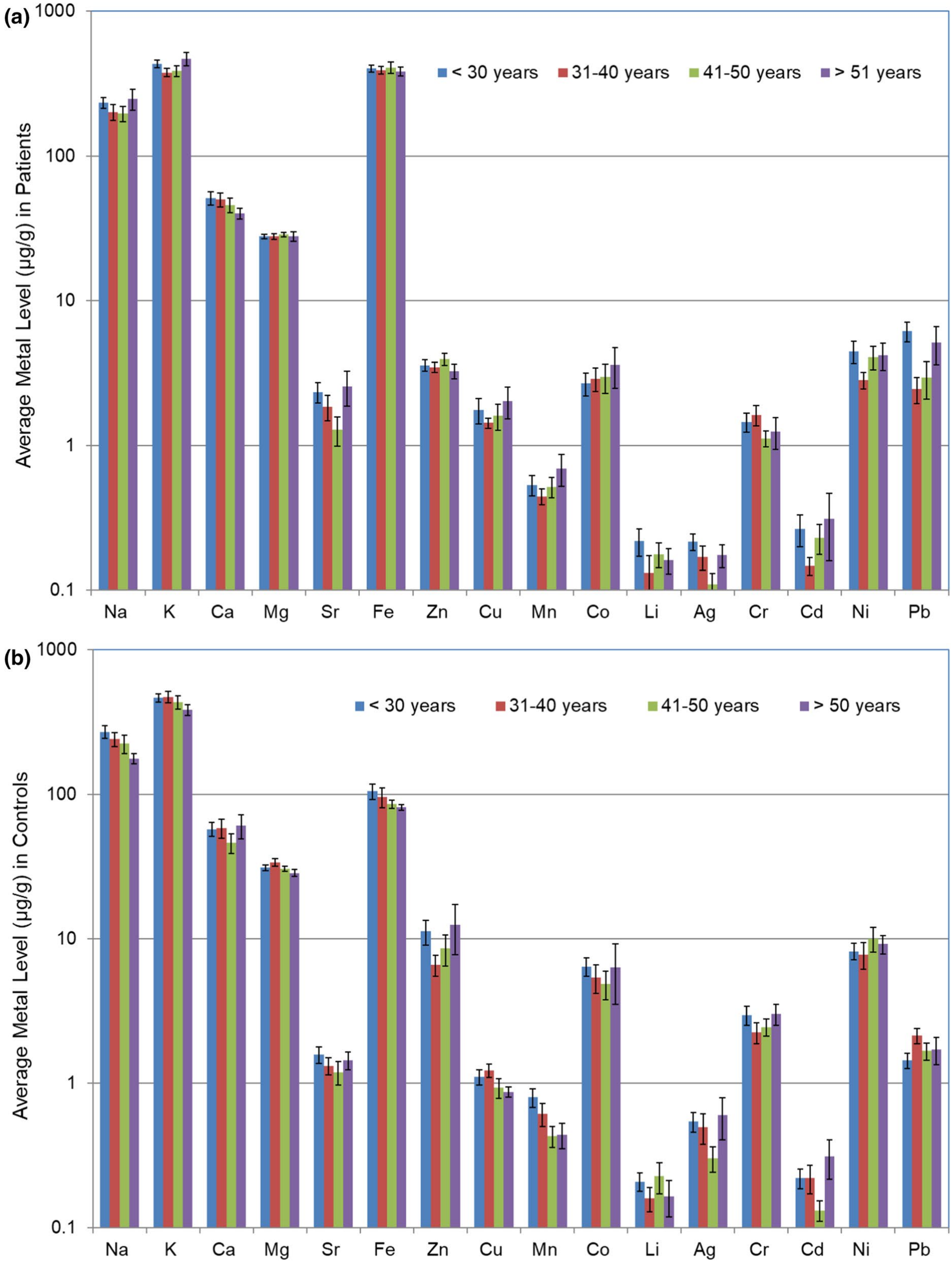


Fig. 2 (continued)


(a)
Average Metal Level (µg/g) in Patients
1000
100
10
1
0.1
< 30 years
31-40 years
41-50 years
> 51 years
Na K Ca Mg Sr Fe Zn Cu Mn Co Li Ag Cr Cd Ni Pb
(b)
Average Metal Level (µg/g) in Controls
1000
100
10
1
0.1
< 30 years
31-40 years
41-50 years
> 50 years
Na K Ca Mg Sr Fe Zn Cu Mn Co Li Ag Cr Cd Ni Pb

◂**Fig. 3** Comparison of the average concentrations (µg/g, ± SE) of selected macroelements, and essential/toxic trace metals in the blood of various age groups of **a** laryngeal cancer patients and **b** counterpart controls

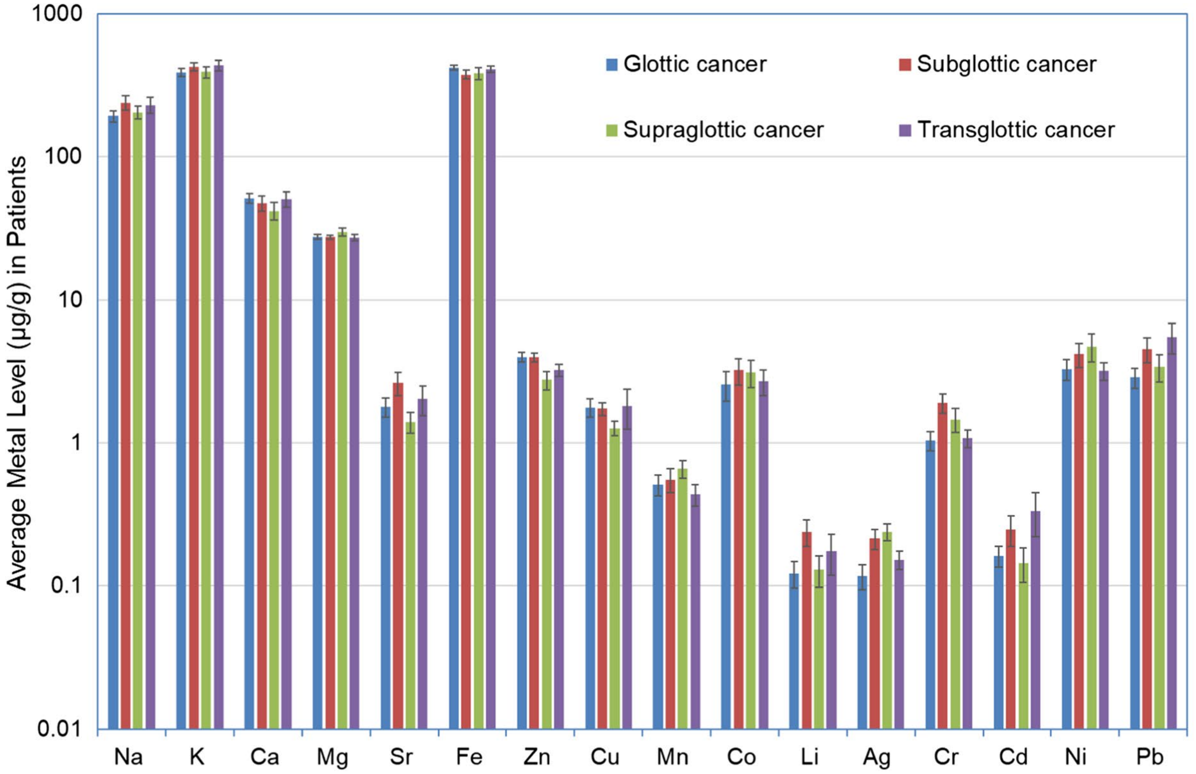
However, almost comparable levels of Na, K, Mg, Fe, Zn, Li, and Pb were found in the vegetarian and non-vegetarian patients. In comparison to vegetarian controls, the non-vegetarian counterparts displayed relatively high levels of Co and Li while the mean levels of Zn, Cu, Ag, Cr, Cd, and Ni in vegetarian controls were considerably higher than the non-vegetarian donors. The measured levels of Na, K, Ca, Mg, Sr, Fe, Mn, and Pb in both control groups were nearly comparable. Diet has an important influence on the onset and progression of malignancy; consumption of healthy diet rich in fruits and green vegetables can lower the incidence of various diseases (Martn-León et al. 2021). It is crucial to take a balanced diet for healthy body otherwise it can lead to serious health issues including blood pressure, heart diseases and in certain cases it can lead to carcinogenesis (Andrews and Johnson., 2020; Khan et al. 2020).

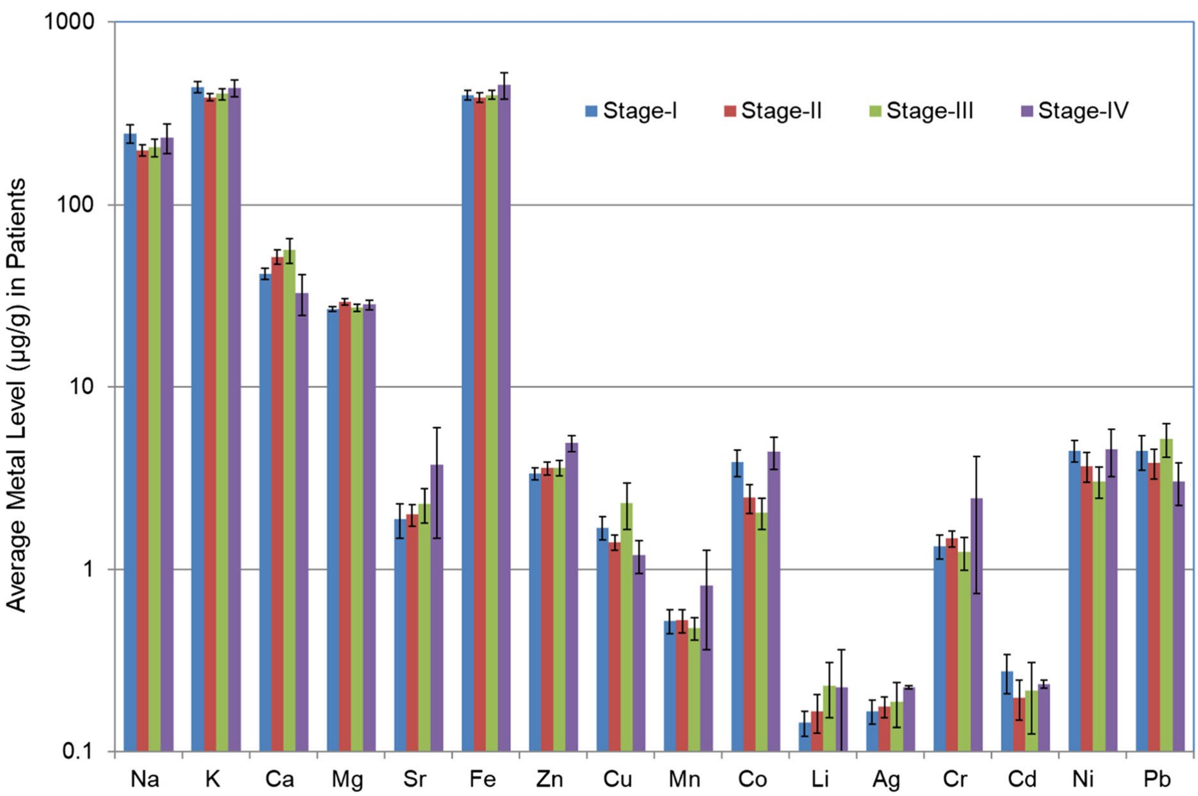
Figure 2d shows smoking-based comparison of the mean metal levels in the patients and controls. The comparative assessment revealed that smoking patients had higher levels of Sr, Cu, Co, Li, Cd, Ni, and Pb than non-smoking patients which showed relatively higher contents of Fe and Zn. The mean levels of Na, K, Ca, Mg, Mn, Ag, and Cr were nearly comparable in both smoking and non-smoking patients. In the case of controls, average levels of Ca, Sr, Co, and Ag were found to be considerably higher in smoking subjects, while non-smoking subjects showed significantly higher levels of Na, Fe, Zn, Cu, Cd, Ni, and Pb. The mean levels of K, Mg, Mn, and Cr were nearly comparable in both control groups. Smoking can cause changes in DNA, leading to the unrestrained cell growth and ultimately turns into a tumour (Bao et al. 2021). Smoking can also weaken the immune system thereby reducing the capability to fight against tumour. It has been reported that smoking is one of the main causes of oral cancer (Jităreanud et al. 2021).

Average levels of the macroelements and essential/toxic trace metals in both donor groups were also compared to appraise the age-related differences, and for this purpose the donors were divided into four age groups: < 30, 31–40, 41–50, and > 51 years. Figure 3a shows the age-based comparison of the patients, which revealed that patients under 30 years had significantly higher levels of Ni and Pb, while those of 31–40 years exhibited relatively higher levels of Cr. Nonetheless, mean levels of Zn were considerably higher in the patients between 41 and 50 years of age whereas the patients over 50 years of age showed significantly higher levels of Sr, Cu, Mn, Co, and Cd. The age-based investigation of the controls as shown in Fig. 3b shows that the subjects under 30 years of age had relatively higher contributions of Na, Sr, Mn, Co, and Cr, whereas those of 31–40 years of age displayed higher levels of Cu and Pb. Nevertheless, 41–50 years group of controls exhibited rather higher levels of Li and Ni but > 50 years controls showed significantly elevated levels of Ca and Zn. The age-based variations among the metal levels revealed considerable divergences between the patients and controls, which can be attributed to the essential and toxic metal imbalances in the patients. It has been reported that the risk of cancer in old age is quite higher than in young ones since the majority of the factors which stimulate the onset of cancer are usually coincide at the old age (Bade and Cruz 2020; Zhang et al. 2022; Kowalska and Milnerowicz 2016).

Comparison of the metal levels based on types/stages of cancer

Figure 4 shows comparison of the average levels of the macroelements and trace metals in the blood of laryngeal cancer patients of various types (glottic, subglottic, supraglottic, transglottic) and stages (I, II, III, and IV). Mean levels Zn in glottic cancer patients were found to be significantly higher than other cancer types while the average levels of Na, Sr, Li, and Cr were comparatively higher in the subglottic cancer patients. Similarly, medullary supraglottic cancer patients exhibited considerably higher level of Mn but the patients with transglottic carcinoma showed noticeably higher levels of Pb and Cd than other cancer types in the present study. Average levels K, Ca, Mg, and Fe were found to be almost comparable in the four types of laryngeal cancer patients included in this investigation (Fig. 4). The patients diagnosed at stage I showed relatively higher levels of Na, K, and Ni but moderately lower levels of Li, Ag, Mn, Zn, and Sr. The essential metals displayed considerable contributions at stage I, while most of the toxic metals


Glottic cancer
Subglottic cancer
Supraglottic cancer
Transglottic cancer
Average Metal Level (μg/g) in Patients
1000
100
10
1
0.1
0.01
Na
K
Ca
Mg
Sr
Fe
Zn
Cu
Mn
Co
Li
Ag
Cr
Cd
Ni
Pb


Stage-I
Stage-II
Stage-III
Stage-IV
Average Metal Level (μg/g) in Patients
1000
100
10
1
0.1
Na
K
Ca
Mg
Sr
Fe
Zn
Cu
Mn
Co
Li
Ag
Cr
Cd
Ni
Pb

◂**Fig. 4** Comparison of the average levels of selected macroelements, and essential/toxic trace metals (μg/g) in the blood of laryngeal cancer patients based on their type of cancer and stages

showed rather reduced levels. Considerably elevated levels of Mg were noted in the patients at stage II, while mean levels of Cu, Ca, and Pb were significantly higher in the patients at stage III. Nevertheless, the patients at stages II and III revealed nearly matching contributions of Na, K, Fe, and Zn, however the patients at stage IV demonstrated fairly higher levels of Sr, Fe, Zn, Mn, Ag, and Cr as shown in Fig. 4. Since distinct biological changes were occurring at different stages or types of cancer, the essential/toxic metal concentrations varied significantly with respect to the types and stages of the cancer (Qayyum and Shah 2019; Klatka et al. 2011).

Conclusion

The current study, which is first of its kind, revealed that the measured concentrations of macroelements and essential/toxic trace metals in the blood of laryngeal cancer patients exhibited significant variations compared to the controls in terms of their relative distribution, mutual correlations and multivariate apportionment. Most of the toxic metals were found to be higher while the essential elements were found in lower concentrations in the patients than the controls. The correlation study revealed that the macroelements, and essential/toxic metals in the patients and controls exhibited diverse associations. PCA and CA indicated considerably divergent apportionment and grouping of the essential/toxic metals in both donor groups. The average levels of the majority of the metals exhibited significant variations based on demographic characteristics of the subjects in both groups. Transglottic carcinoma patients showed substantially higher levels of Pb, and Cd compared with other type of cancers. Similarly, mean levels of Cu, Ca, and Pb in the patients were found to be significantly higher at stage III, whereas relatively higher levels of Sr, Fe, Zn, Mn, Ag, and Cr were found in the patients at stage IV. Accordingly, considerable imbalance in the concentrations of the macroelements, and essential/toxic trace metals in the patients could indicate the onset and development of cancer.

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Author contributions KB: methodology, formal analysis, investigation, data curation, writing—original draft preparation, MHS: conceptualisation, visualisation, resources, supervision, project administration, funding acquisition, writing—review and editing. All the authors have read and agreed to the published version of the manuscript.

Declarations

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, Islamabad REF. NO. QAUC-2017-A77) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

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