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Assessment of essential and toxic elemental concentrations in tumor and non-tumor tissues with risk of colorectal carcinoma in Pakistan

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ABSTRACT

Background: Colorectal tumor is a major cause of cancer morbidity and mortality both in USA and around the globe. Exposure to environmental toxicants such as toxic trace elements has been implicated in colorectal malignancy. However, data linking them to this cancer are generally lacking.
Methods: Accordingly, the current study was to investigate the distribution, correlation and chemometric evaluation of 20 elements (Ca, Na, Mg, K, Zn, Fe, Ag, Co, Pb, Sn, Ni, Cr, Sr, Mn, Li, Se, Cd, Cu, Hg and As) in the tumor tissues (n = 147) and adjacent non tumor tissues (n = 147) of same colorectal patients which were analyzed by flame atomic absorption spectrophometry employing nitric acid-perchloric acid based wet digestion method.
Results: On the average, Zn ($p < 0.05$), Ag ($p < 0.001$), Pb ($p < 0.001$), Ni ($p < 0.01$), Cr ($p < 0.005$) and Cd ($p < 0.001$) showed significantly higher levels in the tumor tissues compared with the non tumor tissues of patients, whereas mean levels of Ca ($p < 0.01$), Na ($p < 0.05$), Mg ($p < 0.001$), Fe ($p < 0.001$), Sn ($p < 0.05$) and Se ($p < 0.01$), were significantly elevated in the non tumor tissues than the tissues of tumor patients. Most of the elements revealed markedly disparities in their elemental levels based on food (vegetarian/nonvegetarian) habits and smoking (smoker/nonsmoker) habits of donor groups. The correlation study and multivariate statistical analyses demonstrated some significantly divergent associations and apportionment of the elements in the tumor tissues and non tumor tissues of donors. Noticeably, variations in the elemental levels were also noted for colorectal tumor types (lymphoma, carcinoids tumor and adenocarcinoma) and stages (I, II, III, & IV) in patients.
Conclusion: Overall, the study revealed that disproportions in essential and toxic elemental concentrations in the tissues are involved in pathogenesis of the malignancy. These findings provide the data base that helps to oncologist for diagnosis and prognosis of colorectal malignant patients.

1. Introduction

Colorectal carcinoma is the most common tumor in gastro-intestinal tract, with over one million new incidence cases induces 0.9 million deaths worldwide in 2020 [1]. Colorectal carcinoma ranks the 2nd leading cause of cancer deaths both in the USA and around the globe. In 2020, colorectal tumor estimated 10% of global cancer incidence and 9.4% of cancerous deaths. The global number of new colorectal tumor patients is predicted to reach 3.2 million in 2040 [1,2]. Some researchers have reported that incidence of colorectal tumor in different areas of Pakistan ranged from 4% to 6.8% [3]. Conclusive scientific evidences advocated that improper diet and environmental pollutions are the major contributors in cancer processes induction [4]. The exact etiology of colorectal tumor is not yet clear, leaving behind many facets which are still controversial. However, age, family history, obesity, genetics, physical inactivity, high consumption of alcohol, diet, smoking habits and inflammatory bowel disease etc., are involved [1,5]. Due to extensive industrialization, the increase in human population,

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motorized vehicular traffic, and the augmented use of diesel generators result the exposure to metals has also increased in the population [6]. Accordingly, chronic exposure to environmental contaminants such as toxic trace elements and their accumulation has long been documented as being capable of growing carcinoma incidence among exposed human populations [5,7].

In biological system, a dynamic balance among various trace elements is always existed, which is responsible for many metabolic/physiological processes [8]. Any disorder in trace elemental balance due to inadequate/excessive intake of elements may lead to cell damaging, DNA injuries and imbalance in oxidative burden ultimately cause various types of diseases including cancers [9,10]. It is known that there are some metals which are considered to act not only as carcinogens but also as co-carcinogens and toxicity can be augmented to other non-essential elements of similar atomic predictable that can imitate there activity of a trace [11]. Exposure to reactive oxygen species (ROS), weakening of the antioxidant defense, enzyme inactivation, mutation, angiogenesis, oxidative stress and DNA damage, these elements (As, Cd, Ni, Cr, Pb and Hg) have been classified as a group 1 carcinogenic to humans as notified by the International Agency for Research on Cancer (IRAC) [5,7,12]. Recent data have focused on the relationship among toxic trace elements and colorectal carcinoma in humans, which showed that the trace elements may influence the malignancy of colorectal [10,13].

It is well known that Cd is an environmental toxicant with strong carcinogenic properties and has been linked with colorectal cancer risk [12]. The poisoning production of Cd through ROS is a key factor leads to carcinogenic cell proliferation and inherent DNA lesions ultimately inducing carcinogenicity [14,15]. Actually, Cd displaces other metallic ions (Cu, Fe and Zn) from their respective protein complexes, which in turn generates oxidative stress eventually progressing to tumor development [14]. Nickel mediated toxicity in cancerous cells by generation of oxidative stress, changing in target gene expression, alterations to cells' DNA lead to DNA damage and the targeting of DNA repair pathways [16]. Chronic exposure of Ni can disrupt oxidative phosphorylation, hematotoxicity, immunotoxicity and finally leads to variety of diseases including cancer [17]. Cr and its compounds are identified to have genotoxic, teratogenic, mutagenic and carcinogenic effects on different organs of body [18]. Recent data advocated that Cr is involved in the formation of Cr-DNA adducts with production of ROS, resulting DNA damages inducing carcinogenicity [19]. However, the mechanism behind cancer is still not completely understood. As a part of vitamin B-12, cobalt is beneficial. High exposure level of Co might increase the risk of upper gastrointestinal tract cancer and lungs cancer [20]. The carcinogenicity of Co is due to direct DNA breaks, oxidative DNA breakdown through free radicals synthesis and endocrine disrupters [21].

Recent studies reported that Pb is able to cause genotoxic effects on humans such as chromosome aberration, mutation and DNA synthesis inhibition [22]. It is believed that exposure of Pb enhances susceptibility to carcinoma and may interfere with toxic effects such as interference with the repair mechanism of DNA results in genome instability, impede the ability of cells to develop appropriate and precise responses to environmental genotoxins [7]. In addition, exposure to Pb inhibits the activities of antioxidant enzymes which eventually stimulates the generation of ROS [23]. Epidemiological studies advocated that Pb substitutes Ca and Zn in enzymes involved in DNA processing and repair, as a consequence, an enhancement in genotoxicity [22]. Weak association of Pb exposure and various types of cancers (lung, stomach, kidney and brain) have been establishes as shown by epidemiological studies [5]. Selenium protects cells from damage due to antioxidant properties and anti-carcinogenic properties [24]. By improving the immune cell activity and affecting the expression of gene Bcl-2 and p53, Se has the ability to prevent tumor growth as evidences came from clinical and epidemiological studies [25]. A strong inverse association between Se and colorectal tumor risk has been developed [26]. Elevated Se levels were inversely associated with the risk to develop colorectal carcinoma as examined by Hughes et al. [25]. Zinc is important for optimum function of the immune system and act as cofactor, superoxide dismutase (SOD) [11]. Zinc is a vital constituent/cofactor of over 300 mammalian enzymes and influence on the immune system, cell differentiation and proliferation, DNA and RNA synthesis and repair [27]. Zinc modulates oxidative stress and might help to prevent cancer and is virtually nontoxic even at higher doses [28]. Multiple lines of evidence suggested that changes in Mn and Zn levels are closely related with pathological conditions including colorectal malignancy [7,9,13].

Clinically, deficiency of Fe can result in the clinical manifestation of anaemia and immune compromise [29]. Deficiency of Fe is common in colorectal cancer patients because reduced immune surveillance response and altered tumor immune microenvironment may result progression of tumor [30]. Moreover, overload of Fe can also promote tissue damage lead to malignancy by converting hydrogen peroxide to free radical formation (Fe act as a catalyst) that disturb functions of cellular membranes, breaks DNA strands, lipid peroxidation, protein damage, inactivate enzymes and finally accelerate tumor initiation [31]. Copper is potentially toxic and is associated with tumorigenesis such as activation of the oncogenic, impairment of cancer cell and angiogenic activity as well as aberrant DNA methylation [32]. Cu integrates in many pro-angiogenesis pathways that may be important for carcinogenesis. The association among Cu and colon cancer is stronger as the Cu modulates BRAF signaling and BRAFV600E mutation in colon cancer patients [33]. Mg influences the physiological process of tumor progression including colorectal cancer such as DNA synthesis, DNA repair, insulin sensitivity, the regulation of cell proliferation and apoptosis [34]. It was recently discovered that intake of Mg may be linked with a reduction in the risk of colorectal tumor, but the findings have been inconsistent [35,36].

Assessment of trace elemental levels in human specimens such as tissues and fluids (blood, serum, plasma, hairs, nails and urine) were used to attain information on the nutritional status for diagnosis of diseases, viewing of systemic intoxication and to obtain information on environmental exposure [10,11,13,37]. The cancerous tissue directly shows variations of trace elements that are evoked by the disease. Few studies have been found on elemental concentrations of colorectal tumor tissues and compared it to the controls tissues with inconsistent results [7]. Still, insufficient attention has been paid to existing research on the amount and impact of toxic trace elements on Pakistan's colorectal carcinoma incidence [5]. To address this knowledge gap, the current study was designed to highlight the following: to investigate the concentrations of 20 elements including Ca, Na, Mg, K, Zn, Fe, Ag, Co, Pb, Sn, Ni, Cr, Sr, Mn, Li, Se, Cd, Cu, Hg and As in the tumor affected tissues and adjacent non tumor (healthy) tissues of colorectal patients, to evaluate the mutual variations between the elemental levels by correlation analysis; to appraise the multivariate apportionment between the elements in the tumor tissues and non tumor tissues of the colorectal patients; and to assess the levels of elements with respect to the form/type of tumor and stages/grades of colorectal tumor, thereby investigating whether these toxic trace elements had any presumptive benefits in the diagnosis of malignancy in patients. To our knowledge, this is the first study to examine the potential association among essential/toxic elemental concentrations and colorectal tumor and non tumor tissues in Pakistani patients.

2. Materials and methods

All the colorectal cancerous patients were recruited among 2020–2021. The colorectal carcinoma tissues (147, male= 88 and female 59) received/collected during the postoperative biopsies from the patients visited in Department of Pathology, Faisalabad Medical University Faisalabad. All samples were kept in plastic containers at −20 °C frozen in the refrigerator until examination. All of the tissues were examined morphologically and histologically to confirm the diagnosis of

Table 1
Instrumental operating conditions for the elemental analyses along with their LOD/LOQ and certified vs measured concentrations* (μg/g) of selected elements in standard reference materials.

							Bovine liver, NIST-SRM 1577c		
Metal	Wavelength (nm)	HC lamp current (mA)	Slit width (nm)	Fuel-gas flow rate (L/min.)	Limit of detection (mg/L)	Limit of quantification (mg/L)	Certified level (μg/g)	Measured level (μg/g)	Recovery (%)
Ca	422.7	6	0.5	2	0.002	0.015	131	129	98.4
Na	589.0	6	0.5	1.6	0.002	0.006	0.2033	0.2045	101.2
Mg	285.2	4	0.5	1.6	0.003	0.003	620	620	99.5
K	766.5	5	0.5	1.9	0.004	0.010	1.023	1.019	99.4
Zn	213.9	4	0.5	2	0.002	0.007	181.1	184.5	101.9
Fe	248.3	8	0.2	2	0.003	0.019	197.9	194.2	98.1
Ag	328.1	4	0.5	2.8	0.003	0.005	0.0059	0.0059	100.3
Co	240.7	6	0.2	2.2	0.002	0.015	0.300	0.291	97
Pb	217.0	7	0.3	1.8	0.010	0.038	0.062	0.061	98.4
Sn	286.3	7	0.5	2.5	0.005	0.021	—	—	—
Ni	232.0	4	0.15	1.7	0.008	0.017	0.0445	0.0445	102
Cr	357.9	5	0.5	2.6	0.007	0.015	0.053	0.054	101.9
Sr	460.7	4	0.5	1.6	0.005	0.016	0.0953	0.0945	99.2
Mn	279.5	5	0.4	1.9	0.002	0.011	10.46	10.10	97.1
Li	670.4	4	0.5	1.6	0.003	0.009	0.012	0.012	99.6
Se	196.0	10	1	1.2	0.004	0.007	2.031	1.982	97.5
Cd	228.8	4	0.3	1.8	0.003	0.012	0.097	0.099	102
Cu	324.8	3	0.5	1.8	0.005	0.013	275.2	270.7	98.4
Hg	253.7	5	0.5	1.2	0.015	0.020	0.00536	0.00536	99.7
As	193.7	8	1	1.2	0.006	0.015	0.0196	0.0197	100.5

* Triplicate sub-samples

colorectal tumor. Before inclusion, each subject provided written informed consent and all the subjects signed on the proforma to get participation. The tissues (147, male 67 and female= 80) found no signs and symptoms of cancer (malignancy) or any other disease histologically were used as controls tissues for elemental detection and referred to as colorectal non tumor tissues. These normal/healthy tissues were cut from the adjacent part (at a distance of approximately 10 cm) of removed flap of colorectal tumor tissue, without any macroscopic signs of the disease. These non tumor tissues were checked with histology techniques to confirm the absence of tumor cells. Both the tumor tissues and non tumor tissues samples of colorectal persons were treated in the same way. All the tissues subjects were labeled with relevant codes, related to age, eating habits, drinking habits, smoking habits and social as well as general health status. The data was compiled on a proforma at the time of sampling of each donor. None of the patients from whom the tissues were collected had previously been subjected to radiotherapy/chemotherapy or any supplementation. The present study was reviewed and approved by the Ethics Committee of the Allied Hospital, Faisalabad Medical University Faisalabad Pakistan (UE/CHEM/2021/038). Blood parameters and body mass index (BMI) of the subjects were analyzed in the clinical and special chemistry laboratories of Punjab Institute of Nuclear Medicine Faisalabad Pakistan.

2.1. Sample preparation and digestion

All the tissues samples of colorectal tumor and non tumor of patients were washed with double distilled H_2O to get rid of remains of blood and formalin, which was used to store the tissues samples during the time between operation and analyses. The samples of tumor tissues and non tumor tissues of colorectal patients were frozen immediately after being received from the subjects. Before the digestion, each sample of tissue was cut with a surgical quartz knife and partially thawed, the thick sections of the surrounding fatty tissues, blood vessels, and membranes, which could have been contaminated in any of the earlier stages, were detached carefully. Approximately ~10 g of each homogenized tissue sample of the subjects was separately digested with a specified amount of high-purity nitric acid and perchloric acid mixture. The colorectal cancer and healthy tissue samples were digested with $HNO_3/HClO_4$ (10:10 v/v) mixture with subsequent heating on hot plate to a soft boil. After some time white dense fumes evolved indicating the completion of reaction [38]. At room temperature, digested cancerous tissues and healthy tissues samples were then cooled. These samples were diluted with doubly distilled water to a proper volume. The blank was prepared at the same way.

2.2. Measurement of elements

The mineralized tissues samples were evaluated for selected elements (Ca, Na, Mg, K, Zn, Fe, Ag, Co, Pb, Sn, Ni, Cr, Sr, Mn, Li, Se, Cd, Cu, Hg and As) using flame atomic absorption spectrophotometery technique (Shamidzu AA-670, Japan) with atomization in a graphite furnace, with the amounts of each element expressed in μg/g of sample. Standard reference material (NIST 1577c-bovine liver, Gaithersburg, MD, USA) was used to check the quality assurance/accuracy of the analytical process as shown in Table 1. The analytical recoveries were 97–102% of the samples. Same digestion procedure was adopted to prepare standard reference materials as samples. 3 sub-samples of each sample were treated and run separately onto the spectrophotometer to pool average elemental levels. In an independent laboratory, the samples were also measured for comparison of the results and a maximum of ± 2.5% difference was exhibited. All reagents used were of ultrahigh purity (certified >99.99%) purchased from E-Merck.

2.3. Univariate analysis and multivariate analysis

Statistical analyses were conducted on the elemental data using STATISTICA software [39]. The data distribution and recognition tools applied used in this research work included the pretreatment of data in order to attain normalization by discarding outliers. Univariate analyses of data gave basic statistical parameters, such as range, mean, median, standard deviation (SD), standard error (SE), kurtosis and skewness, along with spearman correlation. Multivariate methods applied in the present work were principal component analysis (PCA) and cluster analysis (CA). These statistical tools have been successfully used to scrutinize the toxic and elemental elements apportionment in human and to distinguish among controls and tumor patients [5].

PCA and CA forms a set of tools, basis of the multivariate statistical methods that allow for the clustering of objects, identification of variability, and appearance of results in figures [40,41]. For data reduction (dimensionality), these statistical techniques are primarily used to seek a

Table 2
Characteristics of study population.

Characteristics	n = 147	%
Age (years)		
Range	33–68	
Mean	54.1	
Tobacco Use/No Use		
Cigarette Smoking	87	59%
No Cigarette smoking	60	41%
Diet		
Non-vegetarian	57	39%
Vegetarian	90	61%
Types of Colorectal Tumours		
Lymphoma	52	35%
Carcinoids Tumour	41	28%
Adenocarcinoma	54	37%
Pathological Stages of Colorectal Tumours		
Stage-I	40	27%
Stage-II	53	36%
Stage-III	31	21%
Stage IV	23	16%

few underlying dimensions that involve patterns of variation between many observed variables [42]. PCA extracts the important information from the dataset and to present this information as a set of summary indices called principal components (PC), which can be more easily visualized, increased interpretability and analyzed but at the same time minimizing information loss [43]. In statistics, it can be apply to analyze the interactions between a set of elements, in order to assign case-specific grades to the elements according to their toxic potentials. PCA revealed exploratory factor analysis of the elemental levels with varimax rotation to reduce data and enhance the interpretability of the identified factors [44]. It can be used to reflect the possible contributing factors toward the elemental concentrations and accordingly assess which elements have a common origin [43].

Cluster analysis is an exploratory analysis that observes values of several variables for each individual and combines similar observations into number of clusters. It is similar in concept to discriminant analysis. If the grouping is not previously known, CA tries to identify homogenous groups of cases more specifically [5]. It is a quantitative form of classification and almost totally an applied rather than a theoretical methodology. The CA revealed graphical display of classifications known as a dendrogram, and a set of decision rules for the assignment of new members into the classifications. The dendrogram directly reveals the correlation among elements in the tissues, reflecting the sources of toxic trace elemental contaminants in the tissues of subjects [5]. Strongly interrelated variables clustered together, regardless of the positive/-negative sign of the relationship. Hierarchical algorithms can be divisive or agglomerative. Cluster analysis is used to identify patients who have cancer diseases with a common cause such as (toxic trace elements may be). PCA and CA can be used to identify contamination sources, be those natural or anthropogenic [40]. A distance function is applied to evaluate if the similarity between objects and a wide variety of clustering algorithms depend on different concepts is available.

3. Results and discussion

3.1. Characteristics of colorectal tumor tissues and non tumor tissues of patients

The demographic characteristics of the colorectal tumor patients are presented in Table 2. The tissues samples were collected from 147 patients on volunteer basis. The average age of the cancerous patients was 54 (Range: 33–68) years in the present work. Among the subjects, 59% of the patients were on cigarette smoking and 41% of the patients were not used cigarette for smoking on continuous basis. Less than half of the cancerous patients (39%) were non vegetarian and (61%) were on vegetarian dietary habits. On the basis of types of colorectal tumor, 35%

Table 3
Statistical distribution parameters for concentrations of selected elements in the tumor tissues and non tumor tissues of colorectal patients (μg/g).

	Colorectal Tumor Tissues (n = 147)							Colorectal Non Tumor Tissues (n = 147)							*p*-value
	Range	Mean	SD	SE	Median	Skewness	Kurtosis	Range	Mean	SD	SE	Median	Skewness	Kurtosis	
Mg	10.14–19.95	15.44	0.227	2.748	15.67	-0.157	-0.954	14–39.61	30.03	0.378	4.578	29.90	-0.559	0.582	< 0.001
Cd	0.018–0.981	0.209	0.014	0.174	0.147	1.782	3.766	0.018–0.363	0.132	0.005	0.061	0.130	0.812	1.800	< 0.001
Mn	0.052–0.599	0.376	0.013	0.159	0.400	-0.302	-1.354	0.052–0.995	0.589	0.020	0.239	0.600	-0.196	-0.923	< 0.05
Ca	26.31–39.77	34.91	0.233	2.824	34.64	-0.225	-0.755	26.31–59.43	46.48	0.510	6.187	46.50	-0.127	0.130	< 0.01
Pb	0.004–1.998	0.894	0.044	0.535	0.834	0.403	-0.879	0.004–0.998	0.532	0.023	0.277	0.520	-0.072	-1.024	< 0.001
Co	1.467–1.177	1.177	0.013	0.152	1.096	0.532	-1.244	1.025–2.441	1.627	0.025	0.298	1.640	-0.089	-1.062	NS
Hg	0.001–0.715	0.043	0.007	0.081	0.028	6.332	44.19	0.004–0.972	0.061	0.008	0.092	0.040	7.658	70.465	NS
Cr	0.08–1.374	0.564	0.029	0.352	0.459	0.771	-0.578	0.036–1.036	0.394	0.019	0.227	0.340	0.767	-0.167	< 0.05
Na	10.08–30.02	22.47	0.417	5.051	22.96	-0.749	-0.115	11.62–43.97	27.58	0.526	6.375	26.10	0.409	-0.703	< 0.05
Cu	0.002–0.098	0.053	0.002	0.027	0.056	-0.115	-1.084	0.001–0.095	0.039	0.002	0.026	0.040	0.098	-1.284	NS
As	0.001–0.008	0.002	0.001	0.001	0.002	1.337	2.511	0.001–0.003	0.003	0.001	0.002	0.003	0.948	-0.096	NS
Fe	1.508–3.962	2.968	0.052	0.629	3.104	-0.879	-0.124	0.001–7.260	4.066	0.112	1.360	3.713	-0.167	-0.464	< 0.001
K	0.444–9.937	5.664	0.192	2.327	5.740	-0.418	-0.334	1.011–8.515	4.264	0.131	1.584	4.315	-0.240	-0.521	NS
Se	0.003–0.782	0.265	0.016	0.189	0.208	0.778	-0.305	0.003–0.995	0.453	0.022	0.265	0.430	0.159	-1.039	< 0.01
Sr	0.004–0.983	0.442	0.025	0.300	0.358	0.365	-1.204	0.100–0.993	0.500	0.029	0.343	0.560	0.046	-1.766	NS
Zn	1.090–7.543	3.949	0.142	1.723	3.250	0.543	-1.098	0.014–6.822	2.097	0.168	2.034	1.035	0.811	-0.960	< 0.05
Ni	0.101–5.020	0.746	0.048	0.578	0.718	5.648	37.82	0.001–0.987	0.423	0.023	0.282	0.402	0.380	-0.928	< 0.01
Li	0.005–0.590	0.280	0.009	0.106	0.257	1.252	1.660	0.025–0.929	0.297	0.017	0.192	0.229	1.895	2.960	NS
Sn	0.036–0.929	0.396	0.018	0.221	0.376	0.603	-0.336	0.024–1.508	0.770	0.029	0.343	0.750	0.033	-0.617	< 0.05
Ag	0.201–2.289	1.219	0.043	0.525	1.198	-0.384	-0.960	0.013–0.950	0.454	0.023	0.279	0.450	0.241	-1.128	< 0.001

of the patients were suffering from lymphoma, while 28% were diagnosed with carcinoids tumour; while the reaming 37% were suffered from adenocarcinoma patients. Among the pathological stages, 36% of tumor patients were at stage II and 27% were at stage I of the colorectal tumor patients. At stage III, 21% of the patients were suffered and 16% patients were at stage IV during induction of the subjects as noted in Table 2. Colonoscopy procedure was opted with each participant.

3.2. Descriptive statistics of essential and toxic elemental concentrations in tissues

The detailed statistic treatment containing range, mean, SD, SE, skewness and kurtosis of the data analysis in the colorectal tumor tissues and colorectal non tumor tissues of patients is given in Table 3. Most elements display broad ranges of concentrations in both donor-groups. Highest average concentrations in cancerous tissues samples of the patients are found for Ca (34.91 μg/g), Na (22.47 μg/g), Mg (15.44 μg/g), followed by K (5.664 μg/g), Zn (3.949 μg/g), Fe (2.968 μg/g), Ag (1.219 μg/g) and Co (1.177 μg/g). Decreasing trend of the elemental levels in the tumor tissues of colorectal patients reveal, on the average basis, following order: Ca > Na > Mg > K > Zn > Fe > Ag > Co> Pb > Ni > Cr > Sr > Sn > Mn > Li > Se > Cd > Cu > Hg > As. Comparatively moderate concentrations were noted for Pb (0.894 μg/g), Ni (0.746 μg/g), Cr (0.564 μg/g), Sr (0.442 μg/g), Sn (0.396 μg/g) and Mn (0.376 μg/g). Lowest levels were found for Li (0.280 μg/g), Se (0.265 μg/g), Cd (0.209 μg/g), Cu (0.053 μg/g), Hg (0.043 μg/g) and As (0.002 μg/g) in the tumour tissues of patients. Higher magnitude of skewness in Hg, Cd, As, Ni and Li concentrations manifested asymmetrical distribution of these elements in the tumour tissues. Ca, Na, K, Zn and Mg exhibited appreciable randomness in their distribution as shown by large SD and SE values. Some of the metals/metalloid including As, Li, Mn, Cr and Cu revealed comparatively normal distribution pattern, as directed by small SD and SE values as shown in Table 3.

In case of non tumor tissues (Table 3), higher average concentration is noted for Ca (46.48 μg/g), Mg (30.03 μg/g), Na (27.58 μg/g), K (4.264 μg/g), Fe (4.066 μg/g), followed by Zn (2.097 μg/g) and Co (1.627 μg/g). Comparatively decreased levels were found of Sn (0.770 μg/g), Mn (0.589 μg/g), Pb (0.532 μg/g), Sr (0.500 μg/g), Ag (0.454 μg/g), Se (0.453 μg/g), Ni (0.423 μg/g), Cr (0.394 μg/g), Li (0.297 μg/g) and Cd (0.132 μg/g). While Hg (0.061 μg/g), Cu (0.039 μg/g) and As (0.003 μg/g) were estimated at the lowest levels in the tissues of controls. The elements in the non tumor tissues of patients showed the following descending order in their average concentrations: Ca > Mg > Na > K > Fe > > Zn > Co > Sn > Mn > Pb > Sr > Ag> Se > Ni > Cr > Li > Cd > Hg > Cu > As (Table 3). Among the elements, Mg, Ca, Co, Na, Fe, K and Zn displayed appreciable randomness in their distribution as revealed by large values of SD and SE. Elements like As, Hg, Cd, Mn, Pb and Cu displayed comparatively normal distribution pattern, as indicated by small values of SD and SE, whereas Na, Mg, Ca, Zn, K and Fe revealed randomness of their concentrations in non tumor tissues. Lower magnitude of skewness in favour of Sr, Cu, Co and Pb manifested nearly symmetrical distribution of these elements in the non tumor tissues. Moderately large asymmetry in the distribution of Hg and Li were demonstrated by maximum kurtosis and skewness values. The distribution study of tumor tissues and non tumor of colorectal patients depicted significant disparities of the elemental levels in the tissues (Table 3).

Average elemental levels in tissues samples of colorectal tumor tissues and colorectal non tumor tissues of patients are compared to find out the significant differences by applying Two-tailed Student t-test (Table 3). Average levels of Cd ($p < 0.001$), Pb ($p < 0.001$), Ag ($p < 0.001$), Ni ($p < 0.01$), Cr ($p < 0.05$) and Zn ($p < 0.05$) revealed significantly higher in the tissues of colorectal tumor patients when compared to non tumor tissues. However, mean Mg ($p < 0.001$), Fe ($p < 0.001$), Ca ($p < 0.01$), Na ($p < 0.05$), Se ($p < 0.01$) and Sn ($p < 0.05$) levels were exhibited to be significantly higher in the tissues of controls. Mean As, Sr

Table 4
Correlation coefficient matrix (*r) of selected elements in the colorectal tumor tissues (below the diagonal) and non tumor tissues (above the diagonal) of patients.

	Mg	Cd	Mn	Ca	Pb	Co	Hg	Cr	Na	Cu	As	Fe	K	Se	Sr	Zn	Ni	Li	Sn	Ag
Mg	1.000	0.080	0.026	0.075	0.068	-0.099	-0.154	0.064	0.017	-0.024	0.175	0.001	-0.121	**0.530**	-0.131	0.010	0.097	0.054	**0.519**	0.007
Cd	0.028	1.000	**0.617**	-0.009	-0.029	0.077	-0.146	0.068	-0.102	-0.050	**0.521**	0.049	-0.083	0.004	0.024	-0.025	-0.048	-0.024	-0.076	-0.043
Mn	0.024	0.090	1.000	**0.553**	-0.033	-0.034	-0.024	-0.120	-0.289	0.022	**0.530**	0.319	-0.159	0.001	0.049	0.076	-0.046	-0.046	-0.177	0.035
Ca	-0.055	**0.635**	**0.628**	1.000	-0.038	-0.126	-0.106	-0.208	-0.296	-0.188	-0.110	0.260	0.070	-0.134	0.060	0.197	-0.065	0.038	-0.176	0.040
Pb	-0.054	-0.031	0.112	**0.618**	1.000	-0.035	0.020	**0.548**	-0.009	**0.735**	0.111	-0.016	-0.042	-0.003	-0.050	0.016	-0.027	0.032	-0.002	-0.022
Co	-0.062	0.061	0.120	-0.058	**0.516**	1.000	-0.099	**0.674**	-0.031	0.058	0.031	0.060	-0.089	**0.513**	-0.123	**0.551**	0.000	-0.078	-0.091	0.040
Hg	-0.031	0.022	0.057	**0.586**	0.053	0.029	1.000	-0.070	0.090	**0.533**	0.093	-0.102	**0.588**	-0.001	0.078	-0.040	0.088	-0.084	0.044	0.036
Cr	0.050	**0.507**	-0.007	0.064	-0.050	0.064	**-0.575**	1.000	**0.566**	0.114	-0.055	-0.290	-0.122	**0.505**	-0.082	-0.006	0.035	-0.260	**0.499**	**0.529**
Na	-0.043	-0.082	**0.587**	0.245	**0.601**	**0.567**	-0.007	-0.021	1.000	-0.012	0.156	-0.229	-0.050	-0.087	0.052	0.025	0.067	**0.547**	**0.543**	0.164
Cu	0.009	-0.089	-0.106	**0.585**	-0.097	-0.063	-0.030	0.031	-0.012	1.000	0.069	-0.035	0.055	-0.093	-0.024	-0.077	-0.168	-0.168	-0.085	0.071
As	-0.085	-0.054	0.121	0.198	**0.531**	**0.597**	0.183	0.012	**-0.505**	0.074	1.000	**0.583**	-0.060	-0.074	0.012	-0.039	0.049	0.086	0.011	-0.014
Fe	-0.059	-0.058	-0.037	-0.107	-0.059	-0.087	-0.043	**0.562**	-0.143	-0.100	-0.009	1.000	-0.011	-0.101	-0.063	0.085	-0.073	0.226	-0.295	-0.160
K	0.056	0.003	-0.121	-0.037	-0.112	0.095	-0.018	**0.571**	0.009	-0.077	**0.532**	0.030	1.000	-0.207	0.047	-0.004	0.001	0.027	0.071	-0.064
Se	0.007	0.001	-0.116	-0.009	**-0.521**	-0.154	0.080	-0.035	-0.053	-0.001	-0.005	0.056	0.110	1.000	0.006	-0.130	0.048	0.013	0.087	-0.060
Sr	0.070	**0.599**	-0.002	0.003	-0.128	-0.064	**0.691**	-0.013	-0.182	0.045	0.020	0.019	-0.085	0.086	1.000	0.170	-0.107	0.094	**0.653**	-0.010
Zn	0.007	-0.041	-0.090	-0.069	-0.089	-0.022	**0.566**	0.010	-0.122	**0.514**	**0.631**	-0.172	-0.044	0.123	**0.520**	1.000	0.037	-0.084	-0.004	-0.040
Ni	0.095	0.000	-0.039	0.038	**0.549**	0.126	-0.108	0.199	0.085	-0.034	0.172	**-0.516**	0.605	-0.024	-0.140	-0.052	1.000	-0.064	0.056	0.022
Li	-0.032	-0.132	**-0.548**	-0.140	0.026	0.063	-0.290	-0.154	**0.748**	**0.581**	-0.104	-0.006	0.078	-0.058	-0.460	-0.244	-0.011	1.000	-0.136	-0.049
Sn	**0.623**	0.513	**0.567**	0.164	0.115	0.180	0.014	-0.054	**0.669**	0.037	**0.516**	-0.104	0.006	-0.159	0.105	0.044	0.008	-0.119	1.000	-0.071
Ag	**0.515**	-0.030	0.038	-0.084	**0.563**	0.007	-0.200	-0.027	-0.052	-0.129	-0.097	0.009	-0.059	-0.152	-0.336	-0.208	0.134	**0.581**	0.048	1.000

* *r*-values are signifcant at $p < 0.05$

Table 5
Principal component analysis of selected elements in the tumor tissues and non tumor tissues of colorectal patients.

	Tumor Tissues						Non Tumor Tissues						
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Eigen value	3.321	2.826	2.486	1.898	1.495	1.243	4.412	3.777	2.562	2.228	1.834	1.346	1.221
Total Variance (%)	19.61	17.63	12.43	11.99	8.214	6.002	23.06	16.88	13.81	10.14	8.668	6.23	4.394
Cumulative Eigen value	3.321	6.147	8.633	10.53	12.03	13.27	4.412	8.189	10.75	12.98	14.81	16.16	17.38
Cumulative Variance (%)	19.61	37.24	49.67	61.66	69.87	75.88	23.06	39.94	53.75	63.89	72.56	78.79	83.19
Mg	–	0.500	–	–	–	–	–	–	–	–	–	0.437	–
Cd	–	–	–	–	–	0.512	–	–	–	–	0.531	–	–
Mn	–	0.479	–	–	–	–	–	–	–	–	0.575	–	–
Ca	–	–	0.505	–	–	–	0.558	–	–	–	–	–	–
Pb	0.548	–	–.	–	–	–	–	–	–	0.559	–	–	–
Co	–	–	0.596	–	–	–	–	–	0.589	–	–	–	–
Hg	–	–	–	–	0.579	–	–	0.597	–	–	–	–	–
Cr	–	–	–	0.626	–	–	–	–	0.612	–	–	–	–
Na	0.608	–	–	–	–	–	–	–	–	–	–	–	0.425
Cu	–	–	0.550	–	–	–	–	–	–	0.518	–	–	–
As	–	–	0.581	–	–	–	–	–	–	–	–	0.749	–
Fe	–	0.588	–	–	–	–	0.801	–	–	–	–	–	–
K	–	–	–	0.542	–		–	-0.564	–	–	–	–	–
Se	–	–	–	–	–	0.594	–		0.511	–	–	–	–
Sr	–	–	–	–	0.550	–	–	0.673	–	–	–	–	–
Zn	–	–	–	0.502	–	–	0.604	–	–	–	–	–	–
Ni	–	–	–	0.630	–	–	–	–	–	–	–	0.508	–
Li	–	0.591	–	–	–	–	0.584	–	–	–	–	–	–
Sn		0.568	–	–	–	–	–	0.518	–	–	–	–	–
Ag	–	0.519	–	–	–	–	–	–	–	–	–	–	0.524

and Li concentrations were nearly comparable in the tumor and non tumor tissues of patients. Significantly elevated levels of Cd, Pb, Cr and Ni in the tissues of colorectal tumor patients advocate considerable association of these elements with the malignancy. Median levels of the elements were also compared by Wilcoxon rank-sum test which showed statistically significant differences ($p < 0.05$) for Pb, K, Zn, Ni and Ag in the tumor tissues and non tumor tissues of colorectal patients (Table 3). Indeed, this comparative study showed imbalances of the elemental levels in colorectal tumor tissues compared to non tumor tissues of patients, which may effect on the emergence and development of colorectal malignancy. Previous findings supported the notion that the median Zn, Cr, Cu, Al, and Pb levels were significantly elevated in cancerous tissues than healthy tissues [7]. Increased levels of Cd, Pb, Ni and Cr were associated with generation of free radical, lipid peroxidation, formation of DNA-strand breaks and tumor growth in cellular systems [21]. Zn and Mn concentrations were found elevated in colorectal cancerous tissues than non-cancerous tissues as shown in some recent studies [10,41]. In colorectal tumor biopsies, the concentration of Mg was significantly higher as compared to adjacent non-tumor tissues [8,45]. Juloski and his coworkers in Serbia found significant elevated K, Cu, Cr, Cd, Mg, Hg Se and Ca levels in tumorous tissues than healthy tissue [10]. These findings were correlated to our studies [8,45]. One study found significantly higher Cu concentration in colorectal tumor tissues as compared to healthy tissues as it was the case in the similar study [41]. Level of K was considerably higher in tumor tissues than non tumor tissues and these findings were consistent with other studies [10, 41]. Levels of Mn, Se, Cu, Fe, Mg and Ca along with elements were significantly higher in tumorous tissues than non tumorous tissues as reported by Lavilla et al., [41].

3.3. Correlation study

The correlation study was applied to investigate the relationship between various elemental pairs in the colorectal tumor tissues and non-tumor tissues of patients as displayed in Table 4 where bold *r*-values are significant at $p < 0.05$. In tumor tissues of colorectal patients (below the diagonal), strong positive correlations were detected between Li-Na (r = 0.748), Sr-Hg (r = 0.691), Sn-Na (r = 0.669), Ca-Cd (r = 0.0.635), Zn-As (r = 0.631), Ca-Mn (r = 0.628), Sn-Mg (r = 0.623), Pb-Ca (0.618), Ni-K (r = 0.605) and Na-Pb (r = 0.0.601). Significant relationship were demonstrated by Sr-Cd (r = 0.599), As-Co (r = 0.597), Na-Mn (r = 0.587), Hg-Ca (r = 0.586), Cu-Ca (r = 0.585), Li-Cu (r = 0.581), Ag-Li (0.581), K-Cr (r = 0.571), Na-Co (r = 0.567), Sn-Mn (r = 0.567), Zn-Hg (0.566), Ag-Pb (0.563), Fe-Cr (r = 0.562), Ni-Pb (r = 0.549), K-As (r = 0.532), As-Pb (r = 0.531), Zn-Sr (r = 0.520), Sn-As (r = 0.516), Co-Pb (r = 0.516), Zn-Cu (r = 0.514) and Cr-Cd (r = 0.507) as presented in Table 4. This directed that these elements in tissues were mutually associated and probably shared common origin in colorectal tumor patients. Nevertheless, some of the elements exhibited significantly negative correlations: Cr-Hg (r = –0.575), Li-Mn (r = –0.548), Pb-Se (r = –0.521), Ni-Fe (r = –0.516), Sn-K (r = –0.514) and As-Na (r = –0.505) thus exhibiting their opposing relationship in the tumor tissues of colorectal patients.

In case of non tumor tissues of patients (above the diagonal), the correlation coefficient matrix of selected elements in Table 4 showed that significantly strong positive correlations were found between Cu-Pb (r = 0.735), and Cr-Co (r = 0.674), Sn-Sr (r = 0.653) and Mn-Cd (r = 0.617). Further, significant correlation were noted for K-Hg (r = 0.588), Fe-As (r = 0.583), Na-Cr (r = 0.566), Ca-Mn (r = 0.553), Zn-Co (r = 0.551), Cr-Pb (r = 0.548), Li-Na (r = 0.547), Sn-Na (r = 0.543), Cu-Hg (r = 0.533), As-Mn (r = 0.530), Se-Mg (r = 0.530), Ag-Cr (r = 0.529), As-Cd (r = 0.521), Sn-Mg (r = 0.519), Se-Co (r = 0.513) and Se-Cr (r = 0.505) in the tissues of controls as shown in Table 5. Some negative correlation was revealed between Na-Ca (r = −0.296), Fe-Cr (r = −0.290) and Fe-Na (r = −0.229). Overall, this correlation study detected significant differences in the tumor tissues and non tumor tissues of colorectal patients in terms of mutual variations between the toxic and essential elements, which may be attributed to the disproportions of the nutrients and trace elements in the patients. Since most of the selected essential/toxic elemental levels were significantly correlated with one another in the tumor tissues of colorectal patients, multivariate methods were used to investigate further the grouping and apportionment of the elements (see below).

3.4. Multivariate analysis of selected elements in the tissues

Elemental apportionment and source identification in the tumor tissues of and non tumor tissues of colorectal patients were performed by PCA and CA [5]. The PC loadings of selected elements in the tumor tissues and non tumor tissues of colorectal patients are given Table 5. In

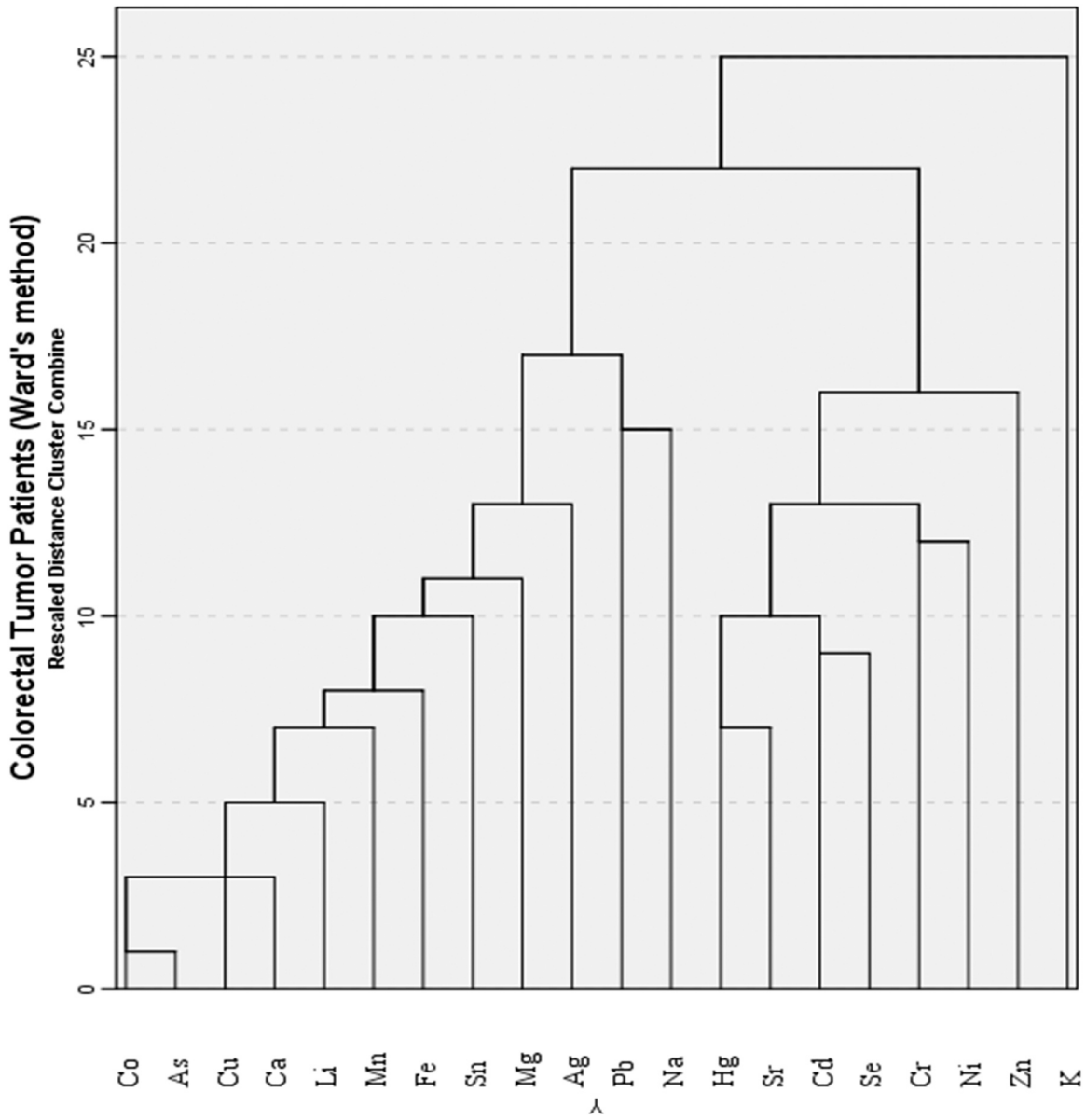

Fig. 1. Cluster analysis of selected elements in the tumor tissues of colorectal patients.

case of tumor patients, six PCs with eigen values greater than 1 were extracted, explaining more than 75% of the cumulative variance of the elemental data. Other small eigenvalues of less than one were not used to get a probable number of contributing source factors. Their corresponding CA based on Ward's method in the tissues of tumour patients are shown in Fig. 1, which exhibited strong clusters of Na–Pb, Mn–Fe–Sn–Mg–Ag–Li, Ca–Cu–As–Co, and K–Zn–Ni–Cr, Sr–Hg and Se–Cd. Accordingly, CA disclosed that toxic elements share common clusters with the essential elements, evincing the imbalances of elements in the tissues of tumor patients. The quantitative information concerning multiple relationships of these elements was evaluated by PCA in which PC 1 revealed higher loadings for Na and Pb while PC 2 exhibited dominant loadings of Ag, Mg, Sn, Li and Fe with joint clusters presented in the CA. These elements can be contributed by dietary habits and environmental pollution like traffic emissions. PC 3 was related to the elevated loadings of As, Co, Ca and Cu, with a strong cluster for these elements given in the CA. This PC showed that these elements can be related to internal body metabolism and nutritional habits of the patients. K, Cr, Zn and Ni revealed major contributor in PC 4 with a cluster in CA as shown in Fig. 5. Likewise, PC 5 represented by higher loadings of Sr and Hg. These elements mostly belong to the anthropogenic pollutants. Highest loadings observed for Cd and Se in PC 6 in the tissues of colorectal tumour patients for which the CA also revealed a strong cluster. These elements can be mostly contributed by food habits as well as anthropogenic contamination.

In case of non tumor tissues of colorectal patients, Table 5 displayed seven principal components (PC) whose eigenvalues are > 1 extracted, manifesting more than 83% of the cumulative variance. The corresponding CA in the tissues samples of controls based on Ward's method is give in Fig. 2, which exposed very strong clusters of Li–Zn–Fe–Ca, K–Hg–Sn–Sr, Se–Cr–Co, Mn–Cd, Cu–Pb, Mg–Ni–As, and Na–Ag. First PC demonstrated elevated loadings of Li, Zn, Fe and Ca, which shared common clusters in CA. Second PC was loaded with K, Sn, Hg and Sr. Similarly third PC consisted of Se, Cr and Co with joint clusters were given in the CA. Likewise, considerable loadings of Mn & Cd and Cu & Pb were associated in fourth and fifth PCs, respectively. Dominant loadings of Ni, Mg and As was displayed by PC 6. The last PC was related with ample loadings of Ag and Na, which were noted with common source of contamination and anthropogenic activities (traffic emissions) as well as with the contributions of food intake. Multivariate statistical methods,

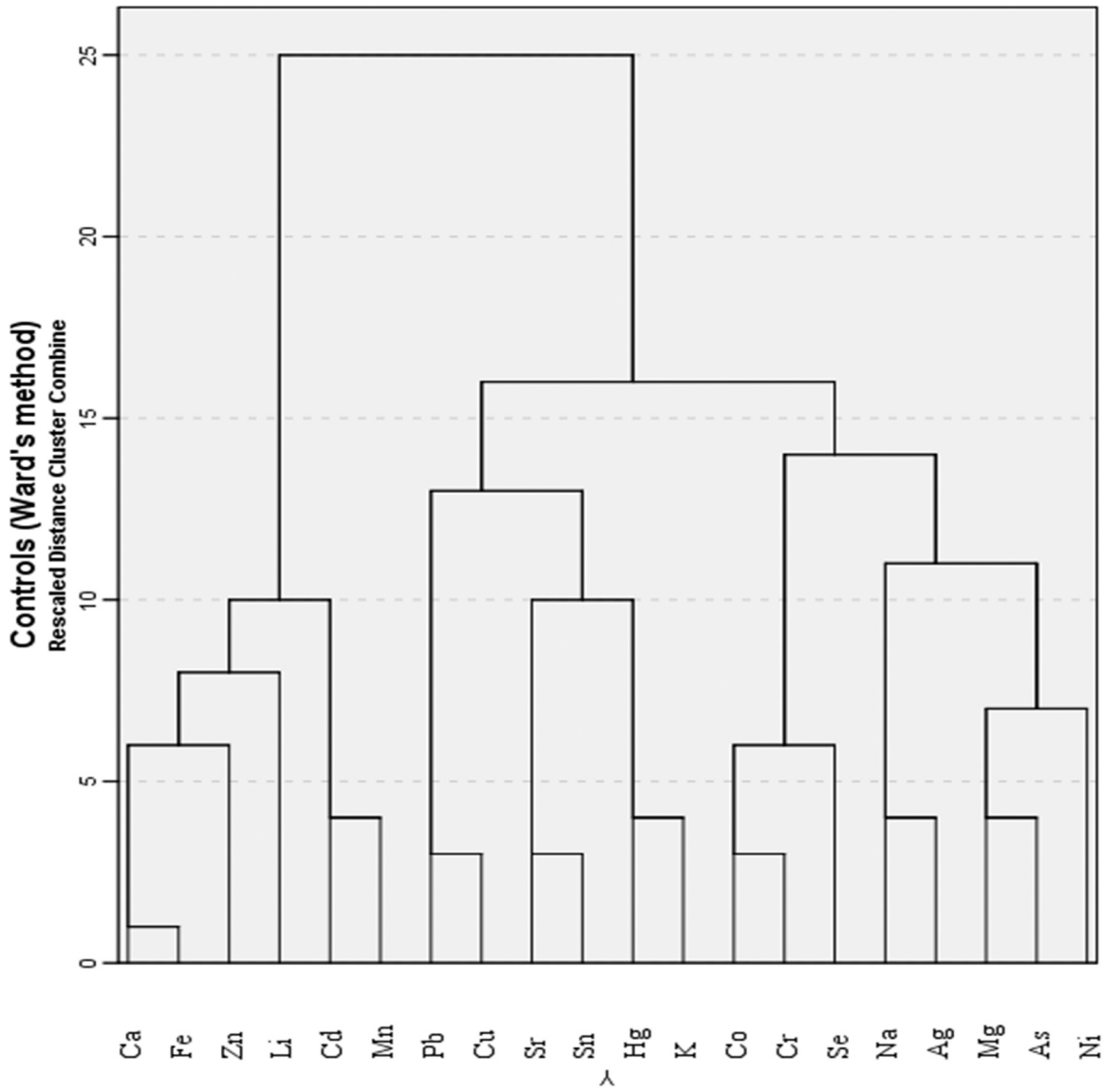

Fig. 2. Cluster analysis of selected elements in the non tumor tissues of colorectal patients.

consequently, advocated noteworthy disparities in elements apportionment in the tumor tissues and non tumor tissues of colorectal patients.

3.5. Comparison of elemental levels in the tissues based on smoking/nonsmoking habits

Cigarette smoking not only as being an increased risk of colorectal tumor but also worsens the prognosis of tumor as well as is a serious public health concern according to American College of Gastroenterology guidelines for colorectal cancer screening 2009 [46]. A huge body of evidence indicates that a significant association of cigarette smoking with colorectal cancer incidence and mortality. Accordingly, cigarette smoking is responsible for millions of deaths worldwide annually [47]. Smoking habits with long term has been reported to have a significantly increase risk of developing colorectal tumor than nonsmokers. However, data were insufficient and conflicting for the association among smoking and colorectal tumor risk. In view of that, the smoking/non-smoking based disparities in mean elemental concentrations (± SE) in the colorectal tumor tissues and colorectal non tumor tissues of patients are shown in Fig. 3. Mean levels of Cd, Pb, Cr, Cu, K, Zn, Ni, Sn and Ag were found to be relatively higher in the tumor tissues than non tumor tissues of patients addicted of smoking, while elevated levels of Mg, Mn, Ca, Co, Hg, Na, As and Se were found in the non tumor tissues of colorectal patients. Non-smoker patients revealed higher levels of Cd, Pb, Cr, Cu, K, Zn, Ni, Sn and Ag in tumor tissues than non tumor tissues of same patients. Nonetheless the measured elemental levels of Mg, Mn, Ca, Co, Hg, As, Fe, Se and Sr were found to be higher in the non tumor tissues than tumor tissues of non smoker patients controls. Average Ag, Li, Ni, Cr and Pb levels were elevated in the tumor tissues than non tumor tissues of non smoker patients, while mean concentrations of Cd, Hg, Se, Sr and Zn were higher in tumor tissues (smoker than non smoker) of patients. Mean concentrations of Se, Fe, Co, Ca, Mn and Mg were relatively higher in non tumor tissues than tumor tissues of non smoker patients (Fig. 3).

3.6. Comparison of elemental levels in tissues based on vegetarian/nonvegetarian habits

Diet is expected to be one of the most important roles in the etiology of colorectal carcinoma. A dietary pattern that is rich in whole grains, vegetables, fruit, fish, legumes, nuts and low in red and processed meat and alcohol has been related to a substantial dwindling in the risk of colorectal tumor [48]. Therefore, the WHO recommends improving dietary quality by increasing consumption of fruit and vegetables, as well as legumes, whole grains and nuts [49]. Subsequently the

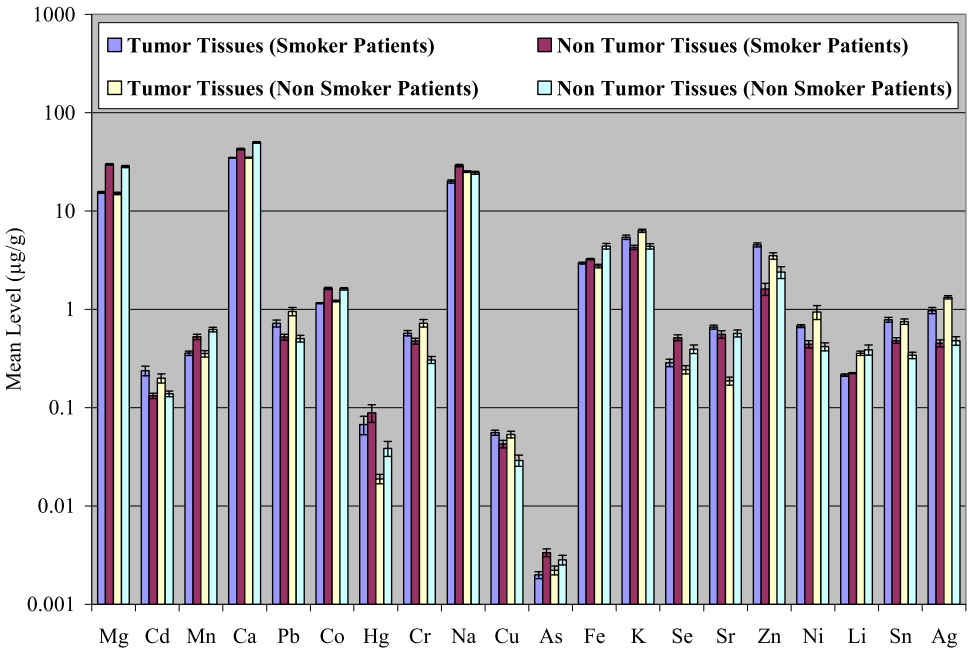

Fig. 3. Comparative average concentrations of elements (± SE) in the tumor tissues and non tumor tissues of colorectal patients based on smoking and nonsmoking habits.

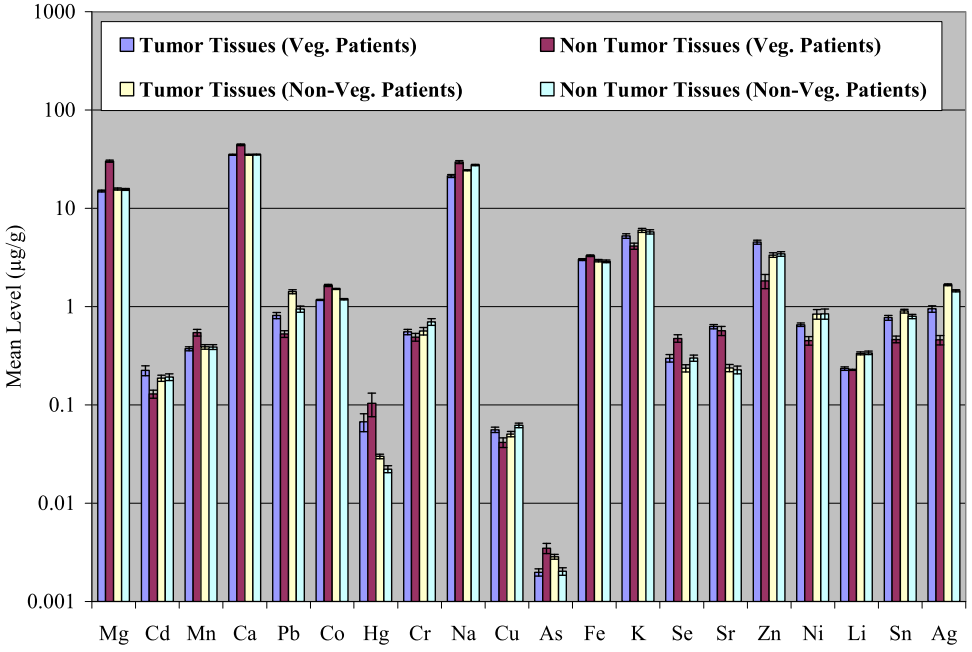

Fig. 4. Comparative average concentrations of elements (± SE) in the tumor tissues and non tumor tissues of colorectal patients based on vegetarian and non vegetarian food habits.

vegetarian/non vegetarian food based comparison in the mean elemental concentrations in the colorectal tumor tissues and colorectal non tumor tissues of patients are shown in Fig. 4. In vegetarian tumor tissues, maximum levels of Cd, Cu, Pb, Zn, Ni, Sn and Ag were detected when compared to non tumour tissues of vegetarian patients. Nonetheless, Mg, Se. Mn, Ca, Co, Hg and Na exhibited maximum in the non tumour tissues than tumor tissues of vegetarian patients. For comparison among tumor and non tumor tissues in the same patients, toxic trace metals were found more contribution than essential metals in the tumor tissues which revealed that toxic trace metals may have a role in the beginning/development of colorectal malignancy. Mean levels of Ag, Sn, As, Hg, Co and Pb were higher in tumor tissues than non tumor tissues of same patients with non vegetarian food habits. Similarly, Cr, Na, Cu and Se were found elevated in the non tumor tissues than tumor tissues of non vegetarian dietary habits. Mean concentrations of Mg, Cd, Mn, Ca, Fe, K, Sr, Zn, Ni and Li were almost same concentrations in the tumor and non tumor tissues of non vegetarian colorectal patients (Fig. 4). Overall, the average elemental contents in the tumor and non tumor tissues of colorectal patients were significantly different in the same organs (colorectal) of patients regarding cigarette smoking habits as well as food habits.

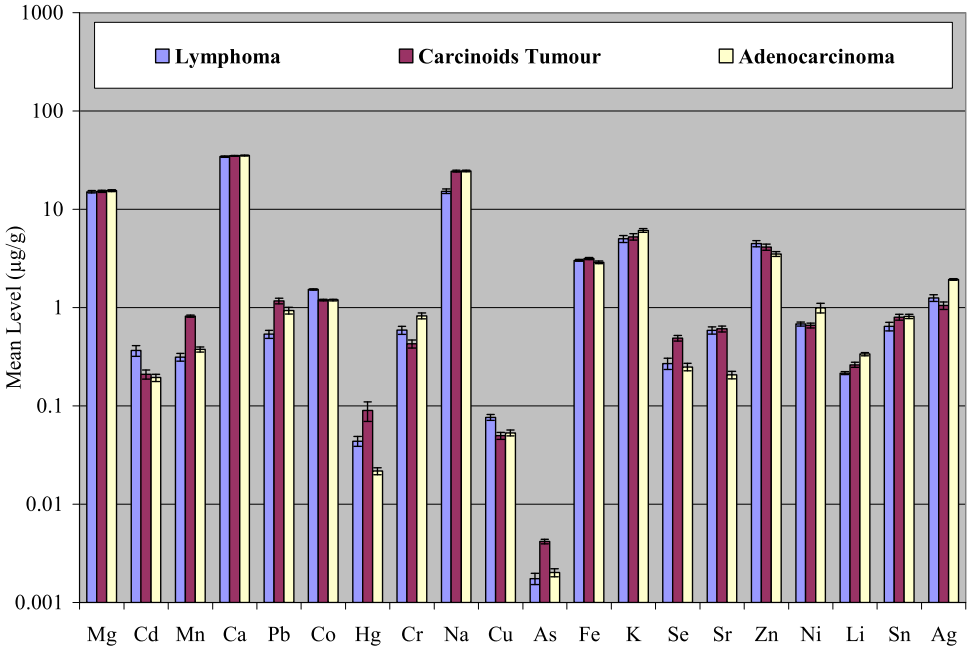

Fig. 5. Comparative average concentrations of elements (± SE) in the tissues based on type of colorectal tumor patients.

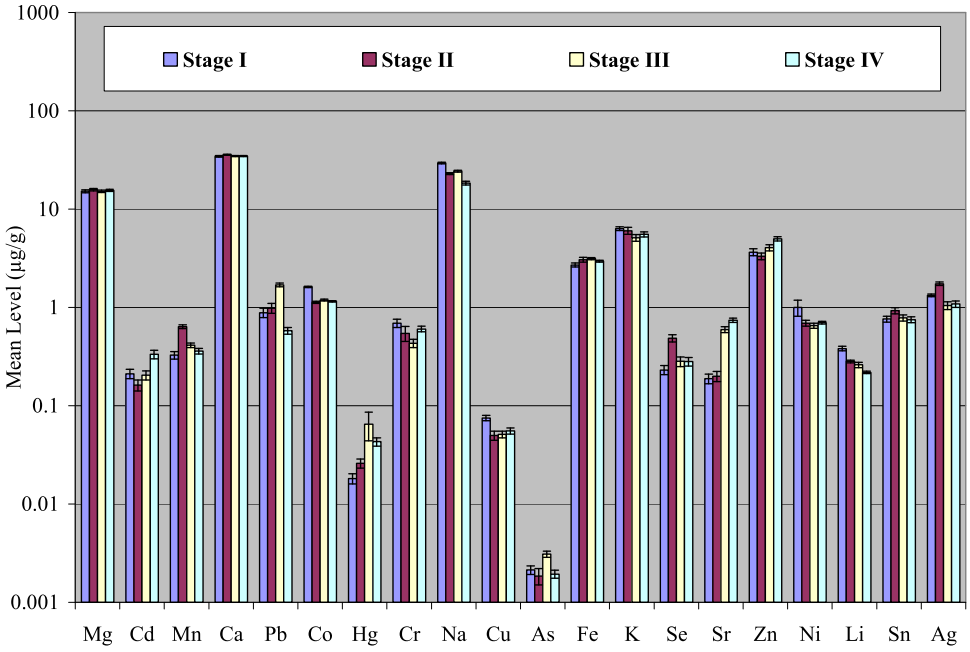

Fig. 6. Comparative average concentrations of elements (± SE) in the tissues based on stage of colorectal tumor patients.

3.7. Comparison of the elemental levels based on types/stages in tumor tissues

Comparative assessment of average elemental levels in the tumor tissues of various types (lymphoma, carcinoids tumor and adenocarcinoma) of the colorectal patients is presented in Fig. 5. Colorectal tumor patients with lymphoma type exhibited relatively elevated levels of Cd, Co and Cu but rather lower levels of Pb, Mn, Na, As, Li, As and Sn. In lymphoma tumor patients, toxic metals exhibited more significant contributions and essential metals showed somewhat less contributions. In the tissues with carcinoids tumor, higher levels of Mn, Pb, Hg, As and Se were observed in the patients. Comparatively elevated mean levels of Cr, Ni, Li and Ag were noticed in the tissues with adenocarcinoma of colorectal patients as shown in Fig. 5. Nonetheless, mean Mg, Ca, Zn and Fe concentrations were more or less similar in the tissues with all types of colorectal tumor patients as shown in Fig. 5.

Comparison of the average elemental levels measured in the tumor tissues of various pathological stages (I, II, III, and IV) of colorectal patients is shown in Fig. 6. At stage I, relatively elevated levels of Co, Cr, Cu, Na, Ni and Li were found in the tissues of colorectal tumour patients. Higher average concentrations of Ag, Sn, Se, and Mn were shown in the tumour tissues at stage II. Similarly mean Pb, Hg and As concentrations showed elevated levels in the tissues at stage III, while considerably higher levels of Cd, Sr and Zn were exhibited by stage IV in colorectal tumor patients. Nonetheless, Mg, Ca and Fe presented almost comparable levels in the tumor tissues of colorectal patients at all four stages as

shown in Fig. 6. This is the first study to examine the associations among toxic/essential elemental concentrations and colorectal carcinoma risk in Pakistani Population. The current study has some limitations. We did lack information on family history, physical activity of colorectal tumor patients. Food intake was self-reported and assessed using a single baseline measurement. We did not have information about occupational exposure. These factors may compromise the accuracy of the analysis. Key strengths of this study include essential/toxic elements were quantified in the tumor tissues at various types and stages of colorectal patients. In the future, prospective studies are needed to explore the causal relationships between toxic trace elements and colorectal malignancy.

4. Conclusions

In the context of the current study, 20 elements showed different concentrations in tumor tissues than non tumor tissues of the same colorectal patients. Average concentrations of Ca ($p < 0.01$), Na ($p < 0.05$), Mg ($p < 0.001$), Fe ($p < 0.001$), Sn ($p < 0.05$) and Se ($p < 0.01$) showed significantly raised levels in the non tumor tissues than tumor tissues, whereas mean Zn ($p < 0.05$), Ag ($p < 0.001$), Pb ($p < 0.001$), Ni ($p < 0.01$), Cr ($p < 0.005$) and Cd ($p < 0.001$) levels revealed significantly higher contributions in the tumor tissues compared to non tumor tissue of the colorectal patients, which is possibly linked to the tumor etiology/growth stage. The correlation study draws attention to the considerable associations among toxic trace and essential elements in the tumor tissues of the colorectal patients that further elucidated in terms of PCA and CA, which exhibited diverse apportionment of the elements in the tumor tissues of the colorectal patients. Disparities of the elements are also observed based on food habits and tobacco/smoking habits. The findings of the present study advocated that tumor tissues may accumulate toxic elements more effectively and that biogenic elements level is disturbed. Further biological mechanism behind it needs to elucidate in terms of pathophysiology as well as identify potential preventive with early-detection strategies.

Ethical approval

This study's protocols were approved by the Ethics Committees of the concerned Institute/Hospital on human experimentation (national and international) as well as with the revised Helsinki Declaration.

Consent to participate

Before the commencement of the study, all colorectal tumor patients were informed face to face about the objectives of the present research work and gave consent form, and all colorectal tumor persons agreed voluntary to participate and signed the performa.

Consent to publish

All the authors agreed and gave consent to publish this article in the present form.

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CRediT authorship contribution statement

The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors. Qayyum MA, Farooq T and Muddassir K designed the research plan and perception of study. Mahmood MHR, Baig A and Bokhari TH ensured sampling, experimentation, and the analysis of results. Qayyum MA, Muddassir K and Baig A statistically analyzed the data as well as the writing of the present article in first form. Ahmad M, Ashraf AR, took part in writing and formatting of the manuscript. Anjum MN and Farooq T contributed to the software, experiment, and visualization. All the authors contributed in the review process of manuscript and editing as well.

Declaration of Competing Interest

All the authors have claimed that they have no conflict of interest.

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