Volume 8, Issue 5, 2018, 3526 - 3538 **ISSN 2069-5837**

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article **Open Access Journal**

Received: 27.08.2018 / Revised: 29.09.2018 / Accepted: 05.10.2018 / Published on-line: 15.10.2018

Disparities of trace elements and electrolytes in oral cancer patients in comparison with healthy subjects

Muhammad Abdul Qayyum [1], Mian Habib-ur-Rahman Mahmood[1], Munir H. Shah [2,*]

[1]Department of Chemistry, University of Education Lahore, Faisalabad Campus Faisalabad 38000, Pakistan
[2]Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan

*corresponding author e-mail address: ***mhshahg@qau.edu.pk; munir_qau@yahoo.com***

ABSTRACT

Oral cancer is increasing worldwide at an alarming rate yet little is known of the impact this increase will have on society. Deficiency or excess of trace elements and electrolytes play a vital role in the development of oral cancer. The aim of the present study was to quantify the elemental and electrolytes levels (Ca, Mg, Sr, K, Na, Li and Co) in the scalp hair, nails and blood of oral cancer patients and counterpart healthy subjects by flame atomic absorption spectrometry. Average concentrations of Na, Sr, Li and Co were significantly higher while Mg levels were lower in the scalp hair and nails of the patients compared to the controls. In the blood, mean levels of Mg, Na and Sr were relatively higher and K, Co and Li levels were lower in the oral cancer patients than healthy donors. The correlation study revealed significantly divergent mutual variations among the elements and electrolytes in oral cancer patients and healthy donors. Most of the elements and electrolytes exhibited significant disparities in their concentrations based on gender, abode, dietary habits and smoking habits of the oral cancer patients and healthy subjects. Variations in the elemental and electrolytes levels were also observed for various cancer types (squamous cell carcinoma and adenocarcinoma) as well as stages (I, II, III and IV) in the patients. Consequently, quantification of elements and electrolytes in the scalp hair, nails and blood of the oral cancer patients demonstrated prolific data; not only as a diagnostic method but also in providing answers pertaining to the treatment.

Keywords: *oral cancer; electrolyte; atomic spectroscopy; statistical analysis; Pakistan.*

1. INTRODUCTION

Oral cancer is the 6th most common cancer in the world and is referred to a group of malignancies including cancers of the mouth, lips, cavity located behind the tonsils and the back of the throat [1]. It is one of the malignancies which are characterized by a high rate of morbidity and mortality worldwide [2]. Two-thirds of the global burden of oral cancer occur in low to middle-income countries such as India, Bangladesh, Sri Lanka and Pakistan [1, 3]. It is the most common cancer in men and may contribute up to 25% of all new cases of cancer in Pakistan [3]. Oral cancer is characterized by histopathological and clinical manifestations and about 90% of oral malignancies in the upper aerodigestive tract are squamous cell carcinomas originating in the tissues that line the mouth and lips [4]. Stage of oral cancer is determined to indicate how far cancer has progressed. Moreover, survival from oral cancer is critically dependent on the clinical stage of cancer at presentation. At an early stage, if undetected this cancer metastasizes in the body leading to death. Commonly, it is classified into the four stages [5]. The aetiology of oral cancer appears to be multifactorial, and strongly related to tobacco smoking (cigarettes, cigars, and pipes), betel quid chewing and excessive alcohol consumption [6]. Others, genetic alterations, viruses (HPS & HPV), immune deficiency (HIV), Syphilis, candida, chronic irritation, exposure to elements and deficiencies in consumption of fresh fruits/vegetables are also implicated in the aetiology of oral cancer [1, 2, 7, 8]. Trace elements and electrolytes have many characteristics which make them an integral part of every biochemical process in body cells, and their imbalances may lead to many physiological disorders such as coronary heart disease, hypertension, autoimmune disease and development of various kinds of cancers including oral cancer [9, 10, 11].

Monitoring and surveillance of the micronutrient profile need to be emphasized when working with cancer patients and can be an inexpensive way to pinpoint improvable risk factors that may be addressed before any treatment [6]. Therefore, increased scientific interest in the elements has led to a search for a reliable biomarker of quantifying and monitoring their levels in human body tissues. Among body parts, blood is considered as the most reliable parameters for the assessment of elemental exposure because the elemental ions reach different parts of the body through blood circulation thus indicating current body burden of various elements [12]. It provides the elemental profiles under any occupational or environmental exposure and ease of sampling makes it one of the most widely used specimens for trace elemental analyses [2]. Hair and nails have been used frequently for the evaluation of environmental and occupational exposure and are recording filaments that can reflect metabolic changes of many elements over long periods of time which may provide post nutritional events [6]. Further, hair and nails exhibited attractive diagnostic materials due to simplicity/non-invasive of sampling, stable matrices, storage ability over a longer time span and finally trace elemental concentrations are not subjected to rapid fluctuation due to diet, air, and water [12, 13]. On contrary, due to the lack of standardized sample preparation procedures, hair/nails samples are controversial with lack of well-defined reference levels [14]. The growth rate of fingernails and toenails are approximately 3 mm/month and 1 mm/month, respectively and nails require approximately six months to regrow completely [15].

So, it is necessary to wait for their full growth which is a hectic task for sampling [16].

Despite the rapid rising of oral cancer incidence in Pakistan, only a few studies have examined the relationship between the pathological status of oral cancer and elements [2, 6]. Thus, there is an urgent need for evaluation elemental profile in the scalp hair, nails and blood of the patients, so that it may reveal possible associations between oral cancer and elements/electrolytes. Therefore, the aim of present study was to evaluate and compare the concentrations of selected elements and electrolytes (Ca, Mg, Na, K, Sr, Li and Co) in different types and stages of patients with oral carcinoma and their counterpart healthy donors. These elemental levels can also be selected as a preliminary screening tool to provide information needed to develop prevention strategies and likewise can be a complement to other diagnostic procedures.

2. EXPERIMENTAL SECTION

2.1. Study subjects. In the present study, scalp hair, nails and blood samples were collected from the oral cancer patients admitted in Nuclear Oncology & Radiotherapy Institute (NORI) Islamabad, and Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan, on a volunteer basis. The healthy subjects or controls were also selected on a volunteer basis from the same localities matched with the patient's demographic characteristics [2]. Prior to the sample collection, all cancer patients and controls were informed about the aim of the study through a signed consent form. Prior to the sample collection, the protocol of the study was approved by the human ethics committees of the institutes. Histological diagnosis was accomplished by an experienced pathologist with the histological stage determined according to the degree of differentiation of a tumour. The patients included in this study had a single primary tumour and none of them had received pre-operative chemotherapy, radiotherapy and surgery. The subjects were not taking any mineral supplement during the last six months. A questionnaire was filled in order to collect the detailed information related to the subjects such as age, gender, type/stage of disease, abode, dietary habit, smoking habit, socioeconomic status and donor consent at the time of sample collection. Physical examinations were performed to measure the participant's weight, height, blood pressure and biochemical data. Prior to the sample collection, healthy subjects were also undergone a standard routine medical examination [17]. The body mass index (BMI) of the subjects was calculated from the heights and weights using the formula:

BMI (kg/m^2) = Weight (kg)/Height^2 (m^2).

2.2. Scalp hair sample: Collection and preparation. About 3–4 g of scalp hair sample (not dyed, bleached or straightened) was cut from the sub-occipital position of the head with a pair of stainless steel scissors. The hair samples were about one centimeter apart from the scalp and directly stored at room temperature in the plastic bags tagged with identifying numbers. To evade external contamination due to dirt, dust, sweat, detergents and cosmetic treatments the scalp hair samples were subjected to the standardized washing procedure [13]. For this purpose, the long scalp hair samples were cut into suitable pieces (1–2 cm) and mixed thoroughly. The samples were immersed in 50 mL of 5% detergent solution and shaken on an auto-shaker for 30 min at 320 vibrations per minute; repeatedly washed with plentiful water to remove all the detergent. Then 30 mL of Triton X-100 (0.5 %, v/v) was added and then placed the contents on an auto-shaker for 20 min shaking. Once again the samples were washed with excess of doubly distilled water and finally dried in an electric oven for overnight at 70°C. The samples were cooled to room temperature in desiccators before weighing. For elemental analysis, scalp hair samples require an additional digestion step to destroy the organic matrix. The method involved sample digestion with HNO_3 and $HClO_4$. Accurately weighed (~3 g) scalp hair sample was taken in the digestion flask to which 10 mL of HNO_3 was added and heated on a hot plate at 70–80°C for about 30 min. The digestion flask was cooled down at room temperature and then 5.0 mL $HClO_4$ was added and again heated on the hot for a short interval of time until the white dense fumes evolved [12]. Following digestion, the solution was transferred to a 50 mL volumetric flask and diluted with doubly distilled water. A blank digestion (without sample) was also carried out through the complete procedure.

2.3. Nail sample: Collection and preparation. Before sample collection, nails were scrubbed using a nylon brush to remove the surface dust. Then the subjects were requested to clean their hands and feet with medicated soap. Nail samples were cut from fingernails and toenails with a stainless-steel clipper. The nails clippings were stored dry at room temperature in marked zip-lock polythene bags. In the laboratory, nail samples were soaked with 5% (w/v) detergent solution overnight to weaken the bound dirt, followed by shaking on an auto-shaker for 20 min at 320 vibrations per minute and then thoroughly washed. After that, the samples were soaked in acetone followed by shaking on an auto-shaker for 20 minutes for removing nail polish/lotion [12]. The samples were washed with distilled water. Thereafter, 30 mL of Triton X-100 (0.5% v/v) solution was added in each sample; the contents were shaken for 30 min on the auto-shaker and then finally washed with an excess of distilled water. The samples were dried in an electric oven before weighing. For sample digestion, the dried nail sample was accurately weighed up to three decimal and directly placed into the digestion flask to which 10 mL of concentrated HNO_3-$HClO_4$ (5:1, v/v) mixture was added, covered and kept at room temperature for 1 h. Then, the digestion flask was heated on a hot plate at 70°C until white dense fumes evolved. After the digestion was completed, the digestion flask was left to cool at room temperature and the resulting solution was transferred to a volumetric flask, and the volume was adjusted with 0.1 N HNO_3. The samples were finally stored in the refrigerator before analyses [12]. Blank solution without a nail sample was prepared simultaneously through the complete digestion procedures and similar acid matrixes.

2.4. Blood sample: Collection and preparation. Three to five milliliter of blood was drawn from the antecubital vein by using venepuncture method with the help of pre-cleansed syringe. The

blood was immediately transferred to an evacuated polyethylene tube. At all stages of sample collection, maximum care was taken to avoid contamination. Samples were kept in a refrigerator at –15°C until further processing [6]. After accurately weighing, the blood sample was transferred to the digestion flask to which 10 mL of concentrated nitric acid was added and left for 10 min and then added 10 mL of concentrated perchloric acid. The sample was heated on a hot plate until completion of digestion (dense white fumed evolved). Thereafter, the sample was diluted with 0.1 N HNO_3 in a 50 mL volumetric flask. A blank (without blood sample) was also processed in the same way along with each batch of five samples.

2.5. Quantification of the elements/electrolytes. The digested samples were quantified for selected elements and electrolytes (Ca, Mg, Na, K, Sr, Li and Co) on an atomic absorption spectrophotometer (Shimadzu AA-670, Japan) equipped with automatic background correction facility under optimum analytical conditions (Table 1). Three sub-samples of each sample were treated and run separately onto the spectrophotometer to pool the mean levels of elements. Parallel routine check on the accuracy of quantified results was ensured through the use of standard reference material materials (Human hair, GBW 07601; & Bovine muscle, NIST-SRM 8414) and the results are summarised in Table 1. The samples were also analyzed at an independent laboratory for comparison of the results which were in good agreement (±2.5% difference) with each other. All reagents used were of high purity (certified >99.9%) procured from E-Merck or BDH. Working solutions were prepared by serial dilutions of 1000 ppm stock standard solutions just before the analysis of the elements on the instrument [6, 12].

Table 1. Optimum analytical conditions for the elemental analyses along with their detection/quantification limits and certified Vs. measured (±SD) concentrations* (µg/g) of the elements and electrolytes in standard reference materials.

Element		Ca	Mg	Na	K	Sr	Li	Co
Wavelength (nm)		422.7	285.2	589.0	766.5	460.7	670.7	240.7
Slit width (nm)		0.5	0.5	0.5	0.5	0.5	0.5	0.2
Limit of Detection (mg/l)		0.004	0.001	0.002	0.003	0.005	0.003	0.005
Limit of Quantification (mg/l)		0.013	0.004	0.007	0.009	0.016	0.009	0.016
Human Hair, GBW 07601	Certified Level	2900	360	152	20	24	2	0.071
	Measured Level	2869 ± 41.31	357.1 ± 4.610	154.8 ± 7.226	20.47 ± 1.204	23.51 ± 1.533	1.966 ± 0.175	0.072 ± 0.002
	Recovery (%)	98.9	99.2	101.8	102.4	98.0	98.3	101.4
Bovine Muscle Powder, NIST-SRM 8414	Certified Level	145	960	2100	15170	0.052	-	0.007
	Measured Level	143.8 ± 3.014	955.2 ± 10.42	2085 ± 21.61	15210 ± 33.75	0.051 ± 0.002	-	0.007 ± 0.001
	Recovery (%)	99.2	99.5	99.3	100.3	98.1	-	99.2

*Triplicate sub-samples

2.6. Statistical analyses. STATISTICA software was used for the statistical analysis of the elements and electrolytes data [18]. The data distribution and recognition tools applied in this study included pre-treatment of the data to attain normalization by discarding the outliers. The quantified results were subjected to statistical analysis in order to classify the relationship between the measured elements and electrolytes levels. Statistical analysis of the data comprised of the basic distribution parameters including range, mean, median, standard error (SE) and skewness while mutual variations among the elemental and electrolytes levels were computed as correlation coefficients. The elements and electrolytes data for the oral carcinoma patients and healthy donors were compared by applying the Student's *t*-test to assess the significance of difference ($p < 0.05$) of the mean concentrations. Further, Wilcoxon rank-sum test was applied for the comparison of the median levels of the elements and electrolytes among the patients and controls ($p < 0.05$).

3. RESULTS SECTION

3.1. Demographic characteristics. The demographic data for the oral cancer patients (hereafter called 'patients') and healthy donors (hereafter called 'controls') are given in Table 2, which showed that the subjects were closely matched for their ages. The existing range of BMI of the oral cancer patients was 20.97-21.03 kg/m^2 and controls was 24.35-24.55 kg/m^2. About 57-60% healthy donors were male, while 49-57% of oral cancer patients were female. However, 66-72% of the patients belong to vegetarian class while 28-33% of healthy donors were vegetarian in their food habits. Based on the habitat, 59–65% of the subjects were drawn from rural areas. A significant number of the patients (44–63%) and healthy donors (38–70%) were cigarette smoker. About 56-67% of the patients were suffering from squamous cell carcinoma and 33–44% from adenocarcinoma in the present study. Among the oral cancer patients included in the present study, 19-22% were diagnosed at stage-I, 34-40% at stage-II, 22-23% at stage-III and 19-22% at stage-IV (Table 2).

3.2. Body mass index and oral cancer. Body mass index (BMI) has been associated with risk of oral cancer as reported in some previous studies but this association remains to be clarified [19]. Several case control studies found inverse relationship between BMI and oral cancer [20]. Other recent prospective studies reported no association between BMI and oral cancer risk [21, 22]. In this study, BMI of oral cancer patients was significantly lower than healthy donors ($p < 0.001$) as shown in Table 2. Reduced BMI has been associated with oral cancer, possibly reflecting poor nutrition, socioeconomic status, or confounding by smoking [22]. In addition, greater than fifty percent of head and neck cancer patients have significant weight loss at the time of diagnosis and just before the treatment [19]. This weight loss is due to cancer induced dysphagia, odynophagia, anorexia or cancer cachexia and it has a negative effect on survival [23]. High BMI was found to be a protective factor for oral cancer but it is not clear whether weight loss is a risk factor for the disease or caused by oral cancer [24]. Some associations between BMI and head and neck cancer based on sex, tobacco smoking and alcohol drinking have been

reported but it was not examined by cancer site in most of the previous investigations. Therefore, the current literature does not provide a complete picture of the association between BMI and oral cancer. A better understanding of the association between BMI and this specific cancer could lead to disease aetiology. A possible mechanism for the link between lower BMI and increased oral cancer is still unknown but they may involve altered absorption and utilization, elevated oxidative stress and increased numbers of DNA adducts [21].

Table 2. Characteristics of the Subjects.

Characteristics	Scalp Hair		Nails		Blood	
	Patients	Controls	Patients	Controls	Patients	Controls
n	74	74	64	60	86	86
Age (years)						
Range	24–86	24–60	24–86	25–60	24–86	25–65
Mean	57.05	42.24	57.66	43.87	57.19	43.93
BMI (kg/m^2)	21.03	24.49	20.99	24.55	20.97	24.35
Gender						
Female	42(57%)	32 (43%)	36(56%)	26(43%)	42(49%)	34(40%)
Male	32(43%)	42(57%)	28(44%)	34(57%)	44(51%)	52(60%)
Diet						
Vegetarian	52(70%)	50(68%)	42(66%)	40(67%)	62(72%)	62(72%)
Non-vegetarian	22 (30%)	24(32%)	22(34%)	20(33%)	24(28%)	24(28%)
Habitat						
Urban	26 (35%)	24 (32%)	26(41%)	24(40%)	32(37%)	30(35%)
Rural	48 (65%)	50 (68%)	38(59%)	36 (60%)	54 (63%)	56 (65%)
Tobacco Use (Smoking)						
No use	46(62%)	46(62%)	36(56%)	32 (53%)	32 (37%)	26 (30%)
Use	28(38%)	28(38%)	28(44%)	28 (47%)	54 (63%)	60 (70%)
Types of Oral Cancer						
Squamous cell carcinoma	42(57%)	--	36(56%)	--	58(67%)	--
Adenocarcinoma	32 (43%)	--	28 (44%)	--	28 (33%)	--
Stages of Oral cancer						
Stage I	14 (19%)	--	14 (22%)	--	16 (19%)	--
Stage II	30 (40%)	--	22 (34%)	--	34 (39%)	--
Stage III	16 (22%)	--	14 (22%)	--	20 (23%)	--
Stage IV	14 (19%)	--	14 (22%)	--	16 (19%)	--

3.3. Distribution of elements and electrolytes in the scalp hair. Comparative distribution of selected elements and electrolytes in the scalp hair of oral cancer patients and healthy donors is presented in Table 3. In the case of the patients, comparatively higher average concentrations were found for Ca (1177 µg/g), Na (289.6 µg/g) and Mg (209.9 µg/g), followed by relatively lower levels of K (14.66 µg/g), Sr (14.64 µg/g)**,** Co (13.48 µg/g) and Li (1.647 µg/g). On the mean basis, the decreasing trend of the elements and electrolytes levels in the scalp hair of oral cancer patients exhibited the following order. Ca > Na > Mg > K > Sr > Co > Li. Overall, Ca, Mg and Na revealed considerable randomness in their distribution pattern as manifested by large SE values on one hand and markedly dissimilar mean and median levels on the other hand. Furthermore, large skewness values for K, Sr, Li and Co exhibited predominantly asymmetrical distribution.

In the case of healthy donors (Table 3), predominantly higher levels in their scalp hair were noted for Ca (1104 µg/g), Mg (229.4 µg/g) and Na (184 µg/g), followed by relatively lower levels of Sr (20.84 µg/g), K (9.204 µg/g), Co (5.777 µg/g) and Li (0.745 µg/g). The selected elements and electrolytes in the scalp hair of healthy donors revealed the following order in their average levels: Ca > Mg > Na > Sr > K > Co > Li. Some elements (Co, Li, Sr and K) displayed a relatively Gaussian distribution pattern in their concentrations as evidenced by fairly low SE values. Nevertheless, Na, Mg and Ca exhibited asymmetrical distribution as showed by large skewness values. Two-tailed Student's *t*-test of the elemental data showed that mean concentration of Na, K, Li and Co were significantly elevated in the scalp hair of patients, while average levels of Sr was significantly higher in the scalp hair of controls ($p < 0.05$). Similarly, median concentrations of the elements and electrolytes were also compared by Wilcoxon rank sum test which revealed statistically significant differences ($p < 0.05$) for Ca, Na, K, Li, Sr and Co in the scalp hair of the oral cancer patients and healthy donors (Table 3).

3.4. Distribution of elements and electrolytes in the nails. The basic statistical parameters related to the distribution of selected elements and electrolytes levels (µg/g, dry weight) in the nails of oral cancer patients and healthy donors are shown in Table 3. Dominant average contents were observed for Ca (1064 µg/g), Na (246.8 µg/g) and Mg (186.6 µg/g), followed by relatively lower levels of K (53.50 µg/g), Sr (23.69 µg/g), Co (20.01 µg/g) and Li (10.37 µg/g) in oral cancer patients. Most of the elements and electrolytes revealed appreciable randomness in their distribution pattern as manifested by large SE. On the mean basis, the decreasing trend of elements and electrolytes levels in the nails of oral cancer patients exhibited following order: Ca > Na > Mg > K > Sr > Co > Li. Relatively, large dispersion in the concentration was observed in the case of Mg, Na and Ca, whereas large skewness values for Ca and Na exhibited their predominantly asymmetrical distribution in the nails of patients.

In the case of healthy subjects, predominantly higher concentrations were noted for Ca (1241 µg/g), Mg (233.3 µg/g), Na (74.69 µg/g), and K (65.15 µg/g), followed by relatively lower concentrations of Sr (19.35 µg/g), Co (13.68 µg/g) and Li (4.955

µg/g). The selected elements in the nails samples of healthy donors exhibited the following decreasing order in their mean levels: Ca > Mg > Na > K > Sr > Co > Li. Most of the elements and electrolytes revealed non-Gaussian distribution as indicated by elevated SE values except Co, Li and Sr. Nevertheless, Na and Ca manifested asymmetrical distribution as shown by relatively large skewness values (Table 3). Two-tailed Student's *t*-test ($p < 0.05$) of the data showed that there was a significant difference between the contents of Ca, Mg, Na, Co and Li in the nails of patients and healthy donors (Table 3). The average contents of Mg, Ca and Na pointed out significantly higher levels in the nails of oral cancer patients than healthy donors. In contrast, average levels of Co, Li and K were considerably lower in the patients as compared to healthy donors. Wilcoxon rank sum test showed that median levels of Ca and Mg levels were significantly elevated and Na, Sr and Li levels were significantly lower in the nails of the oral cancer patients than controls.

Table 3. Statistical distribution parameters for elements and electrolytes concentrations (µg/g) in the scalp hair, nails and blood of oral cancer patients and healthy donors.

		Patients						Controls						*p*-value
		Min	Max	Mean	Median	SE	Skew	Min	Max	Mean	Median	SE	Skew	
Scalp Hair	Ca	467.7	3834	1177	1189	97.44	2.482	348.1	2180	1104	955.2	68.35	1.104	NS
	Mg	91.20	298.5	209.9	211.4	9.139	-0.116	100.4	353.2	229.4	237.9	14.76	-0.062	NS
	Na	9.700	749.9	289.6	271.3	42.63	0.530	1.650	613.4	184.0	133.9	30.08	0.861	<0.05
	K	1.175	33.94	14.66	14.62	1.737	0.190	1.650	17.68	9.204	8.938	1.051	0.239	<0.05
	Sr	2.250	30.90	14.64	14.30	1.204	0.571	3.950	41.05	20.84	20.25	2.084	0.090	<0.05
	Li	0.200	4.150	1.647	1.400	0.179	0.709	0.100	2.350	0.745	0.600	0.093	1.044	<0.05
	Co	2.200	28.95	13.48	13.23	1.187	0.092	0.100	13.50	5.777	4.250	0.686	0.537	<0.05
Nails	Ca	322.5	2578	1064	836.3	117.7	0.740	203.4	2272	1241	1304	109.9	-0.177	<0.05
	Mg	68.23	373.0	186.6	190.1	14.75	0.319	55.64	381.8	233.3	247.3	15.21	-0.623	<0.05
	Na	23.12	829.5	246.8	133.7	40.11	1.249	4.587	234.2	74.69	57.29	10.27	1.112	<0.05
	K	14.00	128.1	53.50	48.90	5.263	0.838	6.92	141.6	65.15	60.20	6.689	0.601	NS
	Sr	2.523	43.75	23.69	25.00	2.076	-0.093	2.717	42.90	19.35	14.59	2.305	0.492	NS
	Li	1.136	28.80	10.37	9.617	1.341	0.579	1.322	18.12	4.955	3.615	0.689	2.188	<0.05
	Co	4.250	48.84	20.01	14.46	2.812	0.871	2.232	30.94	13.68	13.29	1.399	0.767	<0.05
Blood	Ca	16.64	48.46	37.57	39.01	1.041	-0.896	21.05	66.36	39.78	38.54	1.572	0.744	NS
	Mg	24.80	38.72	30.80	30.99	0.464	0.22	21.48	34.96	27.91	27.70	0.498	0.186	NS
	Na	1399	1995	1684	1666	22.19	0.271	1336	2331	1675	1610	38.38	1.178	NS
	K	380.5	682.7	490.1	483.6	11.39	0.584	329.6	919.5	554.8	528.3	17.38	0.925	<0.05
	Sr	0.098	4.759	1.943	1.848	0.163	0.217	0.076	2.239	1.102	1.110	0.089	0.234	<0.05
	Li	0.016	1.235	0.266	0.198	0.037	2.485	0.048	1.335	0.531	0.564	0.049	0.522	<0.05
	Co	0.073	4.597	1.562	1.502	0.161	1.495	0.256	8.711	3.396	2.742	0.350	0.740	<0.05

*NS-non significant

3.5. Distribution of elements and electrolytes in the blood. Average elemental levels along with the basic statistical parameters pertaining to the distribution of selected elements and electrolytes in the blood of oral cancer patients and healthy donors are shown in Table 3. Comparatively higher average concentrations were found for Na (1684 µg/g) and K (490.1 µg/g), followed by lower levels of Ca (37.57 µg/g) and Mg (30.80 µg/g). Lowest levels were observed for Sr (1.943 µg/g), Co (1.562 µg/g) and Li (0.266 µg/g) in the blood of the patients. Overall, mean levels of elements and electrolytes in the blood of oral cancer patients revealed following order: Na > K > Ca > Mg > Sr > Co > Li. Most of the elements exhibited relatively normal and symmetrical distribution in the blood of patients as manifested by their small SE and skewness values. However, Na and K showed asymmetrical distribution as given by their higher SE and skewness values.

In the blood of healthy donors (Table 3), predominantly elevated mean contents were noted for Na (1675 µg/g), K (554.8 µg/g), Ca (39.78 µg/g) and Mg (27.91 µg/g), followed by relatively lower levels of Co (3.396 µg/g), Sr (1.102 µg/g) and Li (0.531 µg/g). In the blood of healthy donors, the elements and electrolytes revealed following order in their average levels: Na > K > Ca > Mg > Co > Sr > Li. Most of the elements exhibited a symmetrical distribution in their levels as manifested by small skewness values, nonetheless, Na and K revealed random distribution as evidenced by the corresponding higher SE values. Two tailed student's *t*-test showed that mean concentration of Sr was significantly higher while mean contents of Co Li and K were significantly lower ($p < 0.05$) in the blood of the patients compared to the controls. However, the average levels of Ca, Na and Mg exhibited insignificant differences in the blood of two donor groups. Likewise, median concentrations of the elements were also compared by Wilcoxon rank sum test which showed statistically significant differences ($p < 0.05$) for Co, Li and Sr in the blood of oral carcinoma patients and healthy subjects. One of the interesting features of this comparative study revealed imbalance of elemental levels in case of oral cancer patients which exhibited the adverse effects of these elements on the emergence and development of oral malignancy.

3.6. Variations of elements and electrolytes based on demographic characteristics. Comparison in the mean levels of elements and electrolytes (±SE) in the scalp hair, nails and blood of oral cancer patients and controls based on gender, abode, dietary and smoking habits are shown in Figures 1-4 (a–l). Gender-based comparison revealed that the mean levels of Na, K, Li and Co were significantly elevated in the scalp hair of the female patients than the female controls (Figure 1a). However, Sr revealed a considerably higher level in the case of female controls. Furthermore, appreciably higher levels of Li and Co were observed in the male patients than male controls, while Mg and Sr showed a significant rise in the case of the male controls.

Residence-based comparison (Figure 2d) revealed that

mean levels of K, Li and Co were significantly elevated in the scalp hair of urban and rural patients in comparison with urban and rural healthy subjects, while average concentrations of Ca and Sr were significantly higher in urban controls. Similarly, rural patients showed a significant rise in mean levels of Na, Li, Co and K in the scalp hair of rural patients compared to urban healthy subjects, nevertheless, considerably higher Ca and Sr levels were noticed in the scalp hair of urban healthy donors. Rest of the elements manifested insignificant differences in their concentrations in scalp hair of the patients and controls from urban localities.

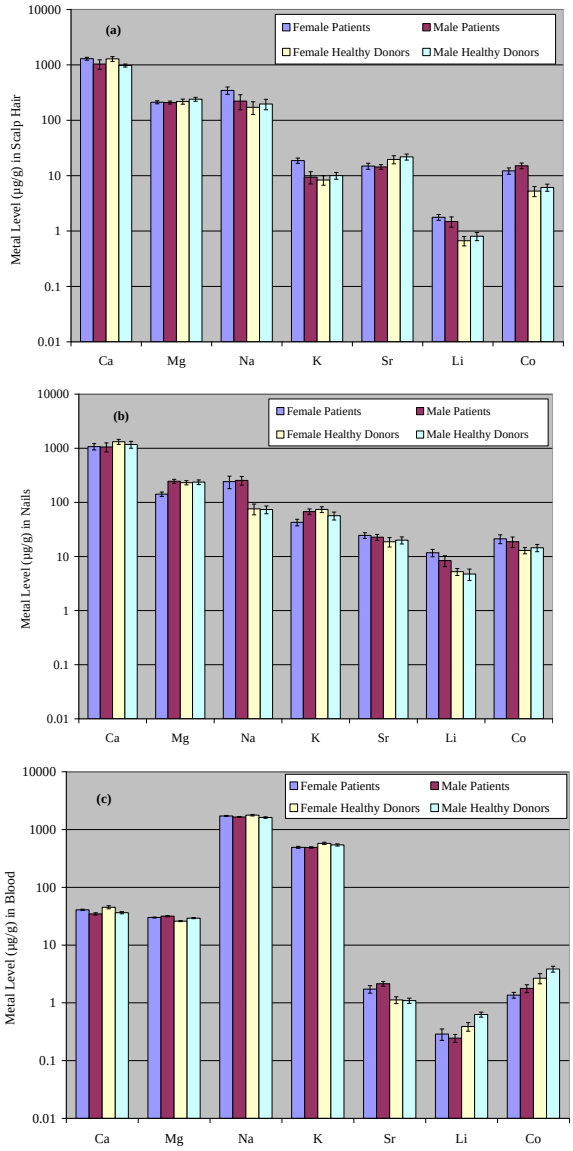

Figure 1. Gender-based variations in mean concentrations of selected elements and electrolytes (±SE), in the scalp hair, nails and blood of oral cancer patients and healthy donors.

In the case of nails, Na, Sr, Li and Co revealed significantly elevated levels in urban and rural patients compared to the urban and rural controls as shown in Figure 2e. In the case of controls, mean concentrations of K and Mg were found at considerably higher levels in the nails of rural controls compared to the rural patients. Likewise, mean concentrations of Ca, Mg and K were elevated in the nails of rural controls than urban patients. Besides, the mean content of Li was appreciably elevated in the nails of urban controls than rural patients. In the case of blood, (Figure 2f) comparatively higher levels were observed for Sr in the patients (urban & rural) as compared to the controls while mean contents of Li and Co were elevated in urban and rural controls. Average concentrations of Li, Sr and Co were appreciably higher in the rural patients than urban patients. Nonetheless, average concentrations of Ca, Mg, Na and K were more or less comparable in the blood of the patients/controls with urban and rural residences.

Diet-based comparison (Figure 3g) revealed that mean levels of Na, K, Li, Co and Sr were considerably higher in the scalp hair of patients (vegetarian & non-vegetarian) than those of healthy donors while average concentration of Sr was found to be appreciably higher in non-vegetarian and vegetarian controls.

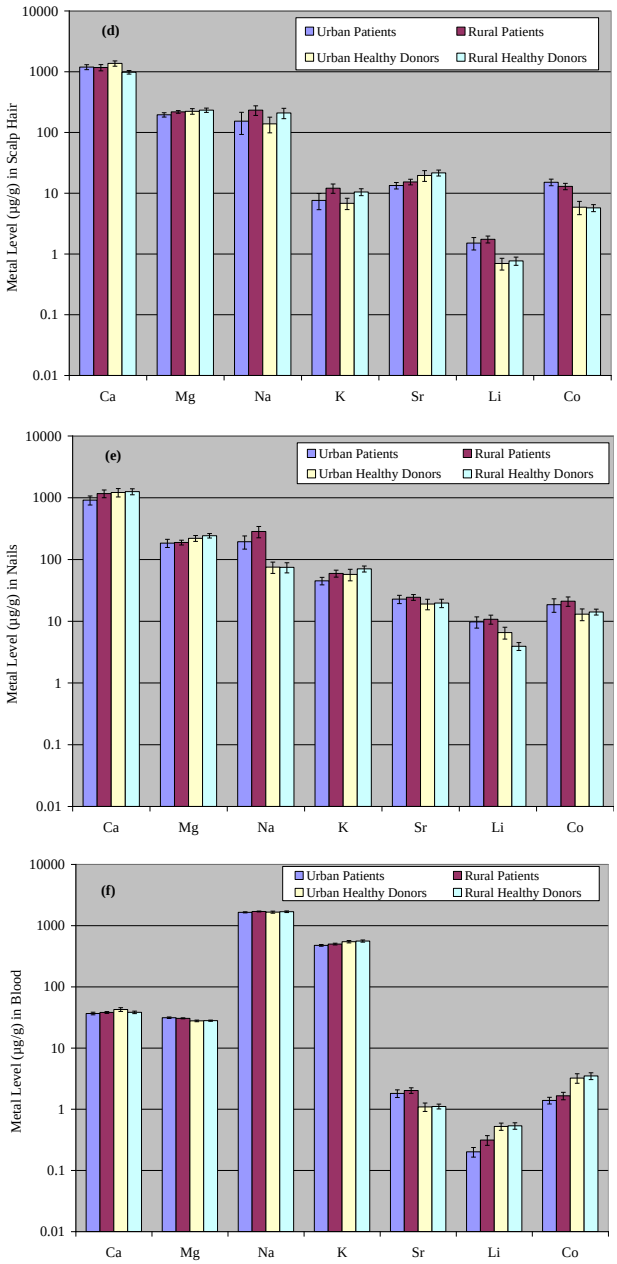

Figure 2. Habitat-based variations in mean concentrations of selected elements and electrolytes (±SE), in the scalp hair, nails and blood of oral cancer patients and healthy donors.

Moreover, non-vegetarian healthy donors exhibited an appreciable rise in mean levels of Mg and Sr compared to the vegetarian controls, whereas Co showed the notably elevated level in the scalp hair of vegetarian controls than non-vegetarian controls. The comparison of the mean concentrations of elements and electrolytes in nail samples of oral cancer patients and controls (Figure 3h) showed considerably higher mean levels of Sr, Li, Co and Na for non-vegetarian and vegetarian patients,

while Mg revealed appreciably higher levels in the case of non-vegetarian and vegetarian controls. Some of the elements (Ca, Sr, Li and Co) manifested appreciably elevated mean levels in the nails of non-vegetarian patients than vegetarian patients. In addition, the mean contents of Mg and Na were relatively higher in vegetarian patients compared to the non-vegetarian patients. Elevated levels of Li and K were noted in the vegetarian controls than non-vegetarian controls, while Na, Sr and Co showed relatively higher levels in the nails of non-vegetarian controls.

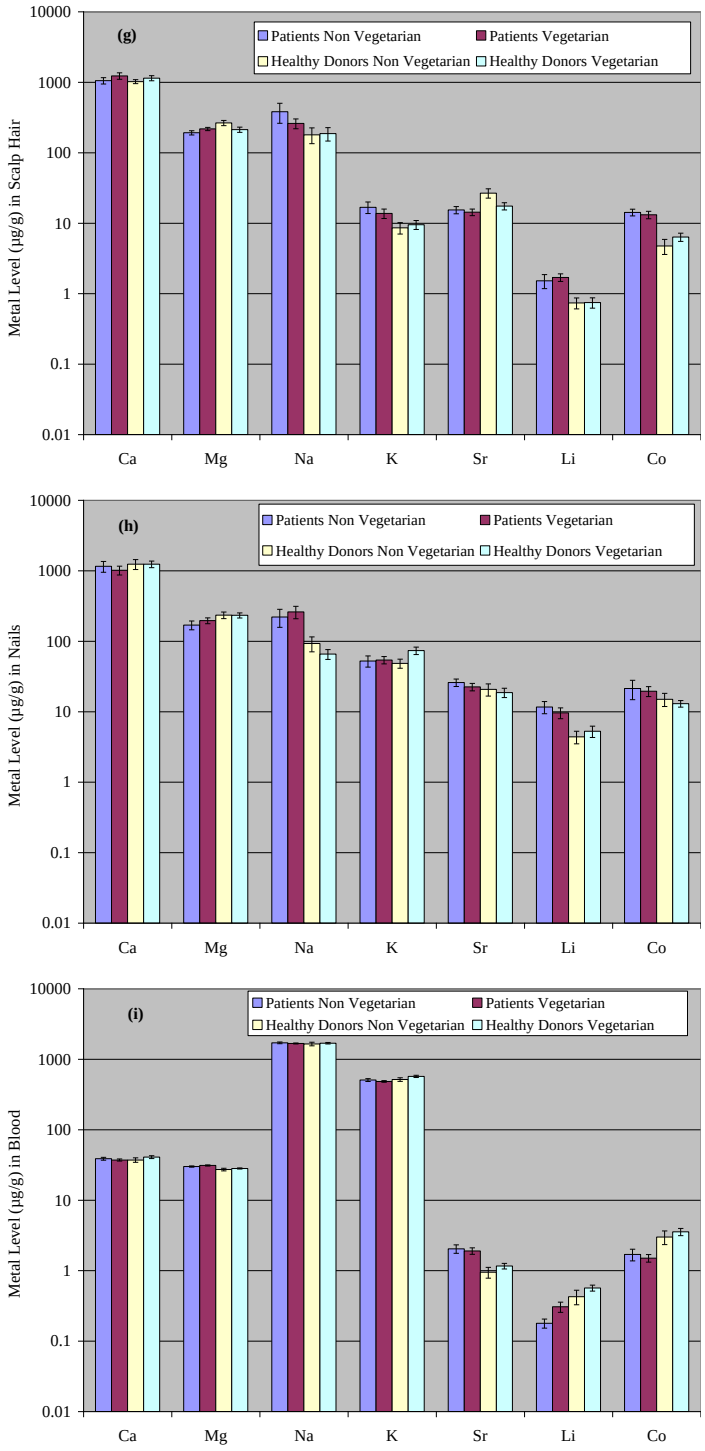

Figure 3. Diet-based variations in mean concentrations of selected elements and electrolytes (±SE), in the scalp hair, nails and blood of oral cancer patients and healthy donors.

In the case of blood (as shown in Figure 3i) Sr was relatively higher while Li & Co levels were lower in non-vegetarian and vegetarian patients than non-vegetarian and vegetarian controls. However, the concentration of K was higher in vegetarian healthy donors as compared to patients with vegetarian food habits. Average levels of remaining elements were not appreciably different in the blood of oral cancer patients and controls (Figure 3i).

Comparison of elemental levels in the scalp hair of smoking and non-smoking patients and controls are shown in Figure 4j. On the mean basis, Ca, Co and Li were considerably higher in the scalp hair of the patients with smoking habits, while notably higher concentrations of Mg, K and Sr were found in the case of smoking controls.

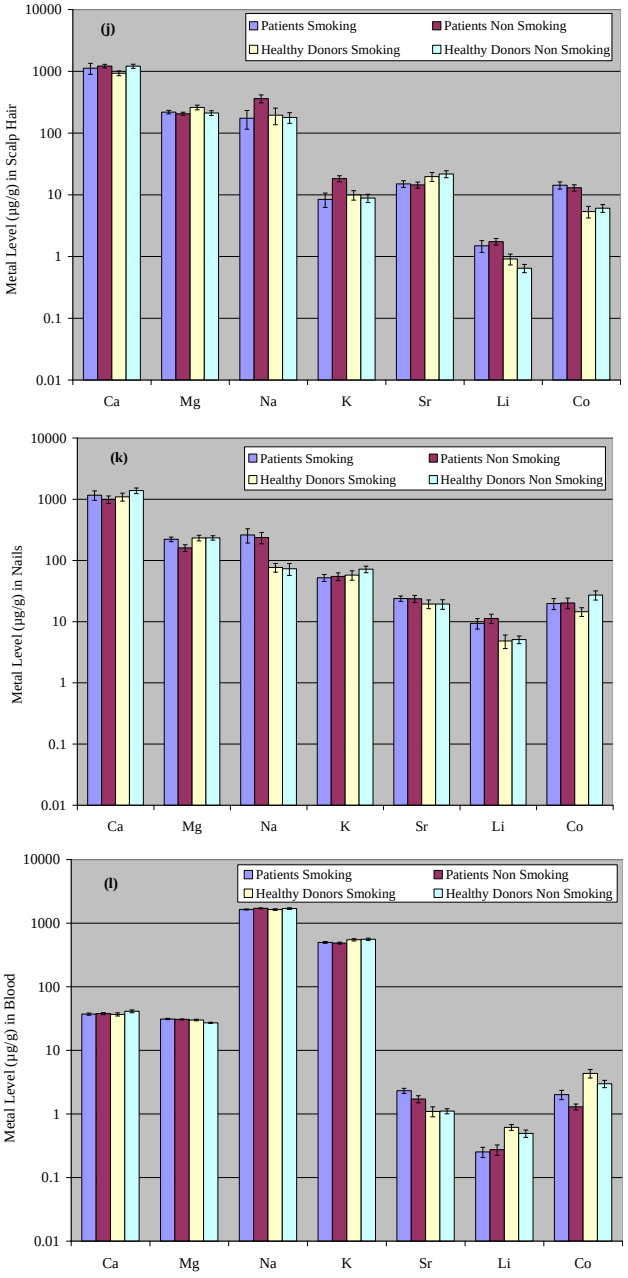

Figure 4. Smoking-based variations in mean concentrations of selected elements and electrolytes (±SE), in the scalp hair, nails and blood of oral cancer patients and healthy donors.

Nonetheless, mean concentrations of Co, Li, K and Na were considerably higher and average Sr content was lower in the scalp hair of non-smoking patients than smoking controls. In addition, mean levels Li and Mg were found to be appreciably higher whereas average concentrations of Ca, Sr and Co were lower in the scalp hair of the smoking controls than non-smoking controls. In the case of nails mean levels of Na, Sr, Li and Co revealed appreciably rise in smoking patients than smoking healthy donors as shown in Figure 4k. Only K revealed marked elevation in its average level for smoking healthy donors, while no

other elements and electrolytes showed any significant variation in their mean levels. Likewise, in the case of non-smoking patients, Na, Li and Sr exhibited higher levels as compared to the non-smoking healthy donors, while mean levels of Co, Ca, Mg and K were comparatively higher in the nails of non-smoking healthy donors. The average concentration of Mg was higher while that of Li was lower in the patients with smoking habits than non-smoking patients.

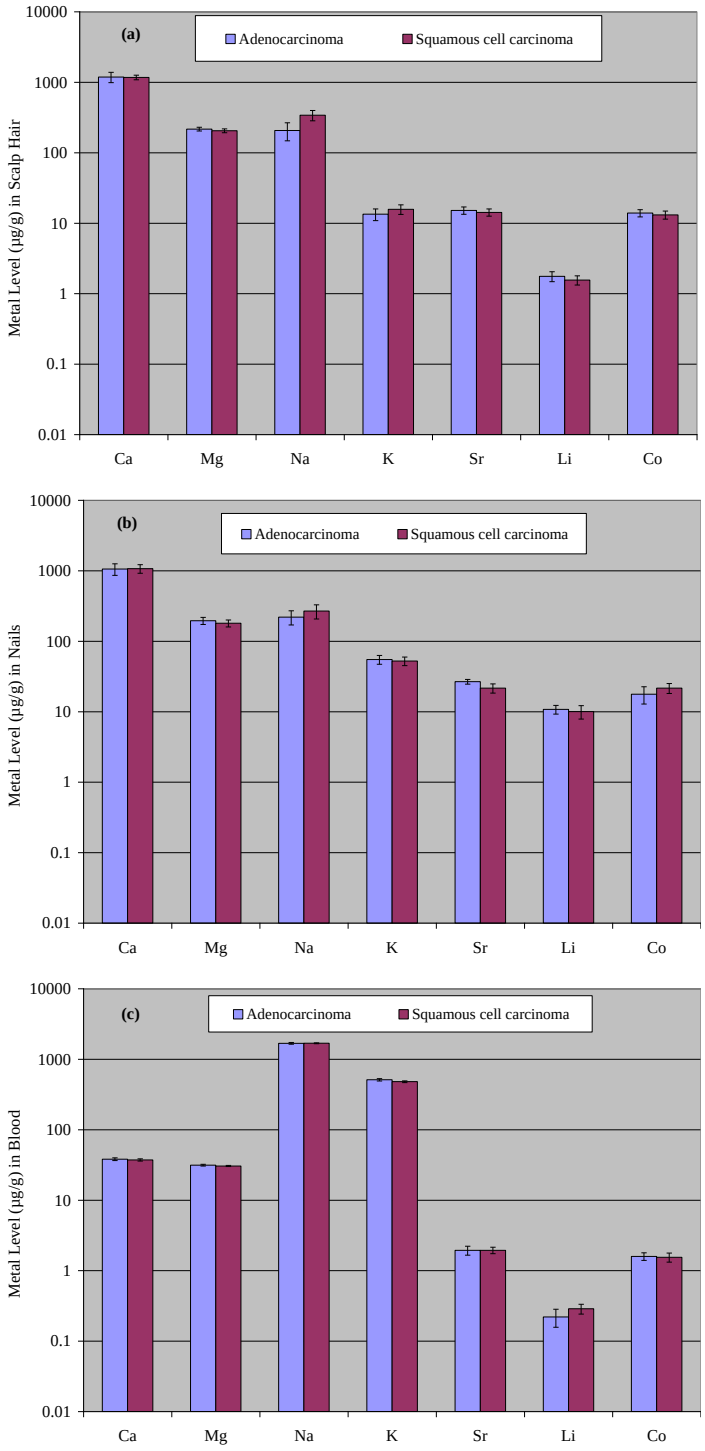

Figure 5. Comparative mean concentrations of selected elements and electrolytes (±SE) in the scalp hair, nails and blood of oral cancer patients based on types.

Mean concentrations of Co, K and Ca were considerably higher in non-smoking controls than patients with smoking habits (Figure 4k). Mean concentrations of Sr and Co were noted to be relatively higher in the blood of smoking patients compared to non-smoking patients, which revealed the appreciably higher average level of Li as noted in Figure 4l. However, mean levels of K and Na showed almost comparable levels in the patients irrespective of smoking or non-smoking habits. Similarly, mean concentrations of Li, Mg and Co were found to be noticeably higher in the blood of smoking controls compared to the non-smoking controls, which exhibited a relatively higher level of Ca in their blood (Figure 4l).

3.7. Variations of the elements and electrolytes based on cancer types. Comparative evaluation of average concentrations (± SE) of the elements and electrolytes in scalp hair, nails and blood of different types of oral cancer patients (i.e., adenocarcinoma and squamous cell carcinoma) is shown in Figure 5 (a–c). In the case of adenocarcinoma, Li revealed very high average concentration while Na and K manifested maximum levels in the scalp hair of squamous cell carcinoma patients as shown in Figure 5a. Rest of the elements were more or less comparable in squamous cell carcinoma and adenocarcinoma patients. In the case of nails, the mean concentration of Sr was significantly higher in adenocarcinoma and average levels of Na and Co were considerably higher in the nails of squamous cell carcinoma patients as shown in Figure 5b. Nonetheless, mean levels of Ca, Mg, K and Li were almost comparable in the nails of adenocarcinoma and squamous cell carcinoma patients. In the blood samples, the markedly elevated level was noticed for Li in the blood of squamous cell carcinoma patients while the remaining elements and electrolytes exhibited almost equivalent contributions in the patients as shown in Figure 5c.

3.8. Variations of the elements and electrolytes based on stages. Comparative assessment of the elements and electrolytes concentrations (± SE) in the scalp hair, nails and blood of oral cancer patients at different stages is shown in Figure 6 (d–f). Mean concentrations of Na and Co in the scalp hair were considerably higher at stage-I, while mean concentration of Li in the scalp hair was found elevated at stage-III of the patients as shown in Figure 6d. It was noted that the average content of Ca was highest at stage-IV in the scalp hair of oral cancer patients. In the case of nails, a higher level of Ca was found in the patients at stage-I, while highest levels of K, Sr and Li were observed in the nails of the patients at stage-III as shown in Figure 6e. Mean content of Mg in nails was found to be highest at stage-IV of the patients. In the case of blood, the average level of Li was maximum at stage-III as displayed in Figure 6f. Some of the concentrations of elements and electrolytes (Ca, Mg, Na, and K) were not appreciably dissimilar at all four stages in the blood of the patients. Mean content of Li in the blood was found lowest at stage-IV of the oral cancer patients.

3.9. Correlation study. The data on mutual correlations in the scalp hair of oral cancer patients and healthy donors are presented in Table 4, wherein the significant *r*-values are shown in bold at $p < 0.05$. In the case of patients, strong positive correlations were found between K-Na (r = 0.879), Sr-Mg (r = 0.548) while a significant relationship was noted for K-Ca (r = 0.399) indicating their probable communal variations/origin in the scalp hair of the patients. Furthermore, some elemental pairs exhibited inverse relationship manifested by significantly negative correlations; Li-Na (r = −0.433), Li-K (r = −0.313) and Co-Mg (r = −0.281). The counterpart data for the controls pointed out strong correlation coefficients between K-Na (r = 0.860) and Sr-Mg (r = 0.745) thus

manifesting mutual variations of these elements. On the other hand, age of the patients revealed a significant inverse correlation with Ca (r = −0.317) in their scalp hair (Table 4).

The data on elemental correlations in the nails of patients and controls are also given in Table 4, wherein the significant *r*-values are shown in bold at $p < 0.05$. In the case of patients, strong positive correlations were noted between Li-Sr (r = 0.727) and K-Mg (r = 0.508), while some significant relationships were observed among Co-Na (r = 0.492), Co-Sr (r = 0.374) and Co-Li (r = 0.368). Additionally, age of the patients showed significant positive correlations with Li (*r* = 0.308) and K (*r* = 0.256) contents in the nails of oral cancer patients. Likewise, in the case of healthy donors (Table 4), positive correlations were found between the following pairs: Co-Mg (r = 0.401), K-Mg (r = 0.333), Co-Li (r = 0.313), Sr-K (r = 0.290) and Sr-Na (r = 0.271). Furthermore, significant inverse correlations were observed among Na-Ca (r = −0.353) and Na-Mg (r = −0.254). Furthermore, age of the oral cancer patients exhibited significant inverse correlations with Na (*r* = –0.255) contents. Some negative correlations were also found but they were not significant.

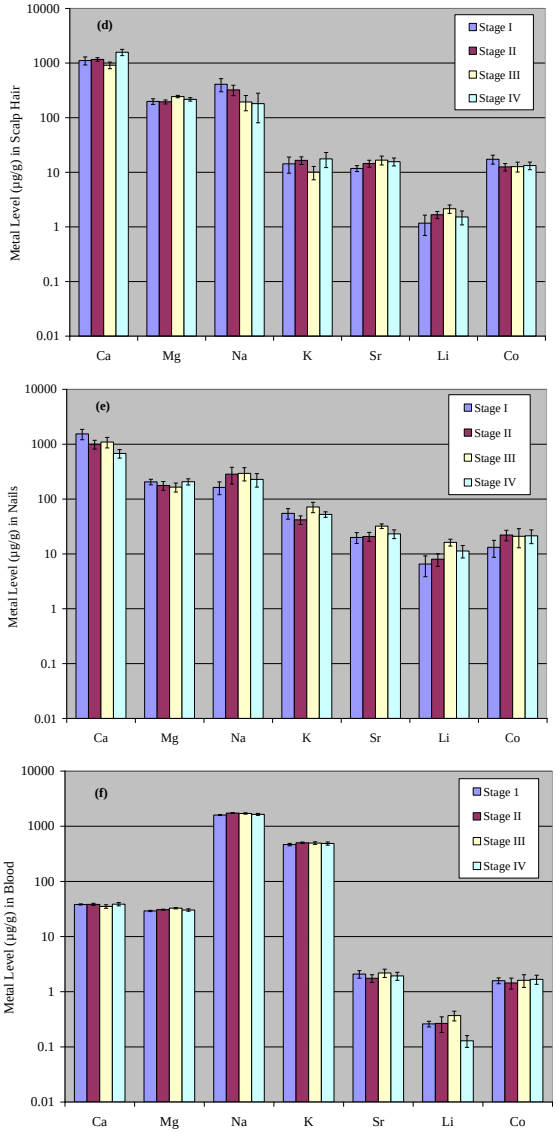

Figure 6. Comparative mean concentrations of selected elements and electrolytes (±SE) in the scalp hair, nails and blood of oral cancer patients based on stages.

In the case of the blood of the oral cancer patients, significant positive relationships were observed between K-Ca (r = 0.425), Sr-K (r = 0.399), Na-Ca (r = 0.383), K-Na (r = 0.305), K-Mg (r = 0.297), Li-Mg (r = 0.297) and Sr-Ca (r = 0.267). In the blood of healthy donors (Table 4), significant associations were found among Li-Mg (r = 0.480), Sr-K (r = 0.463), Co-Sr (r = 0.401), Co-Li (r = 0.384), Li-K (r = 0.331) and K-Mg (r = 0.297) (Table 4). Overall, the correlation results of selected elements and electrolytes for the healthy donors remained significantly dissimilar compared to the oral cancer patients, which may be ascribed to the imbalances of the elements and electrolytes in the patients.

3.10. Elements, oxidative stress and oral cancer. Epidemiological and animal experimental studies have shown an increased cancer incidence associated with chronic exposure to certain elements [25]. Elements undergo redox cycling reactions and have the ability to generate reactive radicals (via the Fenton-like reaction) such as superoxide anion radical and nitric oxide in the biological system [26]. It is generally accepted that ROS via oxidative stress produced by elements eventually causes DNA damage, strand breaks and alterations in the cellular redox balance, whereby insufficient cellular repair mechanisms may involve a wide variety of diseases including oral cancer development [27]. Various studies supported that oxidative stress plays a significant role in oral carcinogenesis [28]. It has long been advocated that cancer cells are under enhanced and persistent oxidative stress due to elevated levels of intracellular ROS production [29]. Ma et al., [30] demonstrated that oxidative stress contributes to the development of oral cancer [31]. Smoking may enhance oxidative stress through the production of ROS which is an important event in the development of oral cancer [32].

*3.10.1. Magnesium***.** Magnesium (Mg) is an important regulator of cell functions, cell proliferation, cell cycle progression and differentiation [9, 33]. Magnesium and Calcium share the same homeostatic control system and antagonizes each other in cell cycle regulation, inflammation and many other physiologic activities [34, 35]. Mg is a necessary cofactor of DNA, RNA and ribosomes [36]. It is required in binding mRNA to polysomal subunits and for the activation of aminoacyl RNA complexes during protein synthesis [37]. Association between Mg and cancer is complex, both excess and deficiency of Mg may produce either carcinogenic or anti-carcinogenic effects [36]. Previous clinical studies supported evidence of the oxidative stress with deficiency of Mg on human pathology [38] such as genomic instability, poor DNA repair and promote apoptosis [39]. Lack of Mg facilitates the uncoupling of oxidative phosphorylation, which leads to loss of an electron in the electron transport chain [40]. Mg deficiency depressed cellmediated immunity and impairs phagocytic activity [41]. Recent data advocated that deficiency of Mg may trigger carcinogenesis by changing the fidelity of DNA replication and increasing membrane permeability [33, 42, 43]. Castiglioni and Maier [44] pointed out that deficiency of Mg can lead to the initiation, proliferation of cancer and hinder treatment [45]. There was a significant decrease in Mg concentration in blood plasma and saliva in oral squamous cell carcinoma patients than normal patients [9]. Shpitzer et al., [45] noted a salivary concentration of Mg was higher in oral cancer patients as compared to controls. The concentration of serum Mg was increased in oral cancer patients as compared to the healthy controls [46]. Nasulewicz et

al., [42] indicated a significant retardation of primary tumour growth in mice receiving Mg-deficient diet. However, Mg repletion caused in these mice significant increase of primary tumour burden [42].

*3.10.2. Cobalt***.** Cobalt (Co) is an essential element, an integral part of vitamin B12 [47] and has a substantial role in the formation of amino acids and neurotransmitters [48]. Cobalt may interact with other elemental ions as in human red cells, e.g., Co shares the transport mechanism with Ca [49]. The uptake is practically irreversible since Co bound in the cytosol is not itself extruded by the Ca pump. Additionally, Co in vitro can replace Fe in the heme group of hemoglobin [50]. Higher concentration of Co becomes toxic to humans. Several studies revealed that Co induced chromosomal aberrations, micronuclei or DNA damage in mammalian cells [51]. Adverse effects of Co were genotoxic and mutagenic in experimental animals [52, 53]. Cobalt is reported to be a potentially carcinogenic compound and has been included recently in group 2A carcinogens *i.e.*, probably carcinogenic to humans [51]. Cobalt seems to produce oxidative stress/damage to lipids, proteins, DNA and carcinogenesis by mediating free radicals via ROS [47, 53, 54]. Cobalt depletes glutathione, resulting in enhanced production of ROS such as catalase [52]. Upon inhalation exposure, the respiratory system is the main target organ of Co in patients with a higher risk of lung cancer [50].

*3.10.3. Sodium***.** Sodium (Na) is a major electrolyte for maintaining body's fluid balance, proper nerve and muscle functions [55]. It also determines membrane potential of cells and participates in the active transport of some molecules across cell membranes. Other electrolytes such as K and Ca interact with Na and influence its physiological effects [56]. Low Na levels occur in a wide variety of medical disorders including cancer [57]. Studies have revealed a marked association with low Na and mortality in lung cancer, gastric cancer, renal cancer and non-Hodgkin's lymphoma patients [58]. However, Dziewulska et al., [46] reported higher Na concentration in the saliva of oral cancer patients than controls [59]. It might be supposed that an elevated Na level could reflect dehydration due to the oral carcinoma itself, as well as smoking and alcohol consumption. Low Na is considered as the most common electrolyte disorder associated with tumour-related conditions [60].

*3.10.4. Potassium***.** Potassium (K) plays a vital role in regular cellular maintenance, cell volume homeostasis and transmission of nerve impulses [61]. It is involved in cellular metabolism, regulating protein synthesis, glucose use and storage [62, 63]. The high or low concentration of K can lead to potentially lethal problems in excitatory tissue, particularly the cardiac muscle. The most important transcellular enzyme involved in K regulation is Na/K ATPase, which maintains the transcellular gradient of Na and K concentrations. β-2-Adrenergic agents increase the activity of Na/K ATPase by binding to cell surface receptors thereby linking K flux to the sympathetic nervous system [64]. Cancer patients face additional risk factors for high K. Side effects of chemotherapy, the breakdown of tumour cells, hormones produced by certain types of tumours and extensive replacement of the adrenal glands by tumours can all result in high K blood levels, according to the manual of clinical oncology [65]. Shpitzer et al., [45] observed a lower average K level K in the saliva of oral cell cancer patients than healthy individuals [46]. The concentration of K in the present investigation is similar to that reported by Shpitzer et al., [45] as well as by Bloniarz et al., [66].

Table 4. Correlation coefficient (r)* matrix of elements and electrolytes in the scalp hair, nails and blood of oral cancer patients (below the diagonal) and healthy donors (above the diagonal).

		Age	Ca	Mg	Na	K	Sr	Li	Co
Scalp Hair	Age	1	**-0.317**	0.150	0.031	-0.113	-0.183	0.026	-0.005
	Ca	0.034	1	-0.163	-0.110	-0.018	-0.125	-0.155	0.030
	Mg	0.102	-0.226	1	-0.116	-0.072	**0.745**	-0.152	-0.161
	Na	-0.219	0.229	-0.011	1	**0.860**	0.021	0.054	0.196
	K	-0.193	**0.399**	0.024	**0.879**	1	-0.077	-0.013	-0.010
	Sr	0.110	-0.081	**0.548**	0.199	0.077	1	0.013	-0.117
	Li	0.003	0.000	-0.092	**-0.443**	**-0.313**	-0.094	1	0.111
	Co	0.088	0.142	**-0.281**	0.066	-0.054	-0.057	0.249	1
Nails	Age	1	**-0.398**	-0.007	-0.234	**-0.467**	-0.048	-0.088	-0.200
	Ca	-0.124	1	-0.007	**-0.353**	0.146	-0.104	-0.187	0.098
	Mg	0.001	-0.079	1	**-0.254**	**0.333**	0.093	0.084	**0.401**
	Na	0.023	0.006	0.158	1	0.080	**0.271**	0.226	0.158
	K	0.182	-0.016	**0.508**	0.247	1	**0.290**	0.057	0.143
	Sr	**0.256**	0.100	0.030	0.046	0.205	1	0.226	0.137
	Li	**0.308**	-0.057	-0.067	-0.087	0.020	**0.727**	1	**0.313**
	Co	-0.284	0.002	0.100	**0.492**	0.143	**0.374**	**0.368**	1
Blood	Age	1	-0.201	**0.427**	**0.296**	0.147	-0.027	0.234	0.017
	Ca	-0.186	1	-0.316	0.125	-0.040	-0.039	0.042	0.174
	Mg	0.043	-0.039	1	0.122	**0.297**	0.184	**0.480**	0.259
	Na	**-0.255**	**0.383**	0.010	1	**0.344**	0.192	0.205	-0.164
	K	0.205	**0.425**	**0.297**	**0.305**	1	**0.463**	**0.331**	0.185
	Sr	-0.012	**0.267**	0.057	0.043	**0.399**	1	0.177	**0.401**
	Li	-0.009	0.191	**0.279**	0.198	0.126	-0.220	1	**0.384**
	Co	0.008	0.164	0.079	-0.050	0.046	0.109	-0.081	1

*r-values > 0.307 or < – 0.307 are significant at $p < 0.001$

*3.10.5. Calcium***.** Calcium (Ca) is an essential element required for critical biological functions including muscle contraction, nerve

conduction, coagulation and bone development [67]. It also takes part in cell signaling, regulating cell proliferation, differentiation and apoptosis [68, 69]. High Ca has been linked to cancer and reported due to the production of circulating hormone like factors by a tumour or from the elaboration of osteolytic factors by these tumours [37]. In experimental studies, increasing the level of Ca decreases cell proliferation and induces differentiation of mammary cells [67]. Malignancy is the most common cause of high Ca seen in patients particularly common in advanced stage cancer [70, 71]. These elevated Ca levels patients with tumour tend to have limited survival for several months. An elevated Ca level is most common in lung cancer, breast cancer, multiple myeloma and lymphoma patients [69]. The concentration of Ca was found highest in the saliva of the oral cancer patients as compared to controls [46]. Another study found that Ca value was elevated in the tissue of oral cancer patients as compared to controls [37]. Previous clinical data advocated high Ca developed in patients with head and neck cancer, but only a few clinical studies have investigated the incidence of this morbidity in large numbers of patients [72].

There are some limitations to the present study. The levels of elements and electrolytes in patients were evaluated after the diagnosis of oral cancer, but before the start of any treatment or therapy. Injuries and infections were reported to alter concentrations of a variety of micronutrients [73]. The size of the study population is relatively small. The study samples were selected randomly and cannot be generalized to the whole population. The potential strong points of the study are that selected elements and electrolytes levels were observed in relation to various clinical types and stages of oral cancer patients. Besides, atomic absorption spectrometry was used to analyze the elements which are more accurate in elemental analysis than traditional calorimetric method [2]. In addition to controlling tobacco smoking and betel quid chewing, approaches to control the disease in Pakistan involve improving the population's living standards and access to health facilities, improving the quality of incidence and mortality data, and studies to investigate other potential etiological causes of oral cancer, such as viruses and occupational exposures, which would help in early detection, management and monitoring the efficacy of treatment. Moreover, the survey should be conducted to determine an actual number of people suffering from oral cancer in Pakistan and to provide basic information for the policymakers to devise health care policies accordingly. Nevertheless, the abnormal levels of elements and electrolytes can be changed with precautionary measures including dietary supplements, change in agricultural approaches, and detoxification for those already burdened, which may help reduce oral cancer risk for a certain population. Future studies can be performed on a large number of well-randomized samples to confirm the correlation between expression of elements/electrolytes and oral cancer.

4. CONCLUSIONS

The current study is based on the measurement of elements and electrolytes (Ca, Mg, Sr, K, Na, Li and Co) in the scalp hair, nails and blood of oral cancer patients in comparison with healthy donors. Mean concentrations of Na, Sr, Li and Co were found to be significantly higher in the scalp hair and nails of oral cancer patients, while average levels of Mg, Na and Sr were significantly elevated in the blood of oral cancer patients compared with the controls. The correlation study exhibited considerable associations between the elements and electrolytes in the case of the patients. Most of the elements and electrolytes showed noticeable disparities in their concentrations based on gender, dietary habits and smoking habits of the subjects. In the case of adenocarcinoma patients, Li level was highest in the scalp hair while Sr was elevated in the nails. Na and K manifested maximum levels in the scalp hair & Co and Na were highest in the nails of squamous cell carcinoma patients. Likewise, stage-based comparison showed significant disparities in the scalp hair/nails/blood of the oral cancer patients. Hence, the disruption in the balance of elements and electrolytes in the scalp hair, nails and blood may possibly indicate the potential diagnostic marker to predict oral cancer progression and its aetiology in patients.

5. REFERENCES

[1] Gupta B., Bray F., Kumar N., Johnson NW., Associations between oral hygiene habits, diet, tobacco and alcohol and risk of oral cancer: a case-control study from India, *Cancer Epidemiology*, 51, 7–14, **2017**.

[2] Arain S.S., Kazi T.G., Afridi H.I., Talpur F.N., Kazi A.G., Brahman K.D., Panhwar A.H., Arain M.S., Scalp hair and blood cadmium levels in association with chewing gutkha, mainpuri, and snuff, among patients with oral cancer in Pakistan, *Journal of Oral Pathology & Medicine*, 44, 707–713, **2105**.

[3] Warnakulasuriya S., Global epidemiology of oral and oropharyngeal cancer, *Oral Oncology*, 45, 309–316, **2009**.

[4] da Silva S.D., Ferlito A., Takes R.P., Brakenhoff R.H., Valentin M.D., Woolgar J.A., Bradford C.R., Rodrigo J.P., Rinaldo A., Hier M.P., Kowalski L.P., Advances and applications of oral cancer basic research, *Oral Oncology*, 47, 783–791, **2011**.

[5] AJCC, American Joint Committee on Cancer, Cancer staging manual, 5th ed., Philadelphia: Lippincott-Raven, **1997**.

[6] Qayyum M.A., Shah M.H., Study of trace metal imbalances in the blood, scalp hair and nails of oral cancer patients from Pakistan, *Science of the Total Environment*, 593–594, 191–201, **2017**.

[7] Su C.C., Tsai K.Y., Hsu Y.Y., Lin Y.Y., Lian I.B., Chronic exposure to heavy metals and risk of oral cancer in Taiwanese males, *Oral Oncology*, 46, 586–90. **2010**.

[8] Kumar M., Nanavati R., Modi T.G., Dobariya C., Oral cancer: etiology and risk factors: a review, *Journal of Cancer Research and Therapeutics*, 12, 458–463, **2016**.

[9] Bagulkar B.B., Chaudhary M., Gawande M., Patil S., Gadbail A., Bagulkar S., Colorimetric determination of magnesium in blood and saliva in oral squamous cell carcinoma and potentially malignant disorders by titan yellow method, *Journal of Orofacial Research*, 3, 240–244, **2013**.

[10] Hashmi G.M.M., Shah M.H., Study of imbalances of essential/toxic metals in the blood of osteoarthritis patients in comparison with healthy subjects, *Biointerface Research in Applied Chemistry*, 7, 1945-1954, **2017**.

[11] Moyer V.A., Vitamin, mineral, and multivitamin supplements for the primary prevention of cardiovascular disease and cancer: US preventive services task force recommendation statement, *Annals of Internal Medicine*, 160, 558–564, **2014**.

[12] Ilyas A., Ahmad H., Shah M.H., Comparative study of elemental concentrations in the scalp hair and nails of myocardial infarction patients versus controls from Pakistan, *Biological Trace Element Research*, 166, 123–135, **2015**.

[13] Czerny B., Krupka K., Ożarowski M., Mrozikiewicz A.S., Screening of trace elements in hair of the female population with different types of cancers in Wielkopolska region of Poland, *The Scientific World Journal*, 2014, 953181, **2014**.
[14] Blazewicz A., Liao K.Y., Liao H.H., Nizinski P., Komsta L., Momcilovic B., Czapla M.J., Michalski R., Alterations of hair and nail content of selected trace elements in non-occupationally exposed patients with chronic depression from different geographical regions, *BioMed Research International*, 2017, 3178784, **2017**.
[15] Yaemsiri S., Hou N., Slining M.M., He K., Growth rate of human fingernails and toenails in healthy American young adults, *Journal of the European Academy of Dermatology and Venereology*, 24, 420–423, **2010**.
[16] Sakamoto M., Chan H.M., Domingo J.L., Oliveira R.B., Kawakami S., Murata K., Significance of finger nail and toe nail mercury concentrations as biomarkers for prenatal methyl mercury exposure in relation to segmental hair mercury concentrations, *Environmental Research*, 136, 289–294, **2015**.
[17] Zowczak M., Iskra M., Torlinski L., Cofta S., Analysis of serum copper and zinc concentrations in cancer patients, *Biological Trace Element Research*, 82, 1–8, **2001**.
[18] StatSoft Inc., STATISTICA for Windows, Computer program manual, Tulsa, OK, **1999**.
[19] Hollander D., Kampman E., Herpen C.M.L, Pretreatment body mass index and head and neck cancer outcome: a review of the literature, *Critical Reviews in Oncology/Hematology*, 96, 328–338, **2015**.
[20] Petrick J.L., Gaudet M.M., Weissler M.C., Funkhouser W.K., Olshan A.F., Body mass index and risk of head and neck cancer by race: the Carolina head and neck cancer epidemiology study, *Annals of Epidemiology*, 24, 160–164, **2014**.
[21] Gaudet M.M., Patel A.P., Sun J., Hildebrand J.S., McCullough M.L., Chen A.Y., Gapstur S.M., Prospective studies of body mass index with head and neck cancer incidence and mortality, *Cancer Epidemiology Biomarkers & Prevention*, 21, 497–503, **2012**.
[22] Hashibe M., Sankaranarayanan R., Thomas G., Kuruvilla B., Mathew B., Somanathan T., Parkin D.M., Zhang Z.F., Body mass index, tobacco chewing, alcohol drinking and the risk of oral submucous fibrosis in Kerala, India, *Cancer Causes and Control*, 13, 55–64, **2002**.
[23] George J., Cannon T., Lai V., Richey L., Zanation A., Hayes D.N., Shores C., Guttridge D., Couch M., Cancer cachexia syndrome in head and neck cancer patients: part II pathophysiology, *Head & Neck*, 29, 497–507, **2007**.
[24] Kabat G.C., Chang C.J., Wynder E.L., The role of tobacco, alcohol use, and body mass index in oral and pharyngeal cancer, *International Journal of Epidemiology*, 23, 1137–1144, **1994**.
[25] Mena S., Ortega A., Estrela J.M., Oxidative stress in environmental-induced carcinogenesis, *Mutation Research*, 674, 36–44, **2009**.
[26] Mulware S.J., Trace elements and carcinogenicity: a subject in review, *Biotech*, 3, 85–96, **2013**.
[27] Das S., Mahapatra S.K., Gautam N., Das A., Roy S., Oxidative stress in lymphocytes, neutrophils, and serum of oral cavity cancer patients: modulatory array of L-glutamine, *Support Care Cancer*, 15, 1399–1405, **2007**.
[28] Choudhari S.K., Chaudhary M., Gadbail A.R., Sharma A., Tekade S., Oxidative and antioxidative mechanisms in oral cancer and precancer: a review, *Oral Oncology*, 50, 10–18, **2014**.
[29] Gupte A., Mumper R.J., Elevated copper and oxidative stress in cancer cells as a target for cancer treatment, *Cancer Treatment Reviews*, 35, 32–46, **2009**.
[30] Ma N., Tagawa T., Hiraku Y., Murata M., Ding X., Kawanishi S., 8-Nitroguanine formation in oral leukoplakia, a premalignant lesion, Nitric Oxide, *Biology and Chemistry*, 14, 137–143, **2006**.
[31] Bahar G., Feinmesser R., Shpitzer T., Popovtzer A., Nagler R.M., Salivary analysis in oral cancer patients DNA and protein oxidation, reactive nitrogen species, and antioxidant profile cancer, *Cancer*, 109, 54–59, **2007**.
[32] Burlakova B.E., Zhizhina G.P., Gurevich S.M., Fatkullina L.D., Kozachenko A.I., Nagler L.G., Zavarykina T.M., Kashcheev V.V., Biomarkers of oxidative stress and smoking in cancer patients, *Journal of Cancer Research and Therapeutics*, 6, 47–53, **2006**.
[33] Anghileri L.J., Magnesium, calcium and cancer, *Magnesium Research*, 22, 247–255, **2009**.
[34] Tao M.H., Dai Q., Millen A.E., Nie J., Edge S.B., Trevisan M., Shields P.G., Freudenheim J.L., Associations of intakes of magnesium and calcium and survival among women with breast cancer: results from Western New York exposures and breast cancer (WEB) study, *American Journal of Cancer Research*, 6, 105–113, **2016**.
[35] Dai Q., Sandler R.S., Barry E.L., Summers R.W., Grau M.V., Baron J.A., Calcium, magnesium, and colorectal cancer, *Epidemiology*, 23, 504–505, **2012**.
[36] Blaszczyk U., Duda-Chodak A., Magnesium: its role in nutrition and carcinogenesis. *RoczPanstwZaklHig*, 64, 165–171, **2013**.
[37] Sarkis S.A., AL-Ani S., Al-Nimr M., Assessment of magnesium and calcium status in oral cancer patients, *Journal of Baghdad College of Dentistry*, 21, 66–69, **2009**.
[38] Zheltova A.A., Kharitonova M.V., Iezhitsa I.N., Spasov A.A., Magnesium deficiency and oxidative stress: an update, *BioMedicine*, 6, 8–14, **2016**.
[39] Wolf F.I., Maier J.A.M., Nasulewicz A., Feillet-Coudray C., Simonacci M., Mazur A., Cittadini A., Magnesium and neoplasia: from carcinogenesis to tumor growth and progression or treatment, *Archives of Biochemistry and Biophysics*, 458, 24–32, **2007**.
[40] Goubern M., Rayssiguier Y., Miroux B., Chapey M.F., Ricquier D., Durlach J., Effect of acute magnesium deficiency on the masking and unmasking of the proton channel of the uncoupling protein in rat brown fat, *Magnesium Research*, 6, 135–143, **1993**.
[41] Tam M., Gomes S., Gonzalez-Gross M., Marcos A., Possible roles of magnesium on the immune system, *European Journal of Clinical Nutrition*, 57, 1193-1197, **2003**.
[42] Nasulewicz A., Wietrzyk J., Wolf F.I., Dzimira S., Madej J., Maier J.A.M., Rayssiguier Y., Mazur A., Opolski A., Magnesium deficiency inhibits primary tumor growth but favors metastasis in mice, *Biochimica et Biophysica Acta*, 1739, 26–32, **2004**.
[43] Seeling M.S., Magnesium in oncogensis and in anticancertreatment, interaction with minerals andvitamins, *Cancer Treatment Research Foundation*, 15, 238–318, **2001**.
[44] Castiglioni S., Maier J., Magnesium and cancer: a dangerous liaison, *Magnesium Research*, 24, 92–100, **2011**.
[45] Shpitzer T., Bahar G., Feinmesser R., Nagler R.M., A comprehensive salivary analysis for oral cancer diagnosis, *Journal of Cancer Research and Clinical Oncology*, 133, 613–617, **2007**.
[46] Dziewulska A., Olszowska J.J., Bachanek T., Grocholewicz K., Salivary mineral composition in patients with oral cancer, *Magnesium Research*, 26, 120–124, **2013**.
[47] Suh M., Thompson C.M., Brorby G.P., Mittal L., Proctor D.M., Inhalation cancer risk assessment of cobalt metal, *Regulatory Toxicology and Pharmacology*, 79, 74–82, **2016**.
[48] Martos F.C., Rojas R.M., Cobalt: Toxicology,Encyclopedia of Food and Health, Academic press is an imprint of Elsevier, The Boulevard, Langford Lane, Kidlington, Oxford OX5 1G 225 Wyman street, Waltham MA 02451, UK, **2016**.
[49] Behl M., Stout M.D., Herbert R.A., Dill J.A., Baker G.L., Hayden B.K., Roycroft J.H., Comparative toxicity and carcinogenicity of soluble and insoluble cobalt compounds, *Toxicology*, 333, 195–205, **2015**.
[50] Nordberg G.F., Fowler B.A., Nordberg M., Friberg L., Handbook on the toxicology of metals, 3rd ed., Elsevier, Academic Press, 30 Corporate Drive, Suite 400, Burlington, MA 01803, USA, **2007**.
[51] Hong H.H., Hoenerhoff M.J., Ton T.V., Herbert R.A., Kissling G.E., Hooth M.J., Behl M., Kras, Egfr, and Tp53 mutations in B6C3F1/N mouse and F344/NTac rat alveolar/bronchiolar carcinomas resulting from chronic inhalation exposure to cobalt metal, *Toxicological Pathology*, 43, 872–882, **2015**.
[52] De Boeck M., Lombaert N., De Backer S., Finsy R., Lison D., Kirsch-Volders M., In vitro genotoxic effects of different combinations of cobalt and metallic carbide particles, *Mutagenesis*, 18, 177–186, **2003**.
[53] Kirkland D., Brock T., Haddouk H., Hargeaves V., Lloyd M., Mc Garry S., New investigations into the genotoxicity of cobalt compounds and their impact on overall assessment of genotoxic risk, *Regulatory Toxicology and Pharmacology*, 73, 311–338, **2015**.
[54] NTP, Report on carcinogens, Monograph on cobalt and cobalt compounds that release cobalt ions in vivo, office of the report on

carcinogens, Division of the National Toxicology Program, US Department of Health and Human Services, National Institutes of Health, Research Triangle Park, NC, **2016**.
[55] Pohl H.R., Wheeler J.S., Murray H.E., Sodium and potassium in health and disease, *Metals Ions in Life Sciences*, 13, 29–47, **2013**.
[56] Adrogue H.J., Madias N.E., Hypernatremia, *New England Journal of Medicine*, 342, 1581–1589, **2000**.
[57] Castillo J.J., Vincent M., Justice E., Diagnosis and management of hyponatremia in cancer patients, *The Oncologist*, 17, 756–765, **2012**.
[58] Rosner M.H., Dalkin A.C., Electrolyte disorders associated with cancer, *Advances in Chronic Kidney Disease*, 21, 7–17, **2014**.
[59] Fuchs P.N., Rogic D., Vidovic-Juras D., Susic M., Milen-Ovic A., Brailo V., Boras V.V., Salivary analytes in patients with oral squamous cell carcinoma, *Collegium Antropologicum*, 35, 359–362, **2011**.
[60] Berardi R., Rinaldi S., Caramanti M., Grohe C., Santoni M., Morgese F., Torniai M., Savini A., Fiordoliva I. Cascinu S., Hyponatremia in cancer patients: time for a new approach, *Critical Reviews in Oncology/Hematology*, 102, 15–25, **2016**.
[61] Udensi U.K., Tchounwou P.B., Potassium homeostasis, oxidative stress, and human disease, *International Journal of Clinical and Experimental Physiology*, 4, 111–122, **2017**.
[62] Crawford A.H., Hyperkalemia: recognition and management of a criticalelectrolyte disturbance, *The Art and Science of Infusion Nursing*, 37, 167–175, **2014**.
[63] Rastergar A., Soleimani M., Hypokalemia and hyperkalemia, *Postgraduate Medical Journal*, 77, 759–764, **2001**.
[64] Porth C.M., Essentials of pathophysiology concepts of altered health states, 3rd ed., Philadelphia, PA, Lippincott, Williams & Wilkins, 786–789, **2010**.
[65] Schubert F., High potassium and cancer, livestrong.com, dated, 26/03/2108, **2018**.
[66] Bloniarz J., Rahnama M., Zareba S., The influence of carcinogenesis in the oral cavity on the level of some bioelements in the saliva, *RoczPanstZaklHig*, 54, 295–300, **2003**.
[67] Cui Y., Rohan T.E., Vitamin D, calcium, and breast cancer risk: a review, *Cancer Epidemiology, Biomarkers & Prevention*, 15, 1427–1437, **2006**.
[68] Peterlik M., Grant W.B., Cross H.S., Calcium, vitamin D and cancer, *Anticancer Research*, 29, 3687–3698, **2009**.
[69] Mirrakhimov A.E., Hypercalcemia of malignancy: an update on pathogenesis and management, *North American Journal of Medical Sciences*, 7, 483–493, **2015**.
[70] Reagan P., Pani A., Rosner M.H., Approach to diagnosis and treatment of hypercalcemia in a patient with malignancy, *American Journal of Kidney Diseases*, 3, 141–147, **2014**.
[71] Stewart A.F., Clinical practice, hypercalcemia associated with cancer, *New England Journal of Medicine*, 352, 373–379, **2005**.
[72] Iwase M., Kurachi Y., Kakuta S., Sakamaki H., Mitsuhashi M.N., Nagumo M., Hypercalcemia in patients with oral squamous cell carcinoma, *Clinical Oral Investigations*, 5, 194–198, **2001**.
[73] Duncan A., Talwar D., McMillan D.C., Stefanowicz F., O'Reilly D.S.J., Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements, *American Journal of Clinical Nutrition*, 95, 64–71, **2012**.

6. ACKNOWLEDGEMENTS

Funding provided by the Higher Education Commission, Government of Pakistan to accomplish this study is thankfully acknowledged. We are thankful to the participants of this study. We are also grateful to the administration of the Nuclear Oncology & Radiation Therapy Institute and Pakistan Institute of Medical Sciences Islamabad, Pakistan for their invaluable help during sample collection. Technical and financial help by Quaid-i-Azam University, Islamabad, Pakistan to execute this project is also acknowledged.