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Comparative assessment of essential/toxic elemental levels in the scalp hair and nails of ovarian cancer patients and controls among Pakistani women

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ABSTRACT

Ovarian cancer is one of the severe gynaecological malignancies in women and exposure to the trace elements may be considered as a risk factor for its development. Selected essential and toxic elements (Ca, Mg, Na, K, Fe, Zn, Cu, Sr, Li, Co, Mn, Ni, Cr, Cd and Pb) were analysed in the scalp hair and nails of ovarian cancer patients and matching controls by atomic absorption spectrometry. Average concentrations of Cr, Li, Mn, Ni and Pb were found to be significantly higher ($p < 0.05$) in the scalp hair and nails of the patients compared with the controls, nevertheless, appreciably higher concentrations of Ca, Fe and Zn were observed in the controls. The correlation study revealed significantly diverse associations among the elements in the scalp hair/nails of the patients and controls. Cluster analysis and principal component analysis were used for the multivariate apportionment of the elements in both donor groups. Variations in the elemental levels were also noted for various cancer types (epithelial cancer, stromal tumour and germ cell tumour) as well as stages (I, II, III and IV) in patients. The study evidenced distinctive variations in the elemental levels in the scalp hair and nails of ovarian cancer patients and controls. Moreover, these results could be of significance in the diagnosis and prognosis of ovarian cancer in patients.

Keywords: *correlation; element; hair; multivariate analysis; nails; ovarian cancer; Pakistan.*

1. INTRODUCTION

The deficiency or excess of trace elements with respect to the human physiological disorders has been found in patients with various diseases, including cancer [1]. Ovarian cancer is the seventh most common type of cancer in women [2]; the leading cause of death among gynaecologic malignancies and the second most commonly diagnosed gynaecologic malignancy worldwide [3]. In Pakistan, the exact incidence is not known but it ranks fourth most common cancer among the females. Amongst the gynaecological malignancies, it is unfortunately being increasingly encountered in Pakistan [4]. In general, ovarian cancers originate in the epithelium of inclusion cysts, which are derived from the surface epithelium [5]. Histological pattern of ovarian cancer is very important to determine because the prognosis depends upon the degree of differentiation. The main histological types of ovarian cancer are epithelial cancer, germ cell tumour and stromal tumour [6]. Likewise, the stage of ovarian cancer is compulsory in categorizing them in malignant tumours because it has different prognoses at each stage which is treated differently. The international federation of gynaecology and obstetrics classified ovarian cancer into four stages [7]. A number of risk factors have been reported for ovarian cancer, e.g., race, age, diet, genetic, obesity, smoking, family history, reproductive history, oestrogen therapy, use of fertile drugs, exposure of metals, etc. [8, 9]. The significance of trace elements in relation to ovarian cancer has been recognized in recent literature worldwide [10, 11].

Measurement of essential and toxic trace elements and their distribution in body tissues have proven useful in assessing the health and nutritional status of the target population [10]. Among the human tissues, hair and nails are widely used as biomarkers for the assessment of toxic chemicals due to the ease of sampling, transportation, storage, preparation for analysis and generally higher chemical concentrations compared to the other biological media, such as, blood, urine, etc., [12, 13, 14]. Elemental levels in the hair and nails reflect their long term exposure and steady state accumulation in the human body through numerous complex processes which are influenced by several factors, such as, environmental quality, age, sex, nourishment, oxidation state of the metals and their binding sites, exposure duration, etc., [15, 16]. Therefore, hair and nails are considered as excellent matrix for the biomonitoring of trace elements [17].

Various studies highlighted a correlation between trace elemental exposure and cancer incidences but, only a few studies have examined the effects of essential and toxic trace elements on the risk of ovarian cancer in patients [18]. Therefore, the present study is based on the measurement of selected essential and toxic elements (Ca, Mg, Na, K, Fe, Zn, Cu, Sr, Li, Co, Mn, Ni, Cr, Cd and Pb) in the scalp hair and nails of ovarian cancer patients in comparison with matching controls. Mutual relationships among the elemental levels were investigated by correlation study, whereas multivariate cluster analysis (CA) and principal component analysis (PCA) were employed for the apportionment of the metals in scalp hair and nails of the two donor groups. Plausible variations in the elemental levels in the scalp hair and nails for different types and stages of the cancer were also evaluated in this study, thereby investigating whether these elemental levels had any presumptive benefits in the diagnosis and/or prognosis of ovarian cancer.

2. EXPERIMENTAL

2.1. Study Population. The scalp hair and nails samples were collected from the newly diagnosed ovarian cancer women admitted in Nuclear Oncology & Radiotherapy Institute (NORI), Islamabad, Pakistan. Subjects were selected on volunteer basis and their ages ranged from 24 and 73 years. Prior to sample collection, the protocol of study was approved by the ethical review committee of the institute. The hair and nails samples were collected from the patients prior to any treatment (i.e., surgery, chemotherapy or radiotherapy) and they were not taking any mineral supplement during past three months. The healthy subjects/controls were also selected on volunteer basis from the same localities with matched age groups, similar socioeconomic status and food habits. The subjects were initially briefed about the purpose and objectives of the study and then a written consent was obtained. A proforma was filled to record the information, such as, age, sex, habitat, ailment duration, food habits, smoking habits, investigations, type of ailment, medicine, hobbies, occupation and tumour type/grade etc., at the time of sample collection from the subjects. Physical examinations were performed in the hospital to measure participant's weight, height, blood pressure and biochemical data.

2.2. Collection and Processing of the Hair Sample. About 3 g of hair was cut from the nape of the neck close to the occipital region of the scalp, as strands 3–5 cm long and directly stored in zip-mouthed polythene bags, duly labelled with relevant codes. The hair samples were thoroughly washed to remove any exogenous impurities to differentiate the endogenous trace elements from the exogenous sources. A variety of washing procedures have been proposed for elemental analysis of hair, but none of them is widely recognized as superior [19]. The ultimate goal of all such methods should be the removal of all exogenous materials loosely adhering with fat, sweat and dirt without changing the endogenous contents of elements in the samples. In the present study, the scalp hair were cut into small pieces of 2–3 cm in length and mixed with 50 mL of detergent solution in a conical flask (250 mL) and shaken on an auto-shaker for 30 minutes at 320 vibrations per minute. The samples were left for 2 h undisturbed then washed with plentiful water until all detergent was leached out. It was followed by the addition of 30 mL Triton X-100 (0.5% v/v) and again shaken on the auto-shaker for 20 minutes. Afterward, the samples were washed with excess of doubly distilled water and finally dried in an electric oven at 70°C for overnight and cooled to room temperature in desiccators [20].

For digestion, an accurately weighed portion (~1 g) of the hair sample was treated with 10 mL of concentrated (65%) nitric acid and heated at 80°C for 10 minutes. It was cooled to room temperature, followed by addition of 5.0 mL of perchloric acid (70%) with subsequent heating to a soft boil until white dense fumes evolved marking the completion of digestion process. Sample was cooled to room temperature and diluted to 50 mL with doubly distilled water [20]. The blank was also prepared the same way but without the hair sample.

2.3. Collection and Processing of the Nails Sample. For collection of nail samples, at first, the subjects were asked to wash their hands and feet thoroughly with distilled water and medicated soap, followed by drying with a clean towel or tissue paper. The nails of the subject were clipped with a stainless steel clipper in order to obtain the measurable quantity. Then samples were stored individually in labelled plastic bags. For washing, the nail samples were scratched with a quartz knife to remove the surface contamination and were placed in conical flasks and soaked in 5% (w/v) detergent solution overnight to weaken the bound dirt and shaken on an auto-shaker for 20 minutes at 320 vibrations per minute followed by washing with plenty of tap water. Any loosely bound impurity was removed manually at this stage. Then acetone (50 mL) was added in the conical flasks containing nails samples and was shaken on the auto-shaker for 20 minutes. This step helped to remove the nail polish, lotion or other such materials. Then the samples were washed with distilled water followed by the addition of 30 mL Triton X-100 (0.5% v/v) solutions and again shaken for 20 minutes. Afterward, the samples were washed with excess of doubly distilled water and finally dried in an electric oven at 70°C for overnight [13].

The dried nail samples (~0.5 g) was taken in the conical flask and concentrated nitric acid (65%) and perchloric acid (70%) (5:1 v/v) were added to the flask. Samples were left undisturbed for 30 minutes at room temperature. After predigesting at room temperature, the nail samples were heated for 1 h (80°C) until white dense fumes evolved. At this time it was observed that the samples were completely digested. Samples were again cooled to room temperature. The digested samples were transferred to volumetric flasks and final volume of 25 mL was adjusted with 0.1 N of HNO_3 [21]. A blank was also prepared in the same way along with each batch but without the nail sample. After dilution the samples were coded and stored in screw tight plastic bottles.

2.4. Quantification of the Elements. The quantitative measurement of selected elements (Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr and Zn) in the digested samples was performed using flame atomic absorption spectrophotometer (Shimadzu AA-670, Japan), with automatic background compensation under optimum analytical conditions as shown in Table 1. All the reagents used were of ultrahigh purity (certified > 99.99%). Doubly distilled water was used throughout the study for the preparation of the samples and standards. Stock solution (1000 mg/L) of each element was used to prepare the fresh working standards just before the analysis. Three sub-samples of each sample were treated and run separately onto the spectrophotometer to pool the mean concentration. The samples were also analysed at an independent laboratory for comparison of the results and a maximum of 2.5% difference was observed in the results of two laboratories. Parallel routine check on the accuracy of quantified results was ensured through the use of standard reference material (Human Hair, GBW 07601 and Bovine Muscle Powder, NIST-SRM 8414) which showed good recoveries (97-103%). Generally,

the contribution of the blank was <5% of the measured elemental levels in the samples.

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 metals in standard reference material.

Metal	Wavelength (nm)	Slit width (nm)	Limit of Detection (mg/l)	Limit of Quantification (mg/l)	Human Hair, GBW 07601			Bovine Muscle Powder, NIST-SRM 8414		
					Certified Level	Measured Level	Recovery (%)	Certified Level	Measured Level	Recovery (%)
Ca	422.7	0.5	0.004	0.013	2900	2869 ± 41.31	98.9	145	143.8 ± 3.014	99.2
Mg	285.2	0.5	0.001	0.004	360	357.1 ± 4.610	99.2	960	955.2 ± 10.42	99.5
Na	589.0	0.5	0.002	0.007	152	154.8 ± 7.226	101.8	2100	2085 ± 21.61	99.3
K	766.5	0.5	0.003	0.009	20	20.47 ± 1.204	102.4	15170	15210 ± 33.75	100.3
Fe	248.3	0.2	0.006	0.018	54	53.76 ± 1.152	99.6	71.2	71.60 ± 1.719	100.6
Zn	213.9	0.5	0.002	0.006	190	187.6 ± 3.704	98.7	142	143.5 ± 3.081	101.1
Cu	324.8	0.5	0.004	0.013	10.6	10.79 ± 0.226	101.8	2.4	2.387 ± 0.051	99.5
Sr	460.7	0.5	0.005	0.016	24	23.51 ± 1.533	98.0	0.052	0.051 ± 0.002	98.1
Li	670.7	0.5	0.003	0.009	2	1.966 ± 0.175	98.3	-	-	-
Co	240.7	0.2	0.005	0.016	0.071	0.072 ± 0.002	101.4	0.007	0.007 ± 0.001	99.2
Mn	279.5	0.4	0.003	0.010	6.3	6.194 ± 0.238	98.3	0.37	0.361 ± 0.023	97.6
Ni	232.0	0.15	0.006	0.019	0.83	0.847 ± 0.051	102.0	0.05	0.051 ± 0.004	102.0
Cr	357.9	0.5	0.006	0.018	0.37	0.362 ± 0.005	97.8	0.071	0.070 ± 0.003	98.6
Cd	228.8	0.3	0.004	0.013	0.11	0.108 ± 0.013	98.2	0.013	0.013 ± 0.002	100.6
Pb	217.0	0.3	0.010	0.029	8.8	8.597 ± 0.271	97.7	0.38	0.372 ± 0.019	97.9

*Triplicate sub-samples

2.5. Statistical Analysis. STATISTICA software was used for the statistical analysis of the elemental data [22]. The quantified results were subjected to univariate and multivariate analysis in order to classify the relationship among the measured element levels. Univariate analysis of the data comprised of the basic statistical parameters, including range, mean, standard error (SE) and skewness while mutual variations in the elemental levels were computed as correlation study. Principal component analysis (PCA) and cluster analysis (CA) were used for the multivariate apportionment of the elemental data and to examine their multiple relationships in the scalp hair and nails of the patients and controls [10].

PCA is a powerful tool to estimate the correlation structure of the variables by finding new variables (principal components, PCs), which are the linear combination of original variables. It reduces data complexity with minimum loss of original information [23]. The first PC explains the maximum possible variation that can be projected onto one dimension; the second PC describes the second and so on [24]. PCA with varimax normalized rotation was applied to data of current study [25]. The magnitude and sign of PC loadings indicate the strength and direction of the correlation between the variable and component. PCA reflects how much each variable contributes to the meaningful variation in the data and to interpret variables relationship [26].

CA is a useful technique, with a purpose of classifying the objects of the system into clusters based on their similarities. It can identify relatively homogenous groups of variables using an algorithm that starts with each variable in a separate cluster and combines variables into agglomerative clusters until only one cluster is left [27]. Results obtained by CA are typically obtained by a dendrogram, which depicts the levels of similarity between the different variables [24]. CA was applied to the data-set using Ward's method which is distinct from all the other methods because an analysis of variance approach is used to evaluate the distances between clusters [26]. The objective of CA is to group objects into clusters such that objects within one cluster share more in common with one another than they do with the objects of other clusters [28].

3. RESULTS AND DISCUSSION

3.1. Characteristics of the Subjects. The demographic characteristics related to the ovarian cancer patients and healthy persons/controls are presented in Table 2. Ovarian cancer was confirmed in the patients histopathologically. The age of the ovarian cancer patients ranged from 24 to 73 years while for healthy subjects it ranged from 26 to 67 years. All the participants involved in this study were female donors and majority of them (>50%) in both groups were vegetarians and 42-44% of the donors were drawn from rural areas. Most of the patients (69%) and healthy donors (80%) were not addicted to tobacco. The relative proportion of the controls was more or less same as those of the patients (Table 2). The patients included in the present study were commonly suffering from epithelial cancer (52-53%), followed by germ cell tumour (25%) and stromal tumour (22-23%). Furthermore, twenty percent (20%) of the patients were diagnosed at stage-I, 23-24% at stage-II, 32% at stage-III and 24-25% at stage-IV of ovarian cancer.

3.2. Distribution and Comparison of the Elemental Levels in Scalp Hair. Basic statistical parameters pertaining to the distribution of selected elements in the scalp hair of ovarian cancer patients and controls are shown in Table 3. Large variations were observed among minimum and maximum concentrations of the elements in both groups. Among all the elements in the scalp hair of patients, Ca (881.9 µg/g), Mg (210.2 µg/g), Zn (205.9 µg/g), Na (149.0 µg/g) and Sr (96.45 µg/g) were the major contributors. However, relatively lower mean levels were observed for Ni (9.192 µg/g), Mn (4.821 µg/g), Co (3.399 µg/g), Cr (2.717 µg/g), Li (1.321µg/g) and Cd (1.202 µg/g). Overall the average elemental

levels in the scalp hair of the patients revealed the following decreasing order: Ca > Mg > Zn > Na > Sr > Pb > Fe > Cu > K > Ni > Mn > Co > Cr > Li > Cd. Most of the elements exhibited random distribution as shown by appreciably large SE and skewness values. Relatively higher dispersion was shown by Ca, Na, Zn and Mg while some of the metals (Cd, Cu, Li, Co and Mn) exhibited moderate spreading. Somewhat lower skewness was noted in favour of Cu, Sr, Li and Cr thus manifesting relatively symmetrical distribution pattern of these metals in the scalp hair of the patients.

Table 2. Characteristics of the subjects.

Characteristics	Scalp Hair		Nails	
	Cancer patients	Controls	Cancer patients	Controls
n	90	88	88	90
Age (years)				
Range	24–73	26–67	24–73	26–67
Mean	47.87	38.54	47.93	38.69
Diet				
Vegetarian	46 (51%)	58 (66%)	44 (50%)	58 (64%)
Non-vegetarian	44 (49%)	30 (34%)	44 (50%)	32 (36%)
Habitat				
Urban	52 (58%)	50 (57%)	50 (57%)	50 (56%)
Rural	38 (42%)	38 (43%)	38 (43%)	40 (44%)
Use of tobacco				
No use	62 (69%)	70 (80%)	62 (70%)	72 (80%)
Use	28 (31%)	18 (20%)	26 (30%)	18 (20%)
Types of ovarian cancer				
Epithelial cancer	48 (53%)	--	46 (52%)	--
Stromal tumour	20 (22%)		20 (23%)	
Germ cell tumour	22 (25%)	--	22 (25%)	--
Stages of ovarian cancer				
Stage-I	18 (20%)	--	18 (20%)	--
Stage-II	22 (24%)	--	20 (23%)	--
Stage-III	28 (32%)	--	28 (32%)	--
Stage-IV	22 (24%)	--	22 (25%)	--

In case of the controls, Ca (1,124 µg/g), Zn (287.4 µg/g), Mg (198.8 µg/g), Sr (63.0 µg/g) and Na (53.78 µg/g) emerged as major contributors, followed by Fe, Cu and Pb at 16.36 µg/g, 11.34 µg/g and 10.57 µg/g, respectively. On the whole, the decreasing trend in the scalp hair of controls revealed following pattern: Ca > Zn > Mg > Sr > Na > Fe > Cu > Pb > Co > K > Ni > Mn > Cr > Cd.> Li. Most of the elements exhibited large dispersion and asymmetry in their distribution as shown by SE and skewness values. Measured levels of Li, Cd, Cr and Mn exhibited relatively normal distribution, as indicated by small SE values. Lower magnitude of skewness in favour of Cr, Cd, Cu, Ca, and Ni manifested almost symmetrical distribution of these metals in the scalp hair of the controls.

Student's *t*-test ($p < 0.05$) of the elemental data showed that there were significant difference between the levels of Ca, Na, K, Sr, Li, Co, Pb, Cd, Mn, Cr and Ni in the scalp hair of the patients and controls. Measured levels of Pb, Cd, Cr, Ni, Mn, Li, Sr, Na and K were significantly higher in the scalp hair of the patients. Almost similar findings were reported by Magalhaes et al., [29] who reported an increase of K level and a decrease of Ca concentration in ovarian cancer patients compared to healthy individuals. However, there was no significant difference in the concentrations of Mg, Fe, Zn and Cu in the scalp hair of the patients and controls.

3.3. Distribution and Comparison of the Elemental Levels in Nails. Average elemental concentrations in the nails of ovarian cancer patients along with the relevant statistical distribution parameters are presented in Table 3. Most of the elements exhibited large spread in their concentrations in the nails of both groups. Among the elements in the nails of the patients, Ca revealed highest mean level at 1,264 µg/g, followed by Na (743.6 µg/g), Mg (120.8 µg/g), Fe (92.94 µg/g) and Zn (85.86 µg/g). However, relatively lower average concentrations were noted for Sr (22.33 µg/g), Co (14.18 µg/g), Li (8.049 µg/g), Mn (6.504 µg/g) and Cu (2.696 µg/g). Nails contents of the elements in the patients revealed following decreasing order: Ca > Na > Mg > Fe > Zn > K > Ni > Pb > Cr > Sr > Co > Li > Mn > Cd > Cu. Comparative dispersion in the elemental data was noted to be higher for Ca, Na, Mg and Fe while Cd, Mn and Cu exhibited relatively Gaussian distribution pattern. Overall, most of the metals exhibited relatively symmetrical distribution in the nails of patients supported by low skewness values.

Basic statistical parameters for the distribution of selected elements in the nails of controls are also shown in Table 3. Highest average concentration was observed for Ca (1,570 µg/g), followed by those of Na (815.8 µg/g), Zn (222.1 µg/g), Fe (130.7 µg/g) and Mg (111.4 µg/g). However, somewhat lower mean levels were noted for Cu (5.743 µg/g), Mn (3.496 µg/g), Li (2.852 µg/g) and Cd (2.394 µg/g). The mean values of metal contents in controls arranged in the following decreasing order: Ca > Na > Zn > Fe > Mg > K > Sr > Pb > Ni > Co > Cr > Cu > Mn > Li > Cd. Predominantly random distribution pattern was observed for Ca, Na, Fe and Mg as manifested by higher SE values. Some of the elements (Cd, Li, Mn and Cu) exhibited relatively lower dispersion and narrow range. Moderately elevated skewness values for Ca, Fe, Li and Pb suggested their asymmetrical distribution, however, rest of the metals showed somewhat symmetrical distribution in the nails of controls as evident by the skewness values.

Student's *t*-test ($p < 0.05$) of the data showed that the measured concentrations of Pb, Cr, Mn, Ni and Li were significantly higher in the nails of the patients compared with the controls. On the other hand, measured levels of Fe, Zn and Cu were significantly higher ($p < 0.05$) in the nails of controls than those noted in the patients; however, rest of the elements exhibited insignificant variations in the nail samples of both groups.

3.4. Correlation Study. Spearman correlation coefficients between the elemental levels measured in the scalp hair of the patients and controls are shown in Table 4, wherein significant *r*-values are shown in bold at $p < 0.001$. In case of the patients, a strong positive correlation was only observed for Na-K ($r = 0.65$). Some other significant correlations were observed between Mg-Sr ($r = 0.47$), Cd-K ($r = 0.43$), Pb-Zn ($r = 0.38$), Cd-Mg ($r = 0.37$) and Cd-Sr ($r = 0.36$). However, significant negative relationship was noted for Ca-Pb ($r = –0.40$) in the scalp hair of patients. On the basis of above observation it can be concluded that essential elements such as K, Mg and Zn were significantly related with the toxic elements such as Cd and Pb thus evidencing the uptake/build-up of these elements in the patients.

Table 3. Statistical distribution of selected element concentrations (μg/g) in the scalp hair and nails of the ovarian cancer patients and controls.

		Patients					Controls					
		Min	**Max**	**Mean**	**SE**	**Skew**	**Min**	**Max**	**Mean**	**SE**	**Skew**	***p*-value**
Scalp Hair	Ca	205.0	1855	881.9	64.40	0.415	715.1	1767	1124	42.77	0.502	<0.05
	Mg	103.6	234.2	210.2	3.879	-2.534	83.95	262.5	198.8	5.213	-1.111	*NS
	Na	7.150	651.3	149.0	25.35	1.556	12.35	313.0	53.78	8.759	3.058	<0.05
	K	1.000	35.65	10.10	1.024	1.484	1.250	18.35	6.060	0.481	1.533	<0.05
	Fe	1.600	36.05	15.45	1.350	0.529	3.932	39.95	16.36	1.222	1.165	*NS
	Zn	105.2	379.2	205.9	10.81	0.643	78.90	657.8	287.4	24.48	0.875	*NS
	Cu	7.546	14.65	10.88	0.276	0.217	5.500	19.65	11.34	0.505	0.643	*NS
	Sr	38.30	178.0	96.45	4.279	0.337	22.13	133.8	63.00	3.596	1.059	<0.05
	Li	0.100	3.050	1.321	0.120	0.301	0.050	1.650	0.604	0.055	0.799	<0.05
	Co	0.050	9.850	3.399	0.397	1.002	0.650	25.60	6.880	0.767	1.783	<0.05
	Mn	0.800	12.95	4.821	0.413	1.194	0.050	9.550	2.454	0.337	1.699	<0.05
	Ni	0.018	22.50	9.192	0.878	0.502	0.150	17.50	5.988	0.849	0.697	<0.05
	Cr	0.600	5.800	2.717	0.191	0.310	0.300	3.950	2.020	0.153	0.363	<0.05
	Cd	0.100	3.500	1.202	0.113	0.712	0.050	1.650	0.688	0.056	0.523	<0.05
	Pb	2.150	65.02	23.56	2.196	1.292	1.550	49.05	10.57	1.156	3.272	<0.05
Nails	Ca	112.1	4128	1264	126.9	1.673	190.6	5468	1570	143.8	2.168	*NS
	Mg	37.15	478.8	120.8	11.40	3.017	5.195	255.8	111.4	10.71	0.477	*NS
	Na	8.750	1906	743.6	94.56	0.300	23.12	1838	815.8	78.01	0.005	*NS
	K	9.524	122.5	47.50	3.415	0.954	11.06	81.03	41.11	2.644	0.260	*NS
	Fe	8.190	318.5	92.94	10.55	1.184	12.34	600.8	130.7	19.21	1.932	<0.05
	Zn	16.25	150.3	85.86	5.070	0.252	28.21	307.5	222.1	9.175	-0.965	<0.05
	Cu	0.161	9.091	2.696	0.345	1.207	0.417	10.92	5.743	0.474	0.118	<0.05
	Sr	1.602	59.93	22.33	1.956	0.627	2.083	67.72	32.11	2.271	-0.048	*NS
	Li	0.117	22.08	8.049	0.824	0.755	0.500	10.23	2.852	0.289	1.870	<0.05
	Co	0.704	32.92	14.18	1.619	0.339	0.272	32.33	11.90	1.252	0.763	*NS
	Mn	0.438	17.39	6.504	0.665	0.759	0.158	8.908	3.496	0.324	0.547	<0.05
	Ni	0.528	81.56	37.70	4.114	0.225	0.158	39.25	14.89	1.663	0.401	<0.05
	Cr	1.724	58.55	25.83	2.486	0.201	0.160	23.11	8.965	0.781	0.776	<0.05
	Cd	0.169	6.771	2.850	0.289	0.531	0.162	6.096	2.394	0.211	0.587	*NS
	Pb	6.771	104.6	30.04	3.244	1.750	2.690	72.32	19.37	2.190	1.633	<0.05

*NS–non significant

However, the correlation study (Table 4) showed that Cu, Mn, Co, Ni, Fe and Cr were not significantly correlated with any of the other elements in the scalp hair of patients, thus evidencing their independent role, irrespective of other elements. Growing body evidence have shown that Pb can substitute for Zn in several proteins that function as transcriptional regulators, including protamine, hence reducing the binding of these proteins to the recognition elements in genomic DNA, a process which suggests an epigenetic involvement of Pb in altered gene expression [30]. These events may be of particular relevance in transplacental exposures and later cancer. Pb-Zn interactions in proteins can also cause post-translational changes in protein structure. The tumour suppressor protein p53 is a Zn-binding protein and thus if Zn is displaced by Pb in p53, may result in a structurally altered form of the protein with functional consequences not different from mutation or deletion of the p53 gene as has been found for Cd [31]. Calcium revealed a significantly negative relationship with Pb, which indicated that the deficiency of Ca in the cancer patients may be linked with the enrichment of Pb contents. The absence of strong association between Ca and Mg in cancer patients was noteworthy, because the elements were well known for their positive correlation in hair [32]. The correlation study revealed that essential metals, such as K, Mg and Zn, were directly related with the toxic metals, such as Cd and Pb, thus evidencing the uptake/build-up of these metals in the patients, which played critical role in the onset and development of the disease [33, 34].

The counterpart data for the controls (Table 4) showed significant direct correlations between Fe-K (r = 0.41), Cu-K (r = 0.39), Li-Co (r = 0.38), Mn-Fe (r = 0.36), Pb-Ni (r = 0.35) and Sr-Na (r = 0.35). A significant negative relationship was noted for Na-Li (r = –0.36) in the scalp hair of controls. Overall, the correlation pattern of trace elements in the scalp hair of controls remains diverse compared to the patients. Also Pb revealed a significant correlation with Ni in controls but in patients it exhibited correlation with Zn. All other metal pairs exhibited insignificant positive or negative relationships, which manifested their independent variations in the scalp hair of controls.

In case of the nail samples of the patients (Table 4), most of the pairs showed non-significant relationships. Strong positive correlations were found between Li-Mn (r = 0.65) and Mn-Cd (r = 0.55), indicating the build-up of these metals in the nails of ovarian cancer patients. In addition, significant correlations were observed between Cr-Li (r = 0.48), Pb-Mn (r = 0.47), Pb-Li (r = 0.43) Ni-Zn (r = 0.42), Ni-Mg (r = 0.41), Na-K (r = 0.39), Ni-Ca (r = 0.37), Ni-Co (r = 0.37) and Mg-Ca (r = 0.35). Some negative correlations were found between Zn-Na (r = –0.46), Cu-Ca (r = –0.35), Ni-Na (r = –0.35) and Cr-Zn (r = –0.35). For the nails of controls, strong positive correlation were found between K-Na (r = 0.65), Cr-Mn (r = 0.57), Mg-Ca (r = 0.56) and Mn-Ni (r = 0.54).

Other notably significant correlations were observed for Cr-Li (r = 0.47), Co-Cd (r = 0.41), Pb-Co (r = 0.40), Mn-Co (r = 0.39), Mn-Li (r = 0.38), Co-K (r = 0.38), Pb-Cd (r = 0.36). However, a significant negative relationship was noted for Li-Mg (r = –0.38) in the nails of controls. The role of Ni was unique in that it showed significant positive correlation with Zn in the nails of patients but in case of controls it was significantly correlated with Mn. Some of the elements (Li-Mn, Na-K, Mg-Ca and Cr-Li) had positive correlations with each other in both donor groups. Likewise, Li and Co were also noted to compete with Cd in both groups, whereas Cd is negatively correlated with Ca in both groups. Similarly, Cr exhibited positive correlation with Mn in the case of controls, while, in case of the patients, it was negatively correlated with Zn. In both groups, it may be worth mentioning that Fe did not show a statistically significant correlation with any of the elements, thus manifesting its independent role in ovarian cancer patients. Consequently, the correlation study revealed significantly dissimilar pattern of mutual dependence among the elements in the nails of the patients and controls.

Table 4. Correlation coefficient (r)* matrix of selected elements in the scalp hair and nails of the ovarian cancer patients (below the diagonal) and controls (above the diagonal).

Scalp Hair															
	Ca	**Mg**	**Na**	**K**	**Fe**	**Zn**	**Cu**	**Sr**	**Li**	**Co**	**Mn**	**Ni**	**Cr**	**Cd**	**Pb**
Ca	1	0.09	-0.02	0.09	-0.11	0.04	-0.14	-0.04	-0.03	0.32	-0.17	0.00	0.12	-0.28	0.14
Mg	0.03	1	-0.07	0.33	0.26	0.04	0.31	0.23	-0.13	0.22	0.16	-0.28	-0.08	0.14	0.03
Na	-0.05	0.06	1	0.16	-0.20	-0.27	-0.10	**0.35**	**-0.36**	-0.11	0.16	-0.08	-0.24	0.00	0.06
K	0.12	0.17	**0.65**	1	**0.41**	0.26	**0.39**	0.34	-0.26	0.15	0.29	0.02	0.10	0.25	0.17
Fe	-0.21	0.28	-0.22	-0.05	1	0.11	0.23	-0.04	-0.17	-0.16	**0.36**	0.17	0.05	0.08	0.24
Zn	-0.12	0.22	0.02	0.10	0.15	1	0.29	0.04	-0.09	0.01	-0.23	0.09	0.18	0.21	0.01
Cu	-0.15	0.00	-0.29	-0.28	0.00	0.04	1	0.24	-0.09	0.03	0.26	-0.04	0.29	0.27	-0.08
Sr	-0.17	**0.47**	0.19	0.14	0.00	-0.04	0.08	1	-0.31	-0.11	0.15	-0.22	-0.06	0.15	0.01
Li	0.18	-0.18	0.15	0.03	-0.19	0.04	-0.05	-0.03	1	**0.38**	0.07	-0.15	0.24	0.01	0.04
Co	-0.06	0.20	-0.29	-0.16	0.10	0.25	-0.02	-0.09	-0.21	1	-0.11	-0.13	0.01	0.17	-0.05
Mn	-0.04	0.20	-0.31	0.04	0.12	-0.30	0.04	0.27	-0.01	0.11	1	-0.07	0.09	-0.04	0.18
Ni	0.04	-0.31	-0.21	-0.22	-0.32	-0.25	0.13	-0.08	0.09	-0.26	-0.08	1	0.17	-0.16	**0.35**
Cr	0.06	-0.03	-0.09	-0.01	-0.03	0.24	-0.19	-0.12	0.25	0.18	0.08	0.10	1	0.27	0.09
Cd	0.09	**0.37**	0.25	**0.43**	0.22	0.04	0.02	**0.36**	0.03	-0.32	0.21	-0.15	-0.12	1	-0.05
Pb	**-0.40**	-0.09	0.10	0.27	0.11	**0.38**	0.04	0.17	-0.12	0.18	-0.01	-0.04	0.02	0.09	1
Nails															
	Ca	**Mg**	**Na**	**K**	**Fe**	**Zn**	**Cu**	**Sr**	**Li**	**Co**	**Mn**	**Ni**	**Cr**	**Cd**	**Pb**
Ca	1	**0.56**	-0.26	-0.10	0.14	0.01	-0.20	-0.03	-0.07	0.13	0.21	0.24	0.17	0.25	0.01
Mg	**0.35**	1	-0.12	0.02	0.32	-0.16	-0.10	0.04	**-0.38**	-0.08	-0.07	-0.15	-0.03	-0.04	-0.18
Na	-0.05	-0.31	1	**0.65**	-0.09	-0.23	0.24	-0.17	-0.13	0.17	-0.05	-0.14	0.01	-0.15	-0.06
K	0.05	0.20	**0.39**	1	0.05	-0.32	-0.06	-0.18	0.07	**0.38**	0.18	-0.13	0.14	0.08	0.20
Fe	-0.16	-0.11	0.27	0.34	1	-0.01	-0.13	0.10	0.23	-0.04	0.16	-0.04	0.30	-0.12	-0.03
Zn	0.11	0.08	**-0.46**	-0.32	-0.21	1	0.01	0.09	-0.02	-0.30	0.13	0.24	0.01	-0.16	0.31
Cu	**-0.35**	-0.19	0.21	0.05	0.31	-0.08	1	0.21	-0.18	-0.12	-0.11	0.10	-0.27	-0.21	0.01
Sr	-0.09	0.08	0.06	0.23	-0.04	-0.27	0.16	1	0.15	0.09	0.00	0.21	-0.08	0.01	-0.11
Li	-0.09	0.05	-0.29	-0.03	0.29	0.15	0.03	0.07	1	0.16	**0.38**	0.27	**0.47**	-0.04	0.14
Co	0.28	-0.04	-0.06	0.13	0.31	0.03	0.17	0.06	0.07	1	**0.39**	0.26	0.21	**0.41**	**0.40**
Mn	0.14	0.14	-0.24	0.03	0.15	0.14	-0.07	0.00	**0.65**	0.10	1	**0.54**	**0.57**	0.08	0.11
Ni	**0.37**	**0.41**	**-0.35**	-0.25	-0.05	**0.42**	-0.11	0.28	0.20	**0.37**	0.06	1	0.16	0.23	0.20
Cr	-0.24	-0.32	0.31	0.25	0.34	**-0.35**	0.31	0.32	**0.48**	-0.14	0.33	-0.27	1	-0.11	0.18
Cd	0.10	-0.16	-0.26	-0.15	0.10	0.06	0.13	0.06	**0.38**	0.17	**0.55**	0.14	0.23	1	**0.36**
Pb	0.11	0.01	-0.10	0.12	0.34	0.07	-0.17	-0.11	**0.43**	0.33	**0.47**	0.26	0.09	0.26	1

*r-values > 0.35 or < – 0.35 are significant at $p < 0.001$

3.5. Multivariate Analysis of the Elemental Levels in Scalp Hair. The PCA of the elements in the scalp hair of the patients and controls, extracted by using varimax normalized rotation on the data-set are shown in Table 5. It yielded seven PCs with eigen values >1, commutatively explaining approximately 80% of total variance of data for patients, and seven PCs for controls, with more than 81% of the cumulative variance. The corresponding CA based on Ward's method is shown in Figure 1(a). In case of patients, PC 1 with 17.5% of the variance showed significant loadings for Na, K and Cd which also revealed a strong cluster in CA as shown in Figure 1. Second PC (15.5% of the variance) showed elevated loadings for Zn, Co and Pb duly supported by their mutual cluster in CA. Third PC with 13.3% of the variance was mostly contributed by Mg, Sr, Mn and Cd which were well-supported by the CA. Likewise, PC 4, 5 and 6 exhibited significant loadings for Li–Cr, Fe–Cu and Mg–Ni, respectively, while PC 7 showed elevated loadings of Ca–Pb. Cluster analysis was in good agreement with PCA findings. The multivariate apportionment indicated mutual associations of the toxic and essential elements in the scalp hair of the patients which may be attributed to the cancer.

The CA of elemental data in the scalp hair of the controls (Figure 1(b)) revealed strong clusters of Sr–Na and Ni–Pb which also showed mutual loadings in PC 2 and PC 4 (Table 4), respectively. Another significant cluster was noted for Cu–Cr–Zn–Cd which showed elevated loadings in PC1, 6 and 7 thus indicating their multiple sources in the controls. The essential metals, K–Fe–Mn–Mg also manifested a strong cluster and

significant loadings in PC 5 while Ca–Li–Co also revealed strong cluster and mutual loadings in PC 3. In case of controls, most of the essential metals shared the common clusters and mutual loadings and thus signifying their vital role in the elemental balance of human body. The multivariate methods thus evidenced noteworthy alterations in the scalp hair elemental contents of the patients in comparison with the controls.

Table 5. Principal component loadings* of selected elements in the scalp hair of ovarian cancer patients and controls.

	Patients							Controls						
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Eigen value	2.62	2.32	1.99	1.65	1.38	1.10	1.00	3.06	2.38	1.94	1.52	1.24	1.05	1.03
% Total Variance	17.5	15.5	13.3	11.0	9.19	7.32	6.69	20.4	15.9	12.9	10.1	8.24	7.02	6.90
% Cumulative Variance	17.5	32.9	46.2	57.2	66.4	73.7	80.4	20.4	36.3	49.2	59.3	67.5	74.5	81.4
Ca	-	-	-	-	-	-	0.91	-	-	0.86	-	-	-	-
Mg	-	-	0.42	-	-	0.71	-	-	-	-	-	0.88	-	-
Na	0.76	-	-	-	-	-	-	-	0.79	-	-	-	-	-
K	0.89	-	-	-	-	-	-	-	0.36	-	0.37	0.47	0.39	0.33
Fe	-	-	-	-	0.68	-	-	-	-	-	-	0.60	-	-
Zn	-	0.82	-	-	-	-	-	0.88	-	-	-	-	-	-
Cu	-	-	-	-	0.86	-	-	0.31	-	-	-	-	0.72	-
Sr	-	-	0.62	-	0.36	-	-	-	0.85	-	-	-	-	-
Li	-	-	-	0.87	-	-	-	-	-	0.67	-	-	-	-
Co	0.33	0.76	-	-	-	-	-	-	-	0.68	-	-	-	0.45
Mn	-	-	0.85	-	-	-	-	-	0.34	-	-	0.58	-	-
Ni	-	-	-	-	-	0.81	-	0.45	-	-	0.62	-	-	-
Cr	-	-	-	0.72	-	-	-	-	-	-	-	-	0.81	-
Cd	0.68	-	0.46	-	-	-	-	-	-	-	-	-	-	0.87
Pb	-	0.68	-	-	-	0.30	0.35	-	-	-	0.84	-	-	-

*PC loading <0.30 were omitted

Table 6. Principal component loadings* of selected elements in the nails of ovarian cancer patients and controls.

	Patients					Controls					
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Eigen value	4.86	2.98	2.10	1.43	1.27	2.84	2.60	2.08	1.73	1.54	1.26
% Total Variance	32.4	19.9	14.0	9.5	8.5	18.9	17.3	13.9	11.5	10.3	8.41
% Cumulative Variance	32.4	52.3	66.3	75.8	84.3	18.9	36.3	50.1	61.7	71.9	80.4
Ca	-	0.80	-	-	-	0.59	-	-	-	-	-
Mg	-	-	0.87	-	-	-	-	-	0.87	-	-
Na	0.46	-	-	-	0.54	-	0.89	-	-	-	-
K	0.48	-	-	-	0.82	-	0.93	-	-	-	-
Fe	0.70	-	-	0.43	-	-	-	-	-	0.78	-
Zn	0.74	-	-	-	0.39	-	-	0.38	-	-	0.76
Cu	0.33	-	-	0.84	-	-	-	-	-	0.54	-
Sr	-	-	-	-	0.89	-	-	0.54	0.38	-	-
Li	0.38	0.43	-	-	0.53	-	-	-	0.83	0.31	-
Co	-	-	-	0.88	-	0.71	-	0.38	-	-	-
Mn	-	0.71	-	-	0.37	-	-	0.83	-	-	-
Ni	0.89	-	-	-	-	-	-	0.83	-	-	-
Cr	0.50	0.43	-	-	0.63	-	-	-	-	0.71	-
Cd	-	-	0.81	-	-	0.90	-	-	-	-	-
Pb	-	0.86	-	0.31	-	0.48	-	-	-	-	0.69

*PC loading <0.30 were omitted

3.6. Multivariate Analysis of the Elemental Levels in Nails. The PCA loadings for the elemental data in the nails of the patients and controls extracted by using varimax normalized rotation are shown in Table 6. In case of the patients, the PCA yielded five PCs with eigen values >1, commutatively explaining more than 84% of total variance while in case of the controls, six PCs were obtained, manifesting more than 80% of the total variance. The corresponding CA based on Ward's method is shown in Figure 1(c). In case of the patients, PC 1 with 32.4% of the variance exhibited highest loadings for Zn, Ni and Fe, supported by a strong cluster in CA, which is mostly contributed by dietary sources/food habits. PC 2 (19.9 % of the variance) showed maximum loadings for Ca, Mn and Pb duly supported by their mutual cluster in CA. These elements can be traced to originate as environmental pollutants and dietary intakes. PC 3 with 14 % of the variance manifested elevated loadings for Mg and Cd, which also revealed a strong cluster in CA, while, PC 4 revealed higher loadings for Cu and Co. Likewise, PC 5 showed higher loadings for K, Sr and Cr; all of these elements constituted one strong cluster in CA.

The CA of elemental data in the nails of the controls (Figure 1(d)) revealed strong clusters of Ca–Cd and Na–K which also showed mutual loadings in PC 1 and PC 2 (Table 5), respectively. Both these clusters were believed to be contributed by dietary sources and environmental exposure, specifically the anthropogenic emissions. PC 3 showed significant loadings for Mn and Ni, which also revealed a strong cluster in CA. Elevated loadings of Mg and Li were shown by PC 4 whereas PC 5, showed higher loadings for Fe, Cr and Cu in the nails of controls. These elements were mostly controlled by internal body metabolism and

linked with nutritional sources. The PCA demonstrated significantly different grouping/origin of the elements in the nails of cancer patients and controls.

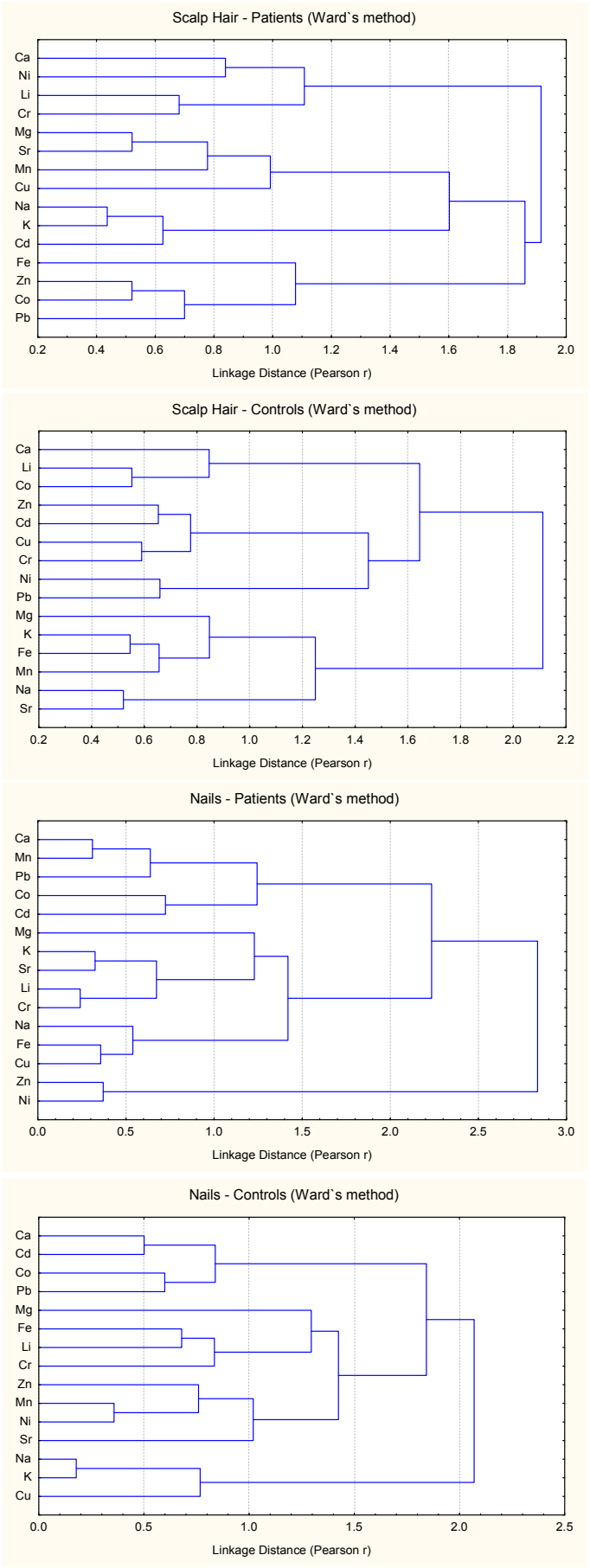

Figure 1. Cluster analysis of the selected elements in the scalp hair and nails of ovarian cancer patients and controls.

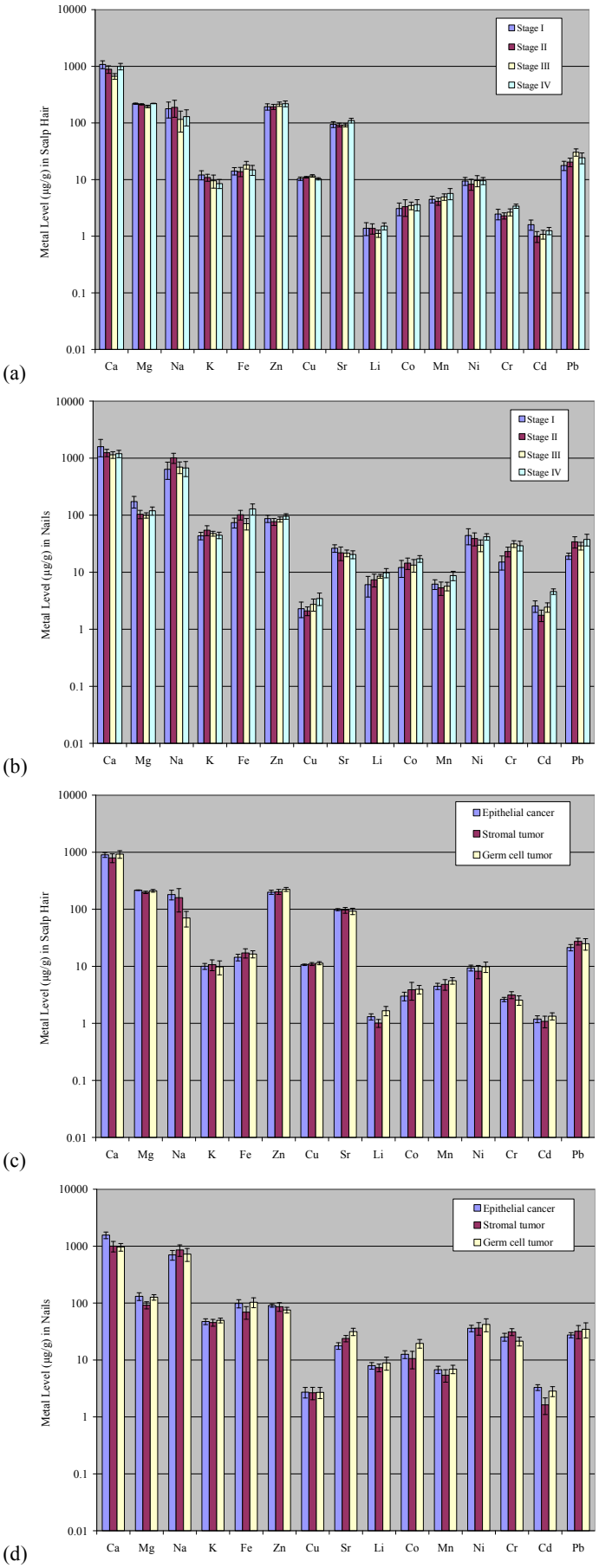

Figure 2. Comparative average concentrations of the selected elements (±SE) in the scalp hair and nails of ovarian cancer patients based on the stages and types of cancer.

The apportionment of elements in the nails of the patients was thus appreciably different from those of controls, which manifested an imbalance of the elements in patients. The multivariate methods may thus be used as a diagnostic tool in the clinical studies and it may provide an alternative technique for the identification of the disease; however, it requires further support and justification by detailed studies comprising larger population segment and more variables.

3.7. Comparison of the Elemental Levels Based on Stages of Ovarian Cancer Patients. Average concentrations of the elements in scalp hair of the patients at different stages are shown in Figure 2(a) for comparative evaluation. Mean levels of Ca, K and Cd were found to be higher at stage-I; nonetheless, measured levels of Na were relatively higher at stage-II. Likewise, average concentrations of Fe and Pb were markedly higher at stage-III while those of Sr, Mn and Cr were comparatively higher at stage-IV. However, mean levels of Mg, Zn, Cu, Li, Co and Ni in the scalp hair of the patients exhibited insignificant differences at various stages.

Comparative elemental levels in the nails of the patients at different stages are also shown in Figure 2(b). Average levels of Cd, Pb, Mn, Co, Li, Cu and Fe were found to be relatively higher at stage-IV while elevated Cr contents were observed at stage-III. It is noteworthy that early stage ovarian cancer is typically asymptomatic which results in most cases presenting with late stage-III or stage-IV cancer (King, 2013). Mean concentrations of Na and K were moderately higher at stage-II while those of Ca, Mg, Sr and Li were noted to be in considerable excess at stage-I. In addition, average levels of Zn in the nails were found to be almost comparable at all four stages. Similar trend was observed for Ca, Na, Co, Mn, Ni, Cd and Pb at stages I and II, while at stage-III and stage-IV, Cd, Mn, Li, K and Mg showed similar trend in the scalp hair and nails of the patients.

3.8. Comparison of the Elemental Levels Based on Types of Ovarian Cancer Patients. A comparative evaluation of mean levels of the elements in the scalp hair of various types of ovarian cancer patients (i.e., epithelial cancer, stromal tumour and germ cell tumour) are shown in Figure 2(c). In case of epithelial cancer patients, Na exhibited highest average concentration, while mean levels of Cr and Pb were higher in scalp hair of stromal tumour patients. Furthermore, Zn, Li, Mn and Cd levels were markedly higher in germ cell tumour patients. The mean concentrations of Ca, Mg, K, Cu and Sr were not appreciably different in the scalp hair of the three types of ovarian cancer patients.

Mean levels of the elements in the nails of various types of ovarian cancer patients are also displayed in Figure 2(d). Average concentrations of K, Cu and Li were not significantly different in the nails of the three types of the patients, whereas, Ca and Zn showed higher concentrations in epithelial cancer patients. Mean levels of Na and Cr were markedly higher in nails of stromal tumour patients while Sr, Co, Ni and Pb levels were relatively higher in the nails of germ cell tumour patients. Conversely, Pb and Sr exhibited lowest concentrations in the nails of epithelial cancer patients while Cd, Mn, Co, Li, Fe and Mg showed lowest contributions in the nails of stromal tumour patients.

3.9. Metals and Oxidative Stress in Ovarian Cancer. Numerous epidemiological studies have shown an increased cancer incidence associated with chronic exposure to certain elements such as Ni, Cr, Pb and Cd [35]. These elements are capable of generating reactive oxygen species (ROS) and are key mediators responsible for lipid peroxidation, protein modification, DNA damage, which lead to mutations in crucial genes thus ultimately leading to cancer [36]. A tightly regulated homeostatic system of elemental ion operates to maintain levels within normal ranges. Disruption of elemental homeostasis may lead to uncontrolled elemental mediated formation of deleterious free radicals which participates in organic and inorganic oxygen radical reactions. In consequence of these oxygen radical reactions, the ROS are produced. Increased production of ROS results from oxidative stress appears to play an important role in the development and progression of ovarian cancer [37]. Signs of oxidative stress in the circulation have been reported in ovarian cancer patients [38], and with the development of oxidative stress, several changes occur such as repeated destruction and repair of the ovarian surface epithelium as well [39]. Moreover, various studies showed that free radicals derived from oxidative stress play significant role in ovarian carcinogenesis [40, 41].

An examination of the tabulated data (Table 3) revealed that, markedly elevated concentrations of some toxic elements in cancer patients compared to the controls indicated a build-up in the elemental levels at the expense of the macronutrients, which exhibited lower concentrations in cancer patients. So the proportional variations of the elements in scalp hair and nails of the patients indicated imbalances of the elements which may be linked with the onset and progress of the ailment. In the present study, trace toxic and essential elemental patterns in scalp hair and nails samples are providing fruitful information not only as a diagnostic procedure but also in providing answers relating to the treatment [12]. Furthermore, the findings of this study are of great importance because they can be utilized as a stepping-stone towards the development of an early screening test for identifying individuals who are at high-risk for the development of ovarian cancer. Several metals including Cd, Ni, Pb and Cr etc., are considered to act not only as carcinogens but also as co-carcinogens and their carcinogenic potential depend mainly on factors such as oxidation states and chemical species [42]. Some of the metals responsible for the formation of ROS resulting from oxidative stress causes carcinogenicity are described below.

3.9.1. Cadmium. Cadmium has no known essential functions and generates toxic effects even at very low doses. It indirectly generates ROS and consequently DNA, lipid and protein oxidation in various cell lines, which lead to DNA damage and hence tumour growth [43]. It interferes with cell proliferation, differentiation and apoptosis pathways [30]. Multiple studies have linked occupational exposure to Cd with lung cancer, leukaemia and prostate cancer, whereas few studies have been associated with Cd exposure for ovarian cancer [30, 44, 45]. Thompson and Bannigan [46] noted that Cd accumulates in the ovarian with age and had been associated with decrements in oocyte development. In addition, Eriksen et al., [33] and Akesson et al., [34] found an association between Cd intake and ovarian cancer. An American study suggested associations between ovarian cancer and Cd

exposure [11, 47]. Other study suggested that Cd exposure was not likely to have substantial role in the development of ovarian cancer [48]. In another study, Cd concentration was significantly higher in the scalp hair of ovarian cancer patients as compared to healthy donors [12]. It was observed in the present work that Cd levels in the scalp hair and nails of ovarian cancer women were elevated than those in the normal women (Table 3).

3.9.2. Lead. Lead is a genotoxic agent and may also generate ROS and cause oxidative damage to DNA and chromosomal aberrations [49, 50]. Oxidative stress can be generated indirectly by non-redox cycling metals such as Pb. Increased Pb contents in dietary intake were linked with cancers of stomach, small intestine, large intestine, ovarian, kidney, lung, myeloma, all lymphomas and all leukemia [51, 52]. Aminolevulinic acid (the heme precursor whose levels were increased by Pb) generated free radicals and had been shown to cause oxidative damage to DNA in Chinese hamster ovarian cells in vitro [53]. It was noted that the concentrations of Pb were high both in scalp hair and nails of ovarian cancer patients as compared to controls in the present study, thus indicated an association between Pb and cancer risk (Table 3).

3.9.3. Nickel. Nickel is a toxic and carcinogenic metal. It is well known to interfere with DNA strand breaks, DNA–protein and DNA–inter strand crosslinks [54]. It also enhances the cytotoxicity and genotoxicity of DNA-damaging agents through inhibition of DNA repair. Growing body of evidence pointed out that Ni exposure causes formation of free radicals in various tissues in both human and animals [55]. A study also found that the chromosomal damage induced by in vitro exposure of Chinese Hamster Ovary cells to carcinogenic Ni compounds preferably occurs in the heterochromatic regions, which contain more proteins and exist in a higher-condensed state [30]. It was described that Ni can block Ca channels and hence Ni releases the stored intracellular Ca via a mechanism underlying the interaction between Ni ions and cell surface receptor [56]. Wadhwa et al., [12] found that the Ni concentration in the scalp hair of ovarian cancer patients was elevated as compared to referent subjects. The results of the present study, involving lower Ca and high Ni concentrations in scalp hair and nails of ovarian females than the non-cancerous females (Table 3) [42], which supported the above mentioned hypothesis.

3.9.4. Chromium. Chromium is an essential nutrient required for sugar and fat metabolism, but excessive Cr can affect human oxidation–reduction, denaturation of protein, precipitation of nucleic acid, interfere with normal enzymatic activity and can lead to DNA damage, which may result in cancer causing gene mutation [57]. It is the only carcinogenic metal species that directly generates ROS by interaction with cellular reductants. It may participate in a "Fenton-like" reaction with H_2O_2 leading to hydroxyl radical production as supported by experiments as well [58]. It has been found to be a strong clastogen in experimental animals producing chromosome aberrations, sister chromosome exchanges, DNA strand breaks and oxidized base damage [56, 59]. Accordingly, higher contents of Cr were also linked to Hodgkin lymphoma, prostate cancer and oral cancer patients [60, 61]. Elevated levels of Cr were observed in the scalp hair and nails of ovarian cancer patients as compared to the controls; it supported the fact that Cr is a carcinogenic agent (Table 3).

3.9.5. Zinc. Zinc is one of the co-factors of antioxidant enzymes such as catalase and superoxide dismutase, an enzyme for cellular protection that removes free radicals. Zinc may play an anticarcinogenic role by stabilizing the structure of DNA, RNA, ribosomes and may influence immune system [62]. Unlike other elements, Zn deficiency, rather than excessive exposure, is often implicated in detrimental effects including oxidative damage to biomolecules, such as, lipids, proteins, DNA and have been shown to continue a pro-carcinogenic factor [63]. It is proved to be an antioxidant or free-radical scavenger in some studies [64]. Many studies reported that long term Zn deficiency enhances production of free radicals in both humans and laboratory animals [37]. Another study reported that scalp hair of patients with ovarian cancer showed lower Zn concentration compared with healthy donors [14]. Similarly reduced Zn concentrations were found in the scalp hair and nails of ovarian cancer patients than controls in the present research (Table 3). Numerous studies have been reported that Zn levels were reduced in lung, breast, gallbladder, colorectal, head and neck cancers as compared to controls [37, 65]. Specific inhibition of tumour growth in Zn deficiency was linked to the importance of this metal for proliferation processes.

3.9.6. Copper. Copper is an essential element with a component of more than 30 enzymes including ceruloplasmin, cytochrome oxidase and ascorbate oxidase etc., in human body. At high concentrations, Cu induced growth proliferation and cancer by damaging DNA with toxic free ROS via oxidative stress and decreasing glutathione levels as well [35]. Ceruloplasmin levels have noted to be high in various types of cancers such as breast, colon and lymphoma [66]. Role of Cu in the formation of tumour angiogenesis has also been studied [67]. The most elevated levels of Cu have been documented in cancer patients suffering from breast, cervical, ovarian, lung, prostate, reticulo-endothelial system, stomach, oral, leukaemia and lymphoma [68, 69]. Conversely, concentration levels of Cu in the scalp hair and nails of ovarian cancer patients were lower than those in controls in the present work (Table 3).

3.9.7. Cobalt. Cobalt is a constituent of vitamin B12 and is involved in preventing and treating pernicious anaemia and also helps in red blood cell production. Although, It is genotoxic or mutagenic and was reported to induce clastogenic effects and sister chromatid exchanges in human lymphocytes [70]. It damages DNA strands and other bimolecules by inducing oxidative stress through the production of ROS. Based on evidence of carcinogenicity in experimental animals, Co and its compounds were included as possible human carcinogens [71]. Soluble Co was reported to block inorganic Ca channels which may interfere with neuromuscular transmissions [72]. Previously published reviews showed that exposure to Co was linked to an increased lung cancer risk, was proven to be genotoxic both *in-vitro* and *in-vivo* in lung cells [73]. Moreover, in the present investigation, it was found that Co contents in scalp hair and nails of ovarian cancer patients were appreciably higher than the counterpart healthy donors indicated an essential role in the development of disease (Table 3).

3.9.8. Iron. Iron is crucial for cellular functions including synthesis of DNA, RNA and proteins, electron transport, cellular respiration, cell proliferation and regulation of gene expression [74]. However, excess of redox active Fe is toxic, aggravates oxidative stress which promotes tissue degeneration and cancer [75]. It is also a nutrient for invading microbial and neoplastic cells. Iron overload has been shown to enhance chemically mediated cutaneous tumour promotion in animals [76]. Recent studies reported association between high levels of Fe and the risk of stomach, colon, breast, liver and uterine cancers, whereas bladder and lung cancers patients possessed low Fe level. [77]. Likewise, in the present study, scalp hair and nails Fe levels were evidently lower in the patients than the healthy donors (Table 3). A decreased concentration of Fe in cancer subjects indicated that the utilization of heme molecule was impaired, because the cancer itself might had been affecting the bone marrow function adversely [24]. Moreover, Iron-restricted erythropoiesis and functional Fe deficiency occur in those with chronic inflammation, renal disease and cancer. This is because inflammatory processes impair the delivery of Fe to red cell precursors, irrespective of Fe stores, with mobilization of Fe from stores insufficient to meet metabolic demands. [78].

Present research revealed significant differences in the concentrations of essential and toxic elements among different cancer stages/types that could lead to a better understanding of the aetiology of the cancer and these results may be valuable practical information that can be applied to clinical medicine. In Pakistan the number of reported ovarian cancer cases is on rise and this type of research study is worth considering. Moreover, it is the first study which assessed the association between ovarian cancer and essential/toxic elements in scalp hair and nails samples among Pakistani women. Until recently, most of the reported studies were limited to evaluate such an association in tissue, plasma and serum and few elements. However, due to the limited sample size, the current analysis may have limited application to assess the role of metals and their interactions in carcinogenesis. The precise mechanism of association of trace elements with ovarian cancer incidence requires further investigation. Limitations of this study primarily relate to the nature of the ecological design. We could not identify the occupational and environmental hazards to each individual. Finally, the study was relatively small. It was observed in the present study that the socioeconomic factors also play a role in higher mortality rates in patients, such as, poor nutrition, no routine check-ups, lack of effective screening approaches, late diagnosis, lack of preventive strategies and unequal access to health care. Screening tests for ovarian cancer should be available in the nearer possible vicinity of the urban and rural population and it should be compulsory for the people to undergo those tests on regular basis. The cost factor of cancer treatment is also very high. Early ovarian cancer mass does not cause obvious symptoms and most of the women present with advanced stage where the prognosis is poor. Earlier detection and treatment may improve the survival rate. In addition, environmental factors such as factories waste having carcinogens or mutagens like toxic trace elements etc. should be properly wasted to make sure that it is harmless for public. The local hygiene facilities are poor in Pakistan and it should be upgraded. Proper data base is required at country level and health organizations should play their role to control/eradicate ovarian cancer among Pakistani women.

4. CONCLUSIONS

The concentrations of essential/toxic elements in the scalp hair and nails of ovarian cancer patients in comparison with the controls were considerably divergent. Mean levels of Cr, Li, Mn, Ni and Pb were found to be significantly higher in the patients compared to the controls but concentrations of Fe, Zn and Cu were considerably lower in the patient than controls, thus indicating the imbalance of these elements in the patients. The average levels also exhibited significant disparities in the scalp hair and nails of the patients and controls based on cancer types and stages. The correlation study revealed appreciably different mutual variations of the elements in the scalp hair and nails of two donor groups. PCA and CA also supported the different apportionment mechanism of the elements in the scalp hair and nails of the patients and controls which evidenced that the body metabolism in the cancer patients was being significantly affected by the elemental concentrations.

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