Biol Trace Elem Res (2011) 144:272–294
DOI 10.1007/s12011-011-9086-x

A Discriminant Analysis of Trace Elements in Scalp Hair of Healthy Controls and Stage-IIIB Non-small Cell Lung Cancer (NSCLC) Patients

Yasemin Benderli Cihan · Sema Öztürk Yıldırım

Received: 20 April 2011 / Accepted: 12 May 2011 /
Published online: 27 May 2011

Abstract Our work aimed at extending the search for the trace elements (TE) abnormalities in patients with lung cancer and in healthy controls who smoke, and also for evidence of a possible association between lung cancer and TE. The analysis of the hair from patients with Stage-IIIB non-small cell lung cancer (group 1) and healthy controls (group 2) were analyzed using the inductively coupled plasma mass spectrometry technique in order to obtain information on the correlation between the lung cancer patients and healthy controls. Sixty-seven one-hair samples in group 1 were individually collected before chemoradiotherapy. For comparison, 74 hair samples were collected from group 2. In group 1, the trace elements present at the highest levels were measured to be Ca, Zn, Sn, Na and Mg, respectively, and they were quantified as 68.2, 53.2, 33.9, 23.3, and 28.9 μg. kg^{-1}, respectively. In group 2, the trace elements present at the highest levels were Zn, Mg, Ca, Fe, and Se, respectively, and they were quantified as 109.7, 31.9, 30.8, 25.0, and 20.1 μg.kg^{-1}. In group 1, the highest levels of Ca, Sn, and Na were 2.03, 1.06, and 1.01 times higher, respectively, compared with group 2. In group 2, Zn, Mg, Fe, and Se were 2, 1.01, 2.7, and 1.6 times higher, respectively, compared with group 1. When the levels of trace elements were compared between groups 1 and 2 using Student's *t* test, the levels of Ag, Au, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Fe, Ga, Hg, K, Ni, Rb, Rh, Sb, Sc, Ti, V, and Zn were found to be statistically different ($p<0.05$). According to Pearson's correlation, the most powerful correlation was found for Cr–As ($r=0.858$) couple in group 1 ($r=0.745$) and for Mn-Cr couple in group 2. The factors obtained according to converted matrix were observed to be as follows: for group 1, first factor, ten variables (Cd, Li, Cs, Ag, Rb, Pd, Ga, Zn, Al, and K); second factor, seven variables (Cr, As, Sn, Co, Ca, Rh, and Fe) and third factor, four variables (Mn, Au, Cu, and Hg). Within the first factor that best describes the overall change, the most important variables are Cd and Li, respectively the first and the second factors. Group 2 contained the following: first factors, six variables, Cr, Mn, Al, Ba,

Y. Benderli Cihan (✉)
Department of Radiation Oncology, Kayseri Education and Research Hospital,
38010 Kocasinan/Kayseri, Turkey
e-mail: cihany@erciyes.edu.tr

S. Öztürk Yıldırım
Department of Physics, Erciyes University Faculty of Sciences, 38039 Kayseri, Turkey

Rb, and Pb; second factor, eight variables, Co, As, Sn, Cd, Hg, Cs, Ca, and Ce; third factor, five variables Na, Ga, Be, B, and Sr; and fourth factor, two variables K and Ag. First, second, third, and fourth factors explain the 36% of the overall change. Within the first factor that best described the overall change, the most important variables were Cr and Mn. In this analysis, we observed that the group 2 trace elements accumulated were heavy metals and that the control group showed both heavy metals and macroelements required for the body. The average trace elements levels in the two groups were evaluated. In addition, the general role of trace elements in the lung carcinogenic processes was discussed. The study revealed that the carcinogenic processes are significantly affecting the trace elements and the trace elements distribution in the hair of lung cancer patients compared with the healthy controls. It was revealed that there was a relation between lung cancer and trace elements, especially heavy metals. Our findings suggest that the heavy metals accumulated in the body may pose a high risk for lung cancer development.

Keywords Lung cancer · Trace elements · ICP/MS · Scalp hair · Healthy controls

Abbreviations

TE	Trace elements
group 1	Lung cancer patients
group 2	Healthy controls
ICP/MS	Inductively coupled plasma mass spectrometry
NSCLC	Non-small cell lung cancer

Introduction

Lung cancer is the most common cancer in men, and cancer itself is the main cause of death in men worldwide [1]. In Turkey, in particular, its incidence has increased, and lung cancer is now the leading cause of cancer deaths in Turkish men. In the report published in 1997 by Cancer Control Department of the Ministry of Health in our country, it was revealed that lung cancer was the first most common cancer among all types of cancer, with a rate of 17.6%. In our country, in which smoking has a high prevalence, lung cancer remains to be an important public health problem [2]. The fact that tobacco smoking is the cause of this alarming trend is well known. The scientific literature has been paid to presence of heavy metals and/or toxic trace elements in tobacco smoke and their possible effects on biochemical processes in the human body. Chronic adverse effects on human health may, in later years, result from prolonged intake of such toxic elements, some of which are powerful carcinogens [1–7].

A large number of epidemiologic studies have been undertaken to identify potential risk factors for lung cancer, among which the association with trace elements has received considerable attention. Various changes trace element contents in different biological samples taken from patients with lung cancer were found accidentally, and development or initiation of lung cancer has not yet been demonstrated. Determining of total concentrations of the TE in serum and tissues is unlikely to be a sufficiently sensitive or specific marker. What might bring about a change in our knowledge is the speciation of the various forms of trace element found in the organism in relation to lung cancer [4–6, 8–10].

The role of trace elements in the development or inhibition of cancer is still unclear, and numerous investigations have been carried out on this subject [4, 5, 8–19]. Many of these studies have focused on heavy elements inducing carcinogenity. Significant differences in the concentration of various trace elements in the blood or different tissues cancerous patients compared with the healthy control have been reported [8–17].

The speciation analysis of trace element concentrations in hair and measurement of trace element levels may provide more specific information. Hair has been considered a valuable specimen in the assessment of nutritional status, environmental and occupational exposure to toxic chemicals, drug abuse, health status, and many other biological and physiological parameters. The relationship between scalp hair trace element levels and the development of tumors in the human body has been reported in recent literature, thus prompting further investigations in this field. Hair trace elements content have also been linked with the possible future development of disease [9, 13, 15, 18].

In recent years, determining trace elements in biological samples has required the use of sensitive and selective techniques such as inductively coupled plasma mass spectrometry (ICP/MS), using sample preparation strategies addressed to shortening and simplifying the stages previous to analysis. Individuals working in an environment, where exposure to heavy metals or environmental toxicity such as cigarettes is likely and unavoidable, are required by their employer to have their hair, blood, or urine analyzed for trace element toxicity on a regular basis. This monitoring has become a mandatory practice implemented in an effort to protect individuals from environmental toxicity [20–22].

No screening techniques that use standard clinical radiology, sputum cytology, or direct biopsies have been proven adequate for lung cancer detection of markers of the preneoplastic phase of carcinogenesis. Attention to developments in tumor biology has now turned to detection of preneoplastic phase of carcinogenesis. A focus on carcinogenesis shifts emphasis toward detection of individual cellular and genetic markers of potentially reversible progression. Validation of carcinogenesis markers requires marker detection in premalignant specimens from individuals who later develop cancer and the absence of the markers from those who remain cancer-free. The hypothesis of this study was that trace elements in hair may be detected as a biomarker of lung cancer [14–22].

In this prospective research, we studied the hypothetical benefit associated with 36 trace elements identified to cancer patients. The main objectives of the present study were, firstly, the present study was conducted in same areas of Anatolia, Turkey. It investigated the status of 36 trace elements in the scalp hair samples of biopsy-confirmed cancer patients and compared with controls. The second main objective of the present study was to find mutual interrelationships among the selected trace elements in scalp hair.

Materials and Methods

Hair Samples

In order to examine the association between TE expression and lung cancer patients and to compare TE levels with healthy control stage-IIIB non-small cell lung cancer (NSCLC; group 1) were treated at the reference Radiation Oncology center in Kayseri Education and Research Hospital, Kayseri, Turkey. Before the start of this study, all controls and patients were informed about the aim of the study; all agreed to precipitate and signed a consent form.

A prospective evaluation of original patients' or controls' files was performed, collecting the following data: physical details, ethnic origin, healthy, dietary habits, age, clinical and pathologic tumor stage, histology, smoking status, and participation in previous clinical trial. Non-smoking individuals were not included. To ensure uniformity, all patients and controls chosen were from the same ethnic background (Anatolia) and geographic origin (living in central of Turkey). The study protocol and ethical aspects were approved by the Ethics Committee of the Research Council, Dean of Research, Erciyes University School of Medical Sciences.

The inclusion criteria for the patients were: (a) cases of lung cancer proved by histopathology, (b) cases not having undergone any specific treatment for lung cancer, (c) cases not suffering from concomitant diseases such as diabetes mellitus and inflammatory conditions such as rheumatoid arthritis, hypertension, or thyroid and liver disorders. Excluding criteria for patients were: (a) cases with other cancers, (b) cases not taking vitamin or mineral supplements during the past year, (c) cases using hair dye, and (d) cases in women. Neither patients nor controls had used any hair cosmetics for 2 weeks before the hair sampling.

About ~3 g of hair was cut from the nape of the neck close to the scalp, in 1.5–2-cm strands long and placed into polyethylene bags provided by the laboratory performing the analysis. To avoid contamination, the handling, cutting, and transfer of hair samples were minimized and carried out using disposable forceps, sterile stainless-steel scissors, and disposable vinyl examination gloves.

ICP/MS

In our study, after the collection of hair samples, individual hair samples were washed in 5% (*w/v*) detergent solution and were rinsed with abundant distilled water to remove the exogenous material. The samples were dried in an electric oven at 50°C, and then, they were cooled in a dryer with desiccators in silica gel at room temperature. Approximately 1 g of hair sample concentrate (65% 10.0 ml) was mixed with nitric acid, and it was heated at 80°C for 10 min. This mixture was obtained at room temperature. Thereafter, with a gradual heating and addition of perchloric acid 5.0 ml, a white intense smoke was observed. Then, the sample was cooled up to room temperature by gradual cooling and was diluted to 50 mL using distilled water. Blank aliquot that does not contain hair was prepared using the same process. All high-purity reagents (>99.99% with approval) used were purchased from E-Merck. For many trace elements, the limit of measurement was recorded as micrograms per kilogram.

Statistical Analysis

The statistical analysis of the results was done using Excel X State (Microsoft Corp., Redmond, WA) computer software and SPSS 13.0 package. Mean and standard deviations of the data were calculated. The variables were evaluated between the people with and without cancer using Student's *t* test. The statistical significance was considered as $p<0.05$. The correlation between the couples of elements was examined using Pearson correlation test. $r>0.50$ was considered to represent a strong correlation. Thereafter, the variables were evaluated with the analyses of factor and clustering.

Results

A total of 141 subjects were included in the study, and all subjects were male. In group 1, there were 67 males with lung cancer with an age of 42–77 years, and mean age was 57.95

±8.30 years. In group 2, there were 74 healthy males with an age of 42–72 years and mean age was 58.17±6.87.

Tables 1 and 2 show minimum, maximum, standard deviation (SD), mean, skewness, and range for trace elements. In the comparison of two groups, the patients with cancer showed higher levels for 28 elements (Al, Au, B, Ba, Bi, Ca, Ce, Co, Cr, Cs, Cu, Ga, Hg,

Table 1 Lung cancer groups values are given in form of range, minimum, maximum, mean, standard deviation, and skewness value (n=67)

Trace elements	Range	Minimum	Maximum	Mean	Standard deviation	Skewness
Ag	3.53	0.00	3.53	0.547	0.696	2.365
Al	97.88	0.05	97.93	16.460	16.313	2.270
As	7.36	0.01	7.37	0.458	1.2692	5.076
Au	32.21	0.08	32.30	2.744	6.471	3.268
B	8.62	0.00	8.62	1.764	2.058	1.485
Ba	8.68	0.08	8.76	1.461	1.972	2.109
Be	0.09	0.00	0.09	0.012	0.017	4.214
Bi	4.20	0.00	4.20	0.872	0.916	1.268
Ca	246.55	7.12	253.67	68.250	61.327	1.344
Cd	0.88	0.01	0.89	0.209	0.176	1.985
Ce	41.70	0.00	41.70	2.724	8.11	3.823
Co	65.22	0.01	65.23	3.131	11.057	4.381
Cr	22.56	0.05	22.61	2.492	4.0213	3.969
Cs	1.20	0.00	1.21	0.264	0.284	1.160
Cu	58.80	0.12	58.91	24.453	18.465	0.233
Fe	44.57	1.10	45.67	9.652	6.643	2.443
Ga	3.14	0.01	3.15	0.288	0.59	3.176
Hg	5.92	0.01	5.93	1.233	1.367	1.620
K	91.90	0.11	92.00	15.322	18.6550	2.011
Li	3.07	0.03	3.10	0.595	0.67	1.537
Mg	158.05	0.88	158.93	28.920	24.2039	2.467
Mn	16.31	0.10	16.41	1.819	2.16	4.949
Na	233.80	0.45	234.24	23.316	30.458	5.248
Ni	2.78	0.13	2.91	1.126	0.800	0.682
Pb	162.95	0.21	163.16	8.577	19.876	7.344
Pd	0.70	0.01	0.72	0.157	0.173	1.477
Rb	5.05	0.00	5.05	0.470	0.7534	3.796
Rh	1.93	0.00	1.93	0.555	0.614	0.796
Sb	2.02	0.02	2.04	0.988	0.77	0.156
Sc	3.14	0.03	3.16	0.134	0.545	5.380
Se	99.81	0.09	99.90	13.703	19.784	2.378
Sn	144.98	0.14	145.12	33.939	24.72	1.632
Sr	7.25	0.00	7.25	1.910	1.9146	1.275
Ti	96.06	0.16	96.22	12.638	19.128	2.127
V	10.05	0.03	10.08	1.835	2.290	2.142
Zn	258.37	3.41	261.79	53.226	60.301	1.478

Table 2 Control groups values are given in form of range, minimum, maximum, mean, standard deviation, and skewness value (n=74)

Trace elements	Range	Minimum	Maximum	Mean	Standard deviation	Skewness
Ag	7.78	0.01	7.78	0.722	1.416	3.538
Al	56.05	0.17	56.22	11.366	12.685	1.831
As	2.94	0.01	2.95	0.558	0.742	1.862
Au	6.52	0.00	6.52	0.687	1.218	3.501
B	9.14	0.00	9.14	1.136	1.915	2.813
Ba	7.71	0.00	7.71	1.396	1.513	1.605
Be	0.37	0.00	0.37	0.038	0.074	2.933
Bi	2.42	0.00	2.42	0.445	0.718	2.014
Ca	89.44	8.90	98.34	30.812	18.809	1.867
Cd	1.80	0.00	1.80	0.316	0.426	1.972
Ce	21.59	0.01	21.60	0.958	2.886	5.774
Co	1.78	0.01	1.79	0.392	0.467	1.154
Cr	6.91	0.08	6.99	0.934	1.016	3.161
Cs	2.12	0.00	2.12	0.246	0.384	3.493
Cu	116.88	0.12	117.00	15.753	16.727	3.373
Fe	97.87	0.25	98.12	25.052	22.929	1.630
Ga	0.91	0.01	0.92	0.250	0.233	1.134
Hg	4.02	0.00	4.02	0.585	0.713	2.121
K	32.08	0.07	32.15	10.701	9.252	.820
Li	2.37	0.03	2.40	0.571	0.586	1.025
Mg	89.54	0.86	90.40	31.921	21.315	1.104
Mn	8.26	0.04	8.30	1.144	1.119	3.756
Na	73.89	0.11	74.00	21.378	15.798	0.799
Ni	2.30	0.06	2.36	0.580	0.547	1.794
Pb	22.38	0.00	22.38	5.090	6.198	1.751
Pd	1.36	0.00	1.36	0.114	0.192	4.195
Rb	0.98	0.00	0.99	0.251	0.300	1.271
Rh	1.93	0.00	1.93	0.414	0.486	1.657
Sb	2.03	0.01	2.04	0.235	0.344	3.131
Sc	0.00	0.03	0.03	0.027	0.000	0.000
Se	78.81	0.17	78.98	20.135	21.042	1.484
Sn	55.84	0.14	55.98	21.509	19.478	0.643
Sr	5.40	0.00	5.40	1.455	1.678	1.324
Ti	20.35	0.03	20.38	2.079	4.464	2.918
V	9.45	0.03	9.48	1.047	1.742	3.537
Zn	320.69	11.30	331.99	109.763	95.334	0.971

K, Li, Mn, Na, Ni, Pb, Pd, Rb, Rh, Sb, Sc, Sn, Sr, Ti, and V) compared with the control group. In addition, the control group showed higher levels for eight elements (Ag, As, Be, Cd, Fe, Mg, Se, and Zn) compared with the group with cancer (Table 3).

In group 1, the highest trace elements were measured to be Ca, Zn, Sn, Na, and Mg, respectively, and they were quantified as 68.250, 53.220, 33.939, 23.316, and

Table 3 Groups values are given in form of mean±SD, *p* value ($p<0.05$)

Trace elements	Group 1 Mean±SD	Group 2 Mean±SD	Signature ($p<0.05$)
Ag	0.547±0.696	0.722±1.416	0.034
Al	16.460±16.313	11.366±12.685	0.182
As	0.458±1.269	0.558±0.742	0.835
Au	2.744±6.471	0.687±1.218	0.000
B	1.764±2.058	1.136±1.915	0.078
Ba	1.461±1.972	1.396±1.513	0.255
Be	0.012±0.017	0.038±0.074	0.000
Bi	0.872±0.916	0.445±0.718	0.007
Ca	68.250±61.327	30.812±18.809	0.000
Cd	0.209±0.176	0.316±0.426	0.000
Ce	2.724±8.110	0.958±2.886	0.002
Co	3.131±11.057	0.392±0.467	0.000
Cr	2.492±4.021	0.934±1.016	0.000
Cs	0.264±0.284	0.246±0.384	0.799
Cu	24.453±18.465	15.753±16.727	0.001
Fe	9.652±6.643	25.052±22.929	0.000
Ga	0.288±0.590	0.250±0.233	0.003
Hg	1.233±1.367	0.585±0.713	0.000
K	15.322±18.655	10.701±9.252	0.000
Li	0.595±0.670	0.571±0.586	0.432
Mg	28.920±24.203	31.921±21.315	0.493
Mn	1.8193±2.160	1.144±1.119	0.058
Na	23.316±30.458	21.378±15.798	0.242
Ni	1.126±0.800	0.580±0.547	0.000
Pb	8.577±19.876	5.090±6.198	0.246
Pd	0.157±0.173	0.114±0.192	0.485
Rb	0.470±0.753	0.251±0.300	0.001
Rh	0.555±0.614	0.414±0.486	0.001
Sb	0.988±0.770	0.235±0.344	0.000
Sc	0.134±0.545	0.027±0.000	0.001
Se	13.703±19.784	20.135±21.042	0.102
Sn	33.939±24.720	21.509±19.478	0.872
Sr	1.910±1.914	1.455±1.678	0.640
Ti	12.638±19.128	2.079±4.464	0.000
V	1.835±2.290	1.047±1.742	0.016
Zn	53.22±60.301	109.763±95.334	0.000

28.920 μg.kg^{-1}, respectively. In group 1, mean levels of trace elements in the hair samples were as follows in descending order: Ca>Zn>Sn>Na>Mg>Cu>K>Se>Al>Ti>Pb>Mn>Fe>Co>Ce>Au>Cr>Sr>B>Ba>Hg>Ni>Bi>Sb>As>Li>Ag>Rb>Rh>Ga>Sc>Cs>Cd>Pd>Be (Table 1).

In group 2, the highest trace elements were Zn, Mg, Ca, Fe, and Se, respectively, and they were quantified as 109.763, 31.921, 30.812, 25.052, and 20.135 μg.kg^{-1}. In group 1, mean levels of trace elements in the hair samples were as follows in descending order: Zn>Mg>Ca>Fe>Se>Sn>Na>Cu>Al>K>Pb>Ti>Ce>Sr>B>Ba>V>Mn>Ag>-Cr>Au>As>Hg>Bi>Li>Ni>Rh>Co>Cd>Cs>Sb>Rb>Ga>Pd>Be>Sc (Table 2). In group 1, Ca, Sn, and Na were 2.03, 1.06, and 1.01 times higher, respectively, compared with group 2. In group 2, Zn, Mg, Fe, and Se were 2, 1.01, 2.7, and 1.6 times higher, respectively, compared with group 1 (Table 3).

When the levels of trace elements were compared between groups 1 and 2 using Student's *t* test, the levels of Ag, Au, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Fe, Ga, Hg, K, Ni, Rb, Rh, Sb, Sc, Ti, and Zn were found to be statistically different ($p<0.05$). The levels measured for Al, As, B, Ba, Cs, Li, Mg, Na, Pb, Pd, Se, Sn, and Sr did not show a statistically significant difference ($p>0.05$). The significant results were obtained for Au, Bi, Ca, Ce, Co, Cr, Cu, Ga, Hg, K, Mn, Ni, Rb, Rh, Sb, Sc, Ti, and V in group 1 and for Ag, Be, Cd, Fe, and Zn in group 2 ($p<0.01$). Table 3 shows arithmetic mean, standard deviation, and *p* values.

In each group, Pearson analysis was performed to investigate the correlation between the trace elements with the couples of element within the same group. It was considered according to positivity or negativity of r. $r>0.50$ was considered as strong correlation. The level of correlation of 0.01 was considered as significant. Tables 4 and 5 show the correlations of trace elements, respectively.

In Table 4, strong correlations were found for the following couples of trace elements in the patients with lung cancer: Be-B ($r=0.354$), Cd-Ag ($r=0.676$), Co-As ($r=0.368$), Cr-As ($r=0.858$), Cr-Co ($r=0.489$), Cs-Ag ($r=0.317$), Cs-Cd ($r=0.633$), Cu-Ca ($r=0.440$), Ga-Co ($r=0.350$), Ga-Cs ($r=0.446$), Hg-Au ($r=0.511$), K-Cd ($r=0.355$), Li-Ag ($r=0.522$), Li-Ba ($r=0.325$), Li-Cd ($r=0.730$), Li-Cs ($r=0.594$), Mn-Ca ($r=0.412$), Ni-B ($r=0.463$), Pb-B ($r=0.441$), Pd-Ba ($r=0.387$), Pd-Cd ($r=0.421$), Pd-Cs ($r=0.437$), Pd-Ga ($r=0.366$), Rh-Ag ($r=0.519$), Rb-Cd ($r=0.558$), Rb-Cs ($r=0.588$), Rb-Li ($r=0.345$), Sn-As ($r=0.390$), Sn-Ca ($r=0.344$), Sn-Cr ($r=0.402$), Sn-Sc ($r=0.415$), Ti- Ce ($r=0.493$), Ti-Na ($r=0.348$), V-Ag ($r=0.334$). A negative correlation was found between Ni-Bi ($r=-0.346$). The strongest positive correlation was between Cr-As ($r=0.858$).

In Table 5, in the control group, the trace elements with strong correlations were as follows: Ba-Al ($r=0.483$), Ba-Al ($r=0.324$), Be-B ($r=0.571$), Cd-Al ($r=0.383$), Cd-As ($r=0.566$), Ce-As ($r=0.421$), Ce-Cd ($r=0.526$), Co-Al ($r=0.310$), Co-As ($r=0.637$), Co-Cd ($r=0.686$), Co-Ce ($r=0.460$), Cr-Al ($r=0.482$), Cr-Ba ($r=0.423$), Cr-Cd ($r=0.361$), Cs-Cd ($r=0.579$), Cs-Co ($r=0.638$), Ga-Cs ($r=0.343$), Hg-Ag ($r=0.342$), Hg-Al ($r=0.328$), Hg-Au ($r=0.313$), Hg-Ca ($r=0.406$), Hg-Cr ($r=0.482$), Hg-Cu ($r=0.306$), K-B ($r=0.483$), K-Be ($r=0.411$), Li-Cd ($r=0.433$), Li-Co ($r=0.412$), Mn-Al ($r=0.419$), Mn-Ba ($r=0.383$), Mn-Ca ($r=0.311$), Mn-Cr ($r=0.745$), Mn-Hg ($r=0.534$), Na-Fe ($r=0.336$), Na-Ga ($r=0.412$), Pb-Ag ($r=0.431$), Pb-Au ($r=0.474$), Pb-Ca ($r=0.349$), Pb-Hg ($r=0.609$), Pb-Mn ($r=0.388$), Pd-Co ($r=0.330$), Pd-Cs ($r=0.489$), Rb-Al ($r=0.325$), Rb-Ba ($r=0.532$), Rb-Cd ($r=0.305$), Rb-Cu ($r=0.379$), Rb-Mn ($r=0.372$), Sb-Rh ($r=0.387$), Se-Rh ($r=0.338$), Sn-B ($r=0.379$), Sn-Co ($r=0.414$), Sn-K ($r=0.364$), Sr-Li ($r=0.308$), Sr-Rb ($r=0.355$), Zn-Al ($r=0.301$), and Zn-Ga ($r=0.306$). Negative correlation was between Sn-Hg ($r=-0.474$) and Na-Ca ($r=-0.374$). The strongest positive correlation was found between Mn-Cr ($r=0.745$).

The results that were not found to be statistically significant according to Pearson correlation were Ag, Al, As, Au, B, Ba, Bi, Ca, Ce, Fe, Mg, Na, Rh, Sb, Sc, Se, Sr, and Zn in group 1 and Ag, Al, As, B, Bi, Ca, Cu, Fe, Mg, Ni, Rh, Sc, Ti, and V in group 2. When

Table 4 Correlation coefficient matrix of trace elements in the scalp hair of lung cancer patients (n=67)

*	Ag	Al	As	Au	B	Ba	Be	Bi	Ca	Cd	Ce	Co	Cr	Cs	Cu	Fe	Ga	Hg	K
Ag	1																		
Al	0.218	1																	
As	−0.008	−0.190	1																
Au	0.145	−0.061	−0.082	1															
B	0.183	0.260*	0.266*	−0.095	1														
Ba	0.081	0.245*	−0.095	−0.075	0.113	1													
Be	−0.112	−0.059	0.098	−0.085	0.354**	0.082	1												
Bi	−0.124	−0.179	−0.201	−0.004	−0.260*	0.069	−0.028	1											
Ca	−0.098	−0.220	0.270*	0.267*	0.062	−0.107	0.182	0.126	1										
Cd	0.676**	0.210	0.054	−0.001	0.296*	0.294*	−0.030	−0.079	−0.009	1									
Ce	−0.059	−0.112	−0.017	−0.040	−0.155	−0.083	−0.029	−0.119	0.111	0.065	1								
Co	0.066	−0.183	0.368**	−0.071	0.003	−0.177	−0.047	−0.050	0.034	0.144	−0.032	1							
Cr	0.050	−0.200	0.858**	−0.020	0.076	−0.207	−0.0.039	−0.162	0.156	0.018	−0.093	0.489**	1						
Cs	0.317**	0.143	0.083	−0.176	0.142	0.240	−0.086	−0.008	−0.134	0.633**	0.137	0.141	−0.008	1					
Cu	−0.211	−0.033	−0.149	0.203	−0.113	−0.139	0.224	0.091	0.440**	−0.058	0.306*	−0.093	−0.187	−0.082	1				
Fe	0.014	0.066	−0.062	−0.140	−0.113	0.013	−0.143	−0.149	−0.251*	−0.097	0.109	−0.179	−0.108	0.132	−0.114	1			
Ga	0.126	0.104	−0.009	−0.108	0.069	0.137	−0.064	−0.054	−0.189	0.295*	−0.123	0.350**	0.066	0.446**	−0.197	−0.049	1		
Hg	0.291*	0.239	−0.111	0.511**	−0.028	0.201	0.136	−0.041	0.072	0.164	−0.032	−0.108	−0.168	−0.043	−0.016	−0.005	0.033	1	
K	0.297*	0.067	−0.027	−0.114	0.292*	0.160	0.221	0.018	0.015	0.335**	−0.160	−0.012	−0.211	0.082	−0.005	−0.043	0.052	0.086	1
Li	0.522**	0.244*	0.103	−0.037	0.264*	0.325**	−0.085	−0.048	−0.142	0.730**	−0.025	−0.186	−0.027	0.594**	−0.130	0.009	0.287*	0.094	0.245*
Mg	0.201	0.221	0.020	−0.121	0.096	0.103	−0.029	0.021	−0.005	0.143	−0.075	−0.131	−0.032	0.118	0.000	0.022	0.000	0.127	0.053
Mn	0.165	0.181	0.051	0.295*	0.045	0.089	0.063	0.069	0.412**	0.301*	0.021	−0.106	−0.032	−0.016	0.250*	−0.112	0.023	0.238	0.067
Na	−0.066	−0.032	0.058	0.008	−0.064	−0.057	0.152	−0.093	0.181	−0.039	0.045	−0.042	0.020	−0.086	−0.100	−0.004	−0.007	0.065	−0.082
Ni	0.219	0.126	0.027	0.124	0.463**	−0.044	0.264*	−0.346**	0.058	0.139	0.194	−0.120	−0.127	0.097	0.144	−0.086	−0.006	0.031	0.088
Pb	−0.087	−0.037	−0.100	0.021	−0.134	−0.023	−0.034	0.441**	0.008	−0.117	0.036	−0.069	−0.088	−0.113	0.047	−0.129	−0.098	−0.078	−0.107
Pd	0.290*	0.145	−0.004	−0.043	−0.061	0.387**	−0.055	0.143	−0.142	0.421**	−0.016	0.268*	0.027	0.437**	−0.195	0.054	0.366**	0.141	0.134
Rb	0.519**	0.045	−0.082	0.013	0.026	0.116	−0.052	−0.076	−0.195	0.558**	0.248*	0.262*	−0.071	0.488**	−0.129	0.067	0.088	0.069	0.188

Table 4 (continued)

*	Ag	Al	As	Au	B	Ba	Be	Bi	Ca	Cd	Ce	Co	Cr	Cs	Cu	Fe	Ga	Hg	K
Rh	−0.069	0.235	−0.091	−0.173	0.006	−0.011	0.001	−0.052	−0.278*	−0.021	0.258*	−0.123	−0.154	0.088	0.105	0.069	−0.051	−0.103	−0.018
Sb	−0.098	−0.107	0.026	−0.123	−0.052	0.175	−0.077	0.052	0.050	0.026	0.013	−0.192	−0.112	0.034	0.052	0.070	−0.181	−0.080	0.040
Sc	0.020	−0.048	−0.046	−0.067	0.000	0.222	−0.039	0.115	0.172	0.026	−0.055	−0.052	−0.041	−0.005	0.019	−0.060	0.219	0.117	0.019
Se	−0.069	−0.092	−0.029	0.294*	−0.044	0.127	0.168	0.157	0.181	−0.009	0.020	0.026	0.032	−0.141	0.141	−0.072	−0.131	0.033	−0.028
Sn	0.212	−0.087	0.390**	−0.017	0.018	−0.004	−0.107	0.014	0.344**	0.217	−0.155	0.308*	0.402**	0.100	0.041	−0.212	0.088	−0.155	−0.043
Sr	−0.055	0.144	−0.068	−0.090	−0.014	0.110	0.019	0.133	−0.102	0.040	−0.047	0.033	−0.104	0.014	−0.115	−0.075	0.151	−0.001	−0.046
Ti	−0.087	−0.070	−0.067	−0.157	−0.107	0.051	−0.010	−0.125	−0.117	0.048	0.493**	−0.141	−0.057	−0.021	−0.008	0.056	−0.112	−0.087	−0.141
V	0.334**	−0.014	−0.058	0.064	−0.098	−0.027	0.032	0.060	0.209	0.173	0.065	0.108	0.094	−0.100	0.193	−0.121	−0.092	−0.052	0.058
Zn	−0.211	−0.208	−0.102	0.146	−0.045	−0.107	0.035	−0.176	0.178	−0.265*	−0.044	−0.088	−0.092	−0.283*	−0.038	−0.108	−0.149	0.022	−0.059

*	Li	Mg	Mn	Na	Ni	Pb	Pd	Rb	Rh	Sb	Sc	Se	Sn	Sr	Ti	V	Zn
Ag																	
Al																	
As																	
Au																	
B																	
Ba																	
Be																	
Bi																	
Ca																	
Cd																	
Ce																	
Co																	
Cr																	
Cs																	
Cu																	
Fe																	

Ga																	
Hg																	
K																	
Li	1																
Mg	0.205	1															
Mn	0.261*	0.156	1														
Na	0.054	−0.008	0.111	1													
Ni	0.084	0.022	0.113	−0.109	1												
Pb	−0.144	0.124	0.068	−0.098	−0.156	1											
Pd	0.262*	−0.018	0.186	−0.094	−0.114	0.122	1										
Rb	0.345**	−0.016	0.011	−0.048	−0.001	−0.110	0.176	1									
Rh	0.129	0.067	−0.104	0.123	0.019	−0.076	−0.038	0.055	1								
Sb	0.072	0.191	0.096	−0.084	−0.027	0.144	0.285*	−0.148	−0.085	1							
Sc	0.048	0.041	0.002	−0.009	0.020	−0.035	0.056	0.005	−0.128	0.061	1						
Se	−0.046	−0.265*	0.071	0.085	0.123	−0.009	0.040	−0.092	−0.110	−0.062	−0.089	1					
Sn	0.213	0.067	0.041	0.072	0.086	−,151	0.007	0.055	−0.043	−0.081	0.415**	0.115	1				
Sr	0.020	0.039	0.236	0.188	−0.002	0.281*	0.166	−0.026	0.048	−0.133	−0.015	−0.023	0.025	1			
Ti	−0.059	0.003	−0.072	0.348**	0.133	−0.036	0.052	−0.048	0.253*	0.115	−0.100	0.133	−0.172	0.207	1		
V	−0.030	−0.203	0.113	−0.076	0.126	−0.023	0.103	0.265*	−0.183	0.129	0.165	0.307*	0.158	−0.200	−0.038	1	
Zn	−0.260*	−0.102	−0.045	0.023	−0.108	−0.007	−0.236	−0.202	−0.167	−0.029	0.099	−0.083	−0.221	−0.049	−0.197	−0.001	1

*p=0.05 correlation is significant (two-tailed)

**p=0.01 correlation is significant (two-tailed)

Table 5 Correlation coefficient matrix of trace elements in the scalp hair of control group (n=74)

*	Ag	Al	As	Au	B	Ba	Be	Bi	Ca	Cd	Ce	Co	Cr	Cs	Cu	Fe	Ga	Hg	K
Ag	1																		
Al	0.054	1																	
As	−0.090	0.165	1																
Au	0.483**	0.078	−0.030	1															
B	−0.025	−0.198	0.281*	−0.190	1														
Ba	−0.128	0.324**	−0.011	0.179	−0.190	1													
Be	−0.034	−0.223	−0.018	−0.184	0.571**	−0.120	1												
Bi	0.025	−0.207	−0.087	−0.110	−0.107	0.000	0.056	1											
Ca	−0.024	0.153	−0.225	0.279*	−0.095	0.189	−0.029	−0.164	1										
Cd	0.037	0.383**	0.566**	0.112	−0.060	0.294*	0.011	0.080	−0.127	1									
Ce	−0.045	0.207	0.421**	0.003	−0.081	0.099	−0.059	−0.054	−0.129	0.526**	1								
Co	−0.116	0.310**	0.637**	−0.050	0.069	0.051	−0.018	−0.061	−0.192	0.686**	0.460**	1							
Cr	−0.035	0.482**	0.134	0.099	−0.208	0.423**	−0.173	0.111	0.295*	0.361**	0.142	0.271*	1						
Cs	0.007	0.121	0.286*	0.091	0.008	0.068	−0.013	0.131	−0.068	0.579**	0.205	0.638**	0.270*	1					
Cu	0.019	0.118	0.010	0.066	0.019	0.127	0.031	−0.186	0.061	0.101	0.060	−0.008	0.211	−0.051	1				
Fe	0.052	0.126	0.026	−0.022	−0.259*	−0.068	−0.136	0.121	−0.069	0.016	0.103	0.078	−0.037	−0.117	−0.163	1			
Ga	0.130	0.011	0.101	0.129	−0.011	0.002	−0.090	−0.077	−0.097	0.120	−0.037	0.118	−0.011	0.343**	−0.133	0.050	1		
Hg	0.342**	0.328**	−0.164	0.313**	−0.226	0.142	−0.216	−0.197	0.406**	−0.062	−0.128	−0.244*	0.482**	−0.181	0.306**	−0.075	0.031	1	
K	0.152	−0.140	0.038	−0.069	0.483**	−0.186	,411**	−0.087	−0.101	−0.122	−0.127	0.019	−0.234*	0.174	−0.079	−0.194	0.262*	−0.085	1
Li	0.261*	0.245*	0.450**	0.124	0.034	0.031	0.102	−0.172	−0.129	0.433**	0.108	0.412**	−0.029	0.135	0.103	0.023	−0.068	0.044	−0.041
Mg	0.169	−0.061	−0.096	0.195	−0.160	−0.042	−0.252*	−0.064	0.149	−0.088	−0.142	−0.034	−0.048	0.025	0.169	0.012	0.103	0.168	0.072
Mn	0.104	0.419**	0.002	0.172	−0.105	0.383**	−0.047	−0.098	0.311**	0.246*	−0.018	0.100	0.745**	0.208	0.254*	−0.240*	−0.065	0.534**	−0.012
Na	0.122	0.069	0.006	0.029	−0.060	−0.141	−0.125	0.072	−0.374**	0.028	−0.021	0.158	−0.035	0.169	−0.054	0.336**	0.412**	−0.100	−0.055
Ni	0.045	−0.092	0.076	0.124	0.125	0.138	0.040	−0.041	0.200	0.016	0.130	−0.004	−0.036	0.054	0.007	−0.154	−0.001	0.031	−0.009
Pb	0.431**	0.287*	−0.108	0.474**	−0.211	0.298*	−0.183	−0.088	0.349**	0.161	0.033	−0.091	0.275*	0.072	0.184	−0.085	0.218	0.609**	0.041
Pd	−0.058	0.074	0.153	−0.008	0.115	0.207	0.153	0.162	0.076	0.282*	0.161	0.330**	0.247*	0.489**	−0.064	−0.214	0.150	−0.047	0.137
Rb	−0.011	0.325**	0.177	−0.098	0.137	0.532**	0.096	−0.261*	0.070	0.305**	0.097	0.107	0.297*	0.024	0.379**	−0.225	−0.160	0.193	0.045

Rh	0.164	−0.215	0.028	−0.180	0.106	−0.069	0.157	0.197	−0.055	0.006	−0.034	−0.025	−0.165	0.006	−0.223	0.094	0.047	−0.152	0.157
Sb	0.089	−0.064	0.153	−0.068	−0.061	−0.003	−0.049	0.089	−0.198	0.053	0.067	0.186	0.080	0.181	−0.229*	−0.050	0.125	−0.079	0.124
Sc	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Se	−0.100	−0.281*	−0.079	−0.187	−0.057	−0.211	0.079	0.209	−0.131	−0.051	−0.073	−0.113	−0.178	−0.053	−0.243*	−0.032	−,055	−0.269*	−0.131
Sn	−0.083	0.036	0.237*	−0.225	0.379**	−0.297*	0.203	0.051	−0.226	0.178	0.118	0.414**	−0.205	0.227	−0.206	0.173	0.126	−0.474**	0.364**
Sr	−0.075	0.247*	0.177	−0.154	0.202	0.083	0.150	−0.238*	0.133	0.210	0.142	0.290*	0.290*	0.011	0.138	−0.138	−0.237*	0.044	−0.082
Ti	−0.017	0.144	−0.157	0.052	−0.087	0.093	−0.055	−0.053	−0.019	−0.080	−0.077	−0.137	−0.110	−0.148	−0.149	−0.046	−0.086	−0.122	0.072
V	−0.128	0.138	0.027	0.199	−0.053	0.109	−0.057	−0.039	0.124	0.048	−0.012	−0.016	0.163	0.132	−0.080	0.104	0.108	0.031	0.046
Zn	0.005	0.301**	0.015	−0.155	−0.035	0.062	−0.136	−0.060	0.029	−0.039	−0.062	0.060	0.169	0.002	0.062	0.020	0.306**	0.111	0.189
*	Li	Mg	Mn	Na	Ni	Pb	Pd	Rb	Rh	Sb	Sc	Se	Sn	Sr	Ti	V	Zn		
Ag																			
Al																			
As																			
Au																			
B																			
Ba																			
Be																			
Bi																			
Ca																			
Cd																			
Ce																			
Co																			
Cr																			
Cs																			
Cu																			
Fe																			
Ga																			
Hg																			
K																			

Table 5 (continued)

*	Li	Mg	Mn	Na	Ni	Pb	Pd	Rb	Rh	Sb	Sc	Se	Sn	Sr	Ti	V	Zn
Li	1																
Mg	0.058	1															
Mn	−0.030	−0.095	1														
Na	0.039	0.075	−0.108	1													
Ni	0.015	−0.025	−0.033	−0.021	1												
Pb	0.068	0.139	0.388**	−0.111	0.130	1											
Pd	0.077	−0.004	0.177	−0.115	0.271*	0.126	1										
Rb	0.273*	−0.087	0.372**	−0.279*	0.094	0.160	0.177	1									
Rh	0.003	0.039	−0.065	0.120	0.217	−0.107	0.246*	−0.043	1								
Sb	0.074	0.095	0.002	0.170	0.264*	0.008	0.282*	−0.118	0.387**	1							
Sc	a	a	a	a	a	a	a	a	a	a	a						
Se	−0.147	−0.059	−0.129	−−0.057	−0.083	−0.209	0.066	−0.190	0.338**	,232*	a	1					
Sn	0.065	−0.094	−0.169	0.134	−0.108	−0.229*	0.201	−0.236*	0.038	0.049	a	−0.011	1				
Sr	0.308**	−0.137	0.278*	−0.219	−0.046	−0.081	0.199	0.355**	−0.032	0.046	a	0.177	0.120	1			
Ti	−−0.168	0.043	−0.066	−0.127	−0.152	0.042	−0.094	−0.104	0.083	0.099	a	0.164	0.102	0.105	1		
V	−0.012	−0.065	0.147	−0.051	−0.014	0.004	0.110	−0.013	−0.103	−0.006	a	−0.052	−0.072	−0.062	−0.105	1	
Zn	−0.097	0.077	0.201	0.119	−0.080	0.095	0.020	0.043	0.115	0.074	a	0.031	0.030	0.046	0.103	−0.023	1

a cannot be computed because at least one of the variables is constant

*p=0.05 correlation is significant at the 0.05 level (two-tailed)

**p=0.01 correlation is significant (two-tailed) the difference between two groups was examined, the trace elements that showed correlation in group 1 but not in group 2 were Cu, Ni, Rh, Ti, and V. The trace elements that showed correlation in group 2 but not in group 1 were Ba, Ce, Na, Sb, Se, Sr, and Zn. In the coupled matching, it was seen that the number and the strength of the correlations were higher in group 2 compared with group 1 (Tables 4 and 5).

A factor analysis was performed to convert the trace elements found to be correlated using Pearson correlation to new data structures which are independent and in less numbers (Tables 6, 7, 8, and 9). Table 6 shows self-value and variance shares declared for the factors obtained from the variables of trace elements in the patients with lung cancer. When the factor analysis was performed for a total of 36 trace elements used in the study, ten factors had a self-value higher than 1.67, and it was seen that these factors explained 63.35% of the total variance. Bartlett's test of sphericity was found to be $p<0.000$. Using the factors obtained by converted matrix, the representation coupling between the factors and the variables was examined. Table 7 contains the factors obtained by converted matrix and their variables. As seen in Table 7, majority of the variables (21 of 36 variables) accumulate in the first, second, and third factors. For group 1, the following results were obtained: first factor, ten variables (Cd, Li, Cs, Ag, Rb, Pd, Ga, Zn, Al, and K); second factor, seven variables (Cr, As, Sn, Co, Ca, Rh, and Fe); and third factor, four variables (Mn, Au, Cu, and Hg). As seen in Table 6, first, second, and third factors explain a part of 25.2% of the total change. Within the first factor that best describes the overall change, the most important variables are Cd and Li, respectively the first and the second factors. In the canonic correlation, when the first function took a high level with 0.878, a good correlation was suggested between the first discriminating function and the set of variables. Within the second factor, the most important variables were Cr and As.

Table 8 shows the self-value and variance shares explained for trace element variables in healthy people; Table 9 shows the distribution of the variables by the factor. In the factor analysis performed in healthy people (group 2), the factor analysis of 35 trace elements, except Sc, was performed, and ten factors were obtained. It was seen that these factors explained 67.04% of the total variance. Kaiser–Meyer–Olkin Measure of Sampling Adequacy was found to be 0.543 and Bartlett's test of sphericity was found to be $p<0.000$. As seen in Table 9, majority of the variables (19 of 34 variables) accumulate in the first, second, and third factors.

Table 6 Principal component loadings for trace elements in the scalp hair of lung cancer patients

Component	Rotation sums of squared loadings		
	Total	Percent of variance	Cumulative percent
1	4,126	11,461	11,461
2	2,747	7,630	19,091
3	2,231	6,198	25,289
4	2,156	5,990	31,279
5	2,141	5,948	37,227
6	2,013	5,592	42,819
7	1,979	5,497	48,316
8	1,963	5,453	53,769
9	1,778	4,939	58,708
10	1,671	4,642	63,350

Values are given in form of percent of variance, cumulative percent, and total variance

Extraction method: principal component analysis

Table 7 Principal component loadings for trace elements in the scalp hair of lung cancer patients

	Component									
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
Cd	0.878									
Li	0.783									
Cs	0.724									
Ag	0.722									
Rb	0.588									
Pd	0.539									
Ga	0.460									
Zn	−0.403									
Al	0.376									
K	0.364									
Cr		0.759								
As		0.704								
Sn		0.639								
Co		0.566								
Ca		0.557								
Rh		−0.380								
Fe		−0.349								
Mn			0.568							
Au			0.556							
Cu			0.528							
Hg			0.472							
Bi				−0.673						
Ni				0.586						
B				0.492						
Pb				−0.484						
Ce					0.707					
Ti					0.599					
Sr					0.057					
V					0.321					
Sb						0.232				
Na							0.454			
Be								0.516		
Mg								−0.476		
Se								0.380		
Ba									0.448	
Sc										0.720

Extraction method: principal component analysis
Ten components extracted

For group 2, the following results were obtained: first factors, six variables Cr, Mn, Al, Ba, Rb, and Pb; second factor, eight variables Co, As, Sn, Cd, Hg, Cs, Ca, and Ce; third factor, five variables Na, Ga, Be, B, and Sr; and fourth factor, two variables K and Ag. First, second, third,

Table 8 Principal component loadings for trace elements in the scalp hair of control groups

Component	Rotation sums of squared loadings		
	Total	Percent of variance	Cumulative percent
1	3,913	11,179	11,179
2	3,595	10,272	21,451
3	2,558	7,309	28,760
4	2,538	7,252	36,012
5	2,211	6,317	42,329
6	2,016	5,759	48,088
7	1,792	5,120	53,207
8	1,701	4,860	58,067
9	1,593	4,551	62,617
10	1,556	4,447	67,064

Values are given in form of percent of variance range, cumulative percent, and total variance

Extraction method: principal component analysis

and fourth factors explain the 36% of the overall change. Within the first factor that best describe the overall change, the most important variables were Cr and Mn. In the canonic correlation, when the first function took a high level with 0.756, a good correlation was suggested between the first discriminating function and the set of variables.

Thereafter, clustering analysis was performed. Clustering analysis is the method used to create subsets of the variables which are similar to each other. Figures 1 and 2 show the clusters of trace elements formed after the clustering analysis realized using the variables. In group 1, cluster 1 included Ag, Al, Ba, Cd, Cs, Ga, Li, Mg, Pd, and Rb; cluster 2 included As, Co, Cr, Sc, Sn; cluster 3 included Au, Ca, Cu, Mn, Hg; cluster 4 included B, K, Be, Ni; cluster 5 included Bi, Pb, and Sr; cluster 6 included Ce, Rh, Na, and Ti; cluster 7 included Fe; cluster 8 included Sb; cluster 9 included Se and V, and cluster 10 included Zn. In group 2, cluster 1 included Ag, Al, Au, Ba, Ca, Cr, Cu, Hg, Mn, Pb, and Rb; cluster 2 included As, Cd, Ce, Co, Cs, Li, Pd, and Sr; cluster 3 included; cluster 4 included Bi, Rh, Sb, and Se; cluster 5 included Fe; cluster 6 included Ga, Na, and Zn; cluster 7 included Mg; cluster 8 included Ni; cluster 9 included Ti, and cluster 10 included V.

It was seen that trace elements of the first, second, and third factors obtained in the factor analysis corresponded to the clustering analysis. In the group with lung cancer, Zn in the first factor, Ca, Rh, and Fe in the second factor and Bi and Pb in fourth factor changed group after the clustering analysis. Accurate rate of grouping for trace elements was 66.6%. In the control group, Mn and Rb in the first factor, Pb and Rh in the second factor, and Sn in the third factor changed group after the clustering analysis. Accurate rate of grouping for trace elements was 81%. In this analysis, we observed that the trace elements accumulated were heavy metals and that the control group showed both heavy metals and macroelements required for the body.

Discussion

Lung cancer is a complicated, multifactorial, and multifaceted disease. Smoking is considered a major risk factor of NSCLC, but factors such as occupational or environmental exposure, household radon, and certain dietary constituents may also have important impact

Table 9 Principal component loadings for trace elements in the scalp hair of control groups

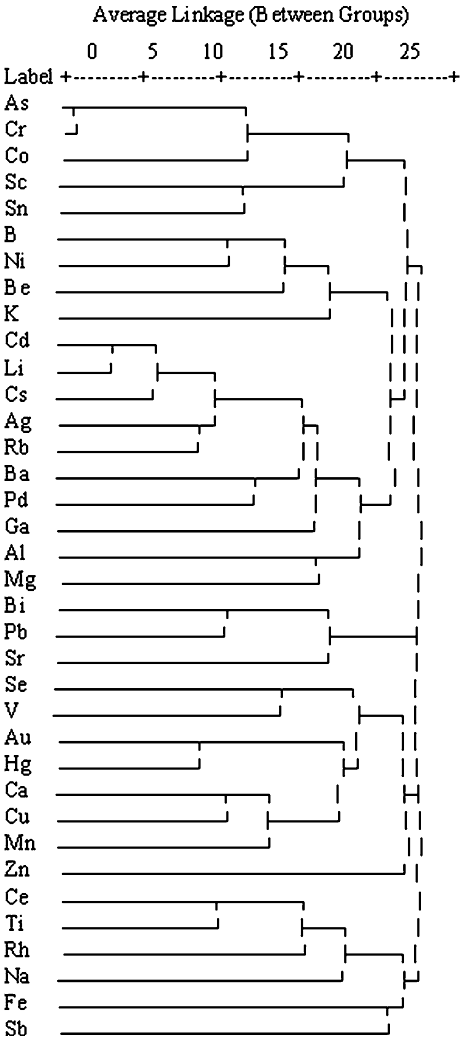
	Component									
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
Cr	0.756									
Mn	0.714									
Al	0.661									
Ba	0.572									
Rb	0.562									
Pb	0.554									
Co		0.783								
As		0.668								
Sn		0.618								
Cd		0.598								
Hg		−0.582								
Cs		0.568								
Ca		−0.451								
Ce		0.441								
Na			0.602							
Ga			0.533							
Be			−0.522							
B			−0.519							
Sr			−0.498							
K				0.679						
Ag				0.436						
Se					0.532					
Pd					0.419					
Sb					0.413					
Li					−0.409					
Rh					0.406					
Zn						0.641				
Ni						−0.478				
V							−0.458			
Ti								0.565		
Cu								−0.398		
Au								0.392		
Bi									0.495	
Fe										0.476
Mg										−0.454

Extraction method: principal component analysis

Ten components extracted

on lung cancer risk. A number of epidemiological studies demonstrated TE relationship between cigarette smoking and environmental exposure to trace elements. Some of trace elements and other biochemically important elements such as As, Al, Cd, Cr, Cu, Pb, Mn, Hg, Ni, Se, and Zn are linked with smoking [1–4].

Fig. 1 Cluster analysis of trace elements in scalp hair of lung cancer patients



The role of TE in the development and inhibition of cancer has a complex character and raises many questions. Several studies have focused on TE relationship between and lung cancer; in these studies, mention is made of changing trace element concentration in the human fluids and tissues of patients with lung cancer caused by an imbalance of trace elements [4, 5, 7, 8, 10, 23–26].

Trace elements are of major importance in physiology and biochemistry in the health of individuals. Many biochemical functions depend on trace elements such as immune activity, protein synthesis, the action hormones, a constituent of enzymes which plays an important role in nucleic acid metabolism, cell replication, tissue repair, etc.; these findings show the necessity of taking broad view of the metabolism of trace elements. It would seem reasonable, therefore, to assume that TE might influence the carcinogenic process [6–8, 19].

It has been recognized for several decades that some trace elements play a definite role in carcinogenesis. Many attempts have been made to investigate element concentrations in

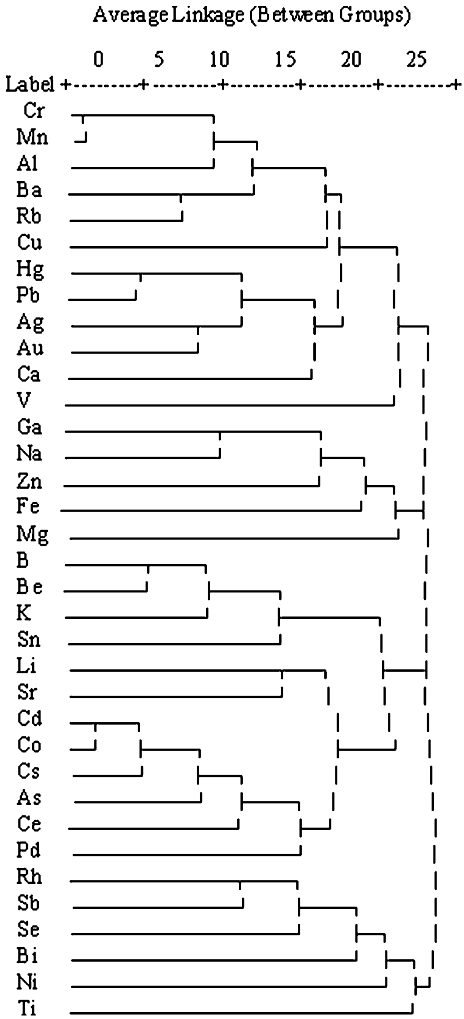


Fig. 2 Cluster analysis of trace elements in scalp hair of control groups

different human fluids and tissues, to find the correlation between element concentration and the kind and clinical stage of tumors [4, 10, 12, 24–28].

The use of biomarkers such as trace element levels in hair, nails, blood, or urine is one way of detecting abnormal levels of these elements in human body. While blood and urine give an idea of recent exposure, hair and nails are proxies of longer time-frame exposures. Analysis of hair is used in various toxicological, clinical, and forensic investigations. The advantages of performing hair analysis over other biological samples are that hair analysis is a less intrusive method, provides a history of drug use, is more resistant to adulteration, can be easily collected and stored, has long shelf-life, and can be easily decontaminated. Exposure levels to environmental and industrial pollutants can be determined by performing an analysis of hair samples [9, 13, 15, 18].

Biomonitoring of trace elements in fluid or tissue samples of lung cancer has been published for many elements at different geographical locations. Many of these papers deal with elements described as toxic or carcinogenic such as Cd [24, 25, 27], Cr [10, 17, 29, 30], Ni [10, 28, 30, 31], or Co [27]. Other publications deal with biomonitoring of essential trace elements, for example, for Se [6, 9, 11, 14, 16, 17, 23], Zn [9, 23], or Cu [9, 23], etc. Epidemiological evidence is also available to document the carcinogenic potential of heavy metals. However, data and some analyses for specific heavy metals with respect to lung cancer are conflicting [10, 24, 25, 27].

No screening techniques that use standard clinical radiology, sputum cytology, or direct biopsies have been proven adequate for lung cancer detection of markers of the preneoplastic phase of carcinogenesis. Attention to developments in tumor biology has now turned to detection of preneoplastic phase of carcinogenesis. A focus on carcinogenesis shifts emphasis toward detection of individual cellular and genetic markers of potentially reversible progression. Validation of carcinogenesis markers requires marker detection in premalignant specimens from individuals who later develop cancer and the absence of the markers from those who remain cancer-free [14–22, 31].

To determine the grade of impact of smoking using the amounts of trace elements obtained from the hair sample of the patients with lung cancer, group 1 and group 2 enrolled the people with same age, same environment, and same characteristics. The highest levels measured were for Ca, Zn, Sn, Na, and Mg in group 1 and for Zn, Mg, Ca, Fe, and Se in group 2. In the control group, it was seen that the levels of Fe and Se required for the body were higher. The significant results were obtained for Au, Bi, Ca, Ce, Co, Cr, Cu, Ga, Hg, K, Mn, Ni, Rb, Rh, Sb, Sc, Ti, and V in group 1 and for Ag, Be, Cd, Fe, and Zn in group 2 ($p<0.05$). This finding shows that, in the group with lung cancer, a statistically significant difference was present for the levels of heavy metals and toxic elements. In our study, differences in some heavy metal (Al, Au, B, Ba, Bi, Ca, Ce, Co, Cr, Cu, Ga, Hg, K, Li, Mn, Na, Ni, Pb, Pd, Rb, Rh, Sb, Sc, Sn, Sr, Ti, and V) levels high were found in lung cancer patients' hair compared with controls. However, Au, Bi, Ca, Ce, Co, Cr, Cu, Ga, Hg, K, Mn, Ni, Rb, Rh, Sb, Sc, and Ti level differences were significantly higher in healthy controls.

In our study, cigarette smoking consumption may explain the accumulation of Co, Sn, Sb, and Ni in lung tumors because these elements were present at higher levels in group 1 than in group 2. Thus, in addition to cigarette smoking, data suggest that environmental/occupational exposure to Co, Sn, Sb, and Ni may be important determinants in Anatolian lung cancer patients. Heavy metal levels circulating in human hair were used as biological indicators of exposure. Therefore, a case–control study was conducted to evaluate the difference in heavy metal content between lung cancer patients and controls. Hence, the relation between lung cancer and trace elements, and possible roles of these elements in carcinogenesis, could not fully be understood by analyzing the trace elements status only. The present study showed that increasing of Cd, Ni, Co, Cr, and Pb oxide fumes by tobacco smoking, causes deficiency of essential elements like Se and Zn. Furthermore, environmental exposure, as well as age, gender, regional origin, and probably also lifestyle, may significantly influence tissue TE levels. The tendency to decrease Se indeed existed according to the severity of the disease. It was shown that Se deficiency may be associated with increased tumors and tumor size.

When the difference between two groups was examined using Pearson correlation analysis, the trace elements that showed correlation in group 1 but not in group 2 were Cu, Ni, Rh, Ti, and V. The trace elements that showed correlation in group 2 but not in group 1 were Ba, Ce, Na, Sb, Se, Sr, and Zn. In the coupled matching, it was seen that the number and the strength of the correlations were higher in group 2 compared with group 1. Group 1 revealed correlation between heavy metals; group 2 revealed correlations between both heavy metals and macroelements required for the body. In the coupled matching, it was seen that the number and the strength of the correlations were higher in group 2 compared with group 1. In group 1, it was thought that, while the correlation in basic elements decreased, the correlation with toxic trace elements might be mostly related to cancer and partly related to the age. It was thought that the trace elements without correlation do not have an impact on other trace elements and that their biological effect might be independent. Among the first factor that best describes the total change in the patients with cancer as the most important variables, the first and second factors were Cd and Li. Among the first factors that best describe the total change in group 2, Cr and Mn were the most important variables. Group 2 contained both heavy metals and trace elements required for the body. The presence of these elements, other than heavy metal, suggests that antioxidant and oxidant capacity of the body might be adequate. When this balance is impaired, it is thought that a trend toward the presentation observed in group 1 increased and that the toxic dose was reached, as a result of some interactions with particular elements. The results obtained in the factor analysis were subject to a clustering analysis. When the trace elements clustered during this analysis were examined, it was seen that heavy metals were clustered and that, in the control group, both heavy metals and macroelements required for the body were clustered. These findings are consistent with the results obtained from the studies that highlighted the importance of heavy metals in the cancer.

In the past few years, increasing consideration has been given to interactions occurring in the organism between TE and bioelements essential for life. These interactions are complex and involve bioelements such as Zn, Cu, Fe, Se, Ca, Ti, and Mg and toxic elements including Co, Ni, Cd, Pb, Sb, Cr, etc. The relevance of element-to-element interactions should be considered in the light of the men's exposure to toxic elements and the common deficiency in the essential elements.

Conclusions

The aim of our research was to show that abnormalities in the trace element content were also observed in patients diagnosed with IIIB non-small cell lung cancer. The present study showed an association between lung cancer and trace elements. Moreover, it showed that concentrations of heavy metals and trace elements such as Li, Ag, Pd, Ti, Co, Ni, As, Sn, etc., in hair may be used as a biomarker of exposure. However, more studies with a larger number of subjects and more homogeneous samples are needed to verify this relationship and to examine the potential associations between the trace elements seen in higher concentrations in the hair of cancer patients.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P (2004) Global cancer statistics. CA Cancer J Clin 55:74–108
2. Republic of Turkey Ministry of Health: Statistics 1999), htpp:/www.saglık.gov.tr/extras/istatistikler/apk2001/092.htm (accessed on: December, 2008).
3. Haiman CA, Stram DO, Wilkens LR, Pike MC, Kolonel LN, Henderson BE et al (2006) Ethnic and racial differences in the smoking-related risk of lung cancer. N Engl J Med 354:333–342
4. Chiba M, Masironi R (1992) Toxic and trace elements in tobacco and tobacco smoke. Bull World Health Organ 70(2):269–275
5. Gromadzinska J, Wasowicz W, Rydzynski K, Szeszenia-Dabrowska N (2003) Oxidative-stress markers in blood of lung cancer patients occupationally exposed to carcinogens. Biol Trace Elem Res 91:203–215
6. Comstock GW, Alberg AJ, Huang HY et al (1997) The risk of developing lung cancer associated with antioxidants in the blood: ascorbic acid, carotenoids, a-tocopherol, selenium, and total peroxyl radical absorbing capacity. Cancer Epidemiol Biomark Prev 6:907–916

7. Trobs M, Renner T, Scherer G et al (2002) Nutrition, antioxidants, and risk factor profile of nonsmokers, passive smokers and smokers of the Prevention Education Program (PEP) in Nuremberg, Germany. Prev Med 34:600–607
8. Gehring L, Leonhardt P, Bigl H, Negretti de Bratter V et al (1998) Serum trace elements in lung cancer. In: Collery P, Bratter P et al (eds) Metal Ions in Biology and Medicine. John Libbey Eurotext, Paris, pp 583–587
9. Piccinini L, Borella P, Bargellini A, Medici IC, Zoboli A (1996) A case–control study on selenium, zinc, and copper in plasma and hair of subjects affected by breast and lung cancer. Biol Trace Elem Res 51:23–30
10. Kollmeier H, Seeman JW, Rothe G, Muller KM, Wittig P (1987) Increased chromium and nickel content in lung tissue and bronchial carcinoma. Am J Ind Med 11:659–669
11. Schrauzer GN, White DA, Schneider CJ (1977) Cancer mortalities correlation studies, IV. Associations with dietary intakes of certain trace elements, notably selenium antagonist. Bioinorg Chem 7:35–56
12. Alatise OI, Schrauzer GN (2010) Lead exposure: a contributing cause of the current breast cancer epidemic in Nigerian women. Biol Trace Elem Res 136:127–139
13. Leung PL, Huang HM (1997) Analysis of trace elements in hair of volunteers suffering from naso-pharyngeal cancer. Biol Trace Elem Res 57:19–25
14. Whanger PD (2004) Selenium and its relationship to cancer: an update dagger. Br J Nurt 91:11–28
15. Kilic E, Ozturk S, Demiroglu A, Yilmaz Z, Yildaz Z, Akkurt M, Saraymen R, Ok E (2004) Analysis of element content in scalp hair of healthy people and breast cancer patients with SEM/EDX method. J NZ Med Assoc 117:1–3
16. Drasch G, Schopfer J, Schrauzer GN (2005) Selenium/cadmium rations in human prostates. Indicators for prostate cancer risk of smokers and non-smokers and relevance to the cancer protective effects of selenium. Biol Trace Elem Res 103:103–107
17. Schrauzer GN (2006) Interactive effects of selenium and chromium on mammary tumor development and growth in MMTV-infected female mice and their relevance to human cancer. Biol Trace Elem Res 109:281–292
18. Kolmogorov Y, Kovaleva V, Gonchar A (2000) Analysis of trace elements in scalp hair of healthy people, hyperplasia and breast cancer patients with XRF method. Nucl Instrum Meth Phys Res A 448:457–460
19. World Health Organization (1996) Trace Elements in Human Nutrition and Health. World Health Organization, Geneva
20. Singh J, McLean JA, Pritchard DE, Montaser A, Patierno SR (1998) Sensitive quantitation of chromium–DNA adducts by inductively coupled plasma mass spectrometry with a direct injection high-efficiency nebulizer. Toxicol Sci 46:260–265
21. Tanner S, Baranov V, Bandura D (2002) Reaction cells and collision cells for ICP-MS: a tutorial review. Spectrochim Acta B 57:1361–1452
22. Shane Elliott, Michael Knowles, Iouri Kalinitchenko A (2004). Change in Direction in ICP-MS, published on Mar, in American Laboratory
23. Oyama T, Kawamoto T, Matsuno K, Osaki T, Matsumoto A, Isse T et al (2003) A case-case study comparing the usefulness of serum trace elements (Cu, Zn and Se) and tumor markers (CEA, SCC and SLX) in non-small cell lung cancer patients. Anticancer Res 23:605–612
24. Lamm SH, Parkinson M, Anderson M, Taylor W (1992) Determinants of lung cancer risk among cadmium-exposed workers. Ann Epidemiol 2:195–211
25. Paakko P, Kokkonen P, Anttila S, Kalliomaki PL (1989) Cadmium and chromium as markers of smoking in human lung tissue. Environ Res 49:197–207
26. Hayers RB (1997) The carcinogenicity of metals in humans. Cancer Causes Control 8:371–385
27. Hengstler JG, Bolm-Audorff U, Faldum A, Janssen K, Reifenrath M, Gotte EW, Jung D, Mayer-Popken S et al (2003) Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. Carcinogenesis 24:63–73
28. Andersen A, Berge SR, Engeland A, Norseth T (1996) Exposure to nickel compounds and smoking in relation to incidence of lung and nasal cancer among nickel refinery workers. Occup Environ Med 53:708–713
29. Rowbotham AL, Levy LS, Shuker LK (2000) Chromium in the environment: an evaluation of exposure of the UK general population and possible adverse health effects. J Toxicol Environ Health B 3:145–178
30. Sorahan T, Burges DC, Hamilton L, Harrington JM (1998) Lung cancer mortality in nickel/chromium platters. Occup Environ Med 55:236–242
31. Silvera SAN, Rohan THE (2007) Trace elements and cancer risk: a review of the epidemiologic evidence. Cancer Causes Control 18:7–27