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ORIGINAL ARTICLE

Do trace element levels have prognostic value in non-small cell lung cancer patients treated with chemoradiotherapy?

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Summary

Purpose: *To determine the prognostic value of trace element levels in cases with locally advanced non-small cell lung cancer (NSCLC).*

Methods: *This prospective study included patients receiving chemoradiotherapy for locally advanced NSCLC and healthy individuals as control group. In both groups, 37 trace element levels in hair were studied by inductively coupled plasma-mass spectrometer technique. Treatment response and survival were evaluated and possible relationship between them and trace element levels was investigated.*

Results: *The mean age was 58.01±7.7 years in the patient group (N=70) and 56.3±9.03 years in the control group (N=85). When trace element levels were compared, it was found that there was significant differences between groups regarding Al, As, Be, Ca, Cr, Hg, Li, Ni, Rh, Sb, Se, Sn, Ti and Zn levels ($p<0.05$; Student's t-test). In the patient group, mean overall survival (OS) and progression-free survival (PFS) were 16.3 and 11.4 months, respectively. After concurrent chemoradiotherapy, complete response (CR) was detected in 7 patients (10%), partial response (PR) in 16 (22.9%), stable disease (SD) in 18 (25.7%) and progression (PD) in 29 patients (41.4%). In univariate and multivariate analyses, Au and Cu levels were significantly associated with PFS ($p=0.017$ and $p=0.020$, respectively).*

Conclusion: *An increase was noticed in heavy metal levels in patients with NSCLC. Copper and gold affected significancy PFS while other trace elements had no significant impact on survival.*

Key words: *lung cancer, chemoradiotherapy, trace element, prognosis*

Introduction

While lung cancer was a rare disease in the beginning of 20th century, it became leading cancer in terms of incidence and as a cause of death, achieving epidemic dimensions later. Today, it comprises 12.8% of all new cancer cases and shows 3% increase annually. It is the leading cause of cancer-related deaths in both men and women [1,2]. Only 15% of all cases with lung cancer survive 5 years or more after diagnosis [3].

Although first-line treatment is surgery in cases with NSCLC, most cases are at advanced stage and inoperable at the time of diagnosis. Extent of disease, performance status, weight loss, gender and serum LDH level are known prognostic factors [1,2,4)]. However, there is an ongoing search for prognostic factors influencing survival, since patients with same stage display great variations in survival. Knowledge about prognostic factors can be helpful to determine optimal treatment modality and better classification of patients into prognostic subgroups.

Better understanding of lung cancer development will allow to develop novel therapeutic strategies using targeting molecules [4]. Definitive identification of molecular markers that determine individuals at risk is a topic which is becoming focus of interest worldwide, and it is thought trace elements will be one of those molecular markers.

Today, it is known that many trace elements in cells, tissues and organs play major role in various biological processes for maintenance of hu-

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man health. There are about 40 trace elements in the human body [5,6]. Understanding the effects of trace elements on human health is not only complicated but also an interesting topic. Deficiency or redundancy of trace elements relative to the requirement of the organism have been linked to many diseases. Since 1960s, it had been known that these elements play a role in the development of lung cancer. However, their role in the development or inhibition of cancer isn't fully elucidated. Preclinical and clinical studies about the effects of trace elements on prognosis, treatment response and survival involve relatively small groups [5,7].

The aim of this prospective study was to determine the prognostic/predictive value of trace element levels in patients receiving chemotherapy due to inoperable NSCLC.

Methods

Demographic characteristics

This prospective study included 70 patients with unresectable-locally advanced NSCLC confirmed by histopathology in the Radiation Oncology Department of Kayseri Teaching Hospital, and 85 healthy individuals as controls. The study was approved by the Ethics Committee of Erciyes University Medical School. The study was conducted in accordance to local ethics regulations and Helsinki Declaration.

Inclusion/exclusion criteria

Included were NSCLC patients with ECOG (Eastern Cooperative Oncology Group) performance status (PS) 0-2, normal renal (serum creatinine≤1.5 mg/dL; creatinine clearence≥60 mg/kg), hepatic (serum bilirubin≤1.6 mg/dL) and bone marrow functions (leukocyte≥4,000/μL; platelets 100,000/μL), but not having pleural fluid to drain, severe cardiac problems (coronary artery disease, congestive heart failure, arrhythmia etc.) or previous radiotherapy/chemotherapy. Patients younger than 18 years, those with small cell lung cancer, severe heart, liver, respiratory and renal failure, those with severe infection such as sepsis, those not eligible for RT, and those with history of a second malignancy were excluded.

All patients and controls gave informed consent. Hair samples were obtained on the day which radiotherapy was initiated. Demographic characteristics such as age, tumor type, tumor size, lymph node status, stage, metastasis status, adjuvant therapies employed, toxicity, and overall and disease-free survival were registered.

Radiotherapy

Two-dimensional treatment planning system using conventional X-ray simulator was used for planning radiotherapy. Radiotherapy was delivered by linear accelerator device (Varian CDX 2300) using 6-18 MV photon energies or Co-60 (gamma beam, 1.3 MeV). Planning target volume (PTV) was estimated to involve primary tumor and draining lymphatics (ipsilateral hilar and mediastinal lymph nodes) with 2 cm margins. The treatment area was irradiated up to 46 Gy from anterior-posterior parallel fields. After 46 Gy, booster doses of 14-20 Gy (in 7-10 fractions) were delivered from parallel fields at oblique plane to gross tumor volume at both sides and involved lymph nodes with 1.5 cm margins and medulla spinalis protection. Dose was defined according to fixed SSD technique. RT was delivered in 5 days a week with conventional fractionation (1.8-2.0 Gy/day). Total radiation dose was 60-66 Gy.

Chemotherapy

Concurrent chemotherapy was initiated on the first day of radiotherapy. Paclitaxel (50 mg/m^2) and carboplatin (AUC 2) were given bi-weekly throughout radiotherapy. Treatment response was assessed according to RECIST (Radiologic Evaluation Criteria in Solid Tumors) criteria one month after completion of chemoradiotherapy. In patients with CR, PR or SD, consolidation chemotherapy including 4 courses of paclitaxel (175 mg/m^2) and carboplatin (AUC 6) were administered in 21-day intervals.

Toxicity assessment

Toxicity related to radiotherapy such as nausea, dysphagia and neutropenia were assessed weekly according to the American NCI-CTC version 2.0 (National Cancer Institute-Common Toxicity Criteria) as follows: grade 1, mild adverse effect; grade 2, moderate adverse effect; grade 3, severe adverse effect; grade 4, life-threatening adverse effect. Chemoradiotherapy was interrupted in case of grade 4 hematological toxicity and grade 3 or above esophagitis during treatment. Chemoradiotherapy was resumed when hematological toxicity was regressed to grade 3 or below, and esophagitis was regressed to grade 2 or below.

Assessment of treatment response

Treatment response was assessed according to RECIST criteria one month after completion of chemoradiotherapy. Briefly, CR was defined as disappearance of all lesions, while PR as regression by 30% or more in measurable lesions or lack of newly developed lesions. SD was defined as regression less than 30% or no change for at least 4 weeks in the size of lesions, while PD as growth more than 20% in measurable tumor areas or appearance of new lesion(s). Follow-up visits were scheduled in 3-month intervals during the first year after treatment; thereafter; in 6-month intervals until the completion of 5 years. In the follow-up visits, all patients were assessed by physical examination, complete blood count, serum biochemistry and imag-

ing modalities including cranial CT/MRI, thoracic and abdominal CT scans or PET-CT scan.

Preparation of hair samples

Hair samples (2-3 cm in length) were taken from the neck region in both treatment-naive patients and controls. After harvesting, samples were washed by deionized water first, followed by 15 mL acetone in a magnetic mixer in order to eliminate debris that would be caused by shampoo or scalp. Samples were rinsed by using 15 mL deionized water for three times. Hair samples were mixed with concentrated nitric acid (65%, 10 mL, weighing 1 g) and heated at 80°C for 10 min. Then, perchloric acid (5 mL) was added while heating the samples gradually, and white, dense smoke was obtained. Samples were gradually cooled to room temperature and diluted up to 50 mL with distilled water. Blank samples were prepared in a similar manner. After resolving samples, Agilent 7500 ICP-M spectrophotometer (Agilent Tech, USA), was calibrated. All reagents used were purchased from E-Merck (USA). Water used in the analysis was refined by using Millipore Synergy 185 device (Agilent Tech, USA). All samples were read in inductively coupled plasma-mass spectrometer (ICP-MS) (Agilent Tech, USA. Results were calculated as µg/kg.

Statistics

Statistical analyses were performed by using Excel X State software (Microsoft Corp., Redmond, WA) and SPSS for Windows 15.0 (SPSS Inc., Chicago, IL, USA). Mean values and standard deviations were calculated. Normality of numeric data was assessed by using Kolmogorov-Smirnov test. Normally distributed numeric data were presented as mean±standard deviation, while those with skewed distribution were presented as median (min-max). Student's t-test and Mann-Whitney U test were used for comparisons between groups. OS and DFS were calculated by using Kaplan-Meier analysis. Log-rank test was used to determine the relationship between variables and OS and PFS. Prognostic factors found to be significant in Cox proportional hazard univariate analysis were included into multivariate analysis. $p<0.05$ was considered as statistically significant.

Results

Table 1 presents the arithmetic mean, standard deviation and p values in patients with lung cancer and healthy individuals. The mean age was 58.01±7.7 years in 70 cases with lung cancer and 56.3±9.03 years in 85 healthy individuals. The levels of 28 elements (Al, Au, B, Ba, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Ga, Hg, K, Li, Mg, Mn, Na, Ni, Rb, Rh, Sb, Sc, Sn, Sr, Ti, V) were higher in patients with cancer when compared to controls. In addition, the levels of Ag, Be, Fe, Pb, Pd, Se, Sr and Zn were higher in controls when compared to patients with cancer. When Student's t-test was used to compare the levels of trace elements, significant differences ($p<0.05$) in the levels of Al, As, Be, Ca, Cr, Hg, Li, Ni, Rh, Sb, Se, Sn, Ti and Zn between groups were noticed.

Table 2 depicts the demographic characteristics and clinical findings of patients with lung cancer. There were 6 women and 64 men with median age of 58 years. Fifteen patients (21.4%) had

Table 1. Trace elements in patient and control groups

Trace element	*Patient group (mean±SD)*	*Control group (mean±SD)*	*p-value*
Ag	0.53±0.66	0.84±1.59	0.136
Al	21.27±20.53	11.5±12.67	0.000
As	1.17±1.83	0.51±0.70	0.003
Au	1.08±3.81	0.74±1.27	0.432
B	1.43±1.69	1.04±1.80	0.168
Ba	1.48±1.76	1.30±1.45	0.512
Be	0.01±0.01	0.03±0.06	0.010
Bi	0.44±0.69	0.40±0.68	0.677
Ca	40.8±43.29	29.73±17.93	0.034
Cd	0.38±0.43	0.29±0.40	0.198
Ce	1.44±3.11	0.86±0.70	0.219
Co	0.48±0.53	0.37±0.44	0.150
Cr	1.84±1.57	0.91±0.97	0.000
Cs	0.30±0.42	0.23±0.36	0.331
Cu	16.40±17.02	15.77±15.94	0.813
Fe	23.57±33.01	27.47±24.07	0.402
Ga	0.29±0.46	0.26±0.24	0.652
Hg	1.05±1.27	0.63±0.72	0.012
K	13.38±16.62	10.94±9.02	0.250
Li	0.84±0.68	0.56±0.56	0.008
Mg	32.69±23.35	34.63±22.87	0.608
Mn	1.22±1.17	1.10±1.05	0.536
Na	28.92±32.26	22.37±16.1	0.105
Ni	0.86±0.75	0.55±0.52	0.004
Pb	4.98±4.55	5.23± 6.20	0.781
Pd	0.09±0.20	0.11±0.18	0.643
Rb	0.27±0.36	0.22±0.28	0.398
Rh	0.65±0.53	0.41±0.47	0.003
Sb	0.95±0.88	0.23±0.32	0.000
Sc	0.12±0.53	0.02±0.00	0.110
Se	5.89±12.60	18.73±20.11	0.000
Sn	29.57±23.28	21.49±19.36	0.021
Sr	1.11±1.69	1.31±1.61	0.464
Ti	7.42±14.04	1.89±4.19	0.001
V	1.03±1.53	0.95±1.64	0.765
Zn	45.71±52.60	109.15±91.50	0.000

SD: standard deviation

Table 2. Pretreatment characteristics of lung cancer patients

Characteristics	*Patients N (%)*
Gender	
Male	64 (91.4)
Female	6 (8.6)
Age (years)	
<58	25 (35.8)
≥58	45 (64.2)
Histology	
SCC	47 (67.1)
AC	15 (21.4)
Other	8 (11.4)
ECOG PS	
0	28 (40)
1	42 (60)
T stage	
1	2 (2.9)
2	9 (12.9)
3	15 (21.4)
4	44 (62.9)
N stage	
0	9 (12.8)
1	6 (8.6)
2	37 (52.9)
3	18 (25.7)
TNM stage	
3A	26 (37.1)
3B	44 (62.9)
Radiotherapy dose (Gy)	
60	10 (14.3)
66	60 (85.6)
Response to treatment	
Complete	7 (10.0)
Partial	16 (22.9)
Stable	18 (25.7)
Progression	29 (41.4)
Grade 3-4 hematologic toxicity	
Leukopenia	7 (10)
Thrombocytopenia	4 (5.7)
Anemia	2 (2.8)
Grade 3 nonhematologic toxicity	
Nausea	16 (10.3)
Vomiting	10 (14.3)
Esophagitis	5 (7.1)

AC: adenocarcinoma, SCC: squamous cell carcinoma, ECOG PS: Eastern Cooperative Oncology Group performance status

T3 disease and 44 (62.9%) T4 disease, while there were 37 patients (52.9%) with N2 disease and 18 patients (25.7%) with N3 disease. According to histopathological evaluation, 15 patients (21.4%) had adenocarcinoma and 47 (67.1%) squamous cell carcinoma. Stage IIIA was found in 37.1% and IIIB in 62.9% of the patients. Median follow-up was 14 months (range 3.5-48). Mean OS was 16.3 months, while local control and PFS were 11.4 and 11.1 months, respectively. CR was achieved in 7 patients (10%), PR in 16 (22.9%), SD in 18 (25.7%) and PD in 29 patients (41.4%). Grade 3-4 hematological and non-hematological toxicity were 18.5% and 31.4%, respectively.

Tables 3 displays OS, locoregional progression-free survival (LRPFS) and PFS. Although OS, PFS and LRPFS were better in younger patients, those with stage IIIA, T1-2 and N0-1 disease, with good performance status, with CR, with adenocarcinoma and male patients, the differences didn't reach statistical significance.

Tables 4 and 5 show univariate and multivariate analysis for OS and PFS according to risk factors. In univariate analysis, no factor was found to be associated to OS but Au and Cu were found to be significantly associated to PFS (p=0.017 and p=0.020, respectively). In multivariate analysis, Cu (OR:1.0; 95%CI:1.0-1.0; p=0.013) and Au (OR:1.0; 95%CI:1.0-1.1; p=0.008) remained associated with PFS.

Discussion

The aim of treatment in stage III NSCLC is both local disease control and prevention of systemic disease dissemination. Chemotherapy contributes to both local and systemic control. Concurrent chemoradiotherapy doesn't only improve locoregional control but it also prevents micrometastases [1-3]. Although addition of consolidation chemotherapy to concurrent chemoradiotherapy increases toxicity, it is shown that it provides a survival advantage. Literature indicates that agents including cisplatin, vindesine, mitomycin, vinblastine, paclitaxel and gemcitabine have been preferred for concurrent chemoradiotherapy with reported median survival of 17 months and 2-year survival rates up to 40% [3,4].

Tumor stage and histopathological subtype are most important prognostic factors in NSCLC [2]. However, there is an ongoing search for prognostic factors influencing survival, since patients with same stage display great variations in survival. To the best of our knowledge, there is no study demonstrating prognostic value of all trace elements in human body in patients receiving chemoradiotherapy for lung cancer in the literature. The present study was conducted to determine the levels of trace elements and to determine whether they are valuable predictors of treatment response and prognosis in NSCLC patients.

In our study, significant differences (p<0.05) in the levels of Al, As, Be, Ca, Cr, Hg, Li, Ni, Rh, Sb, Se, Sn, Ti and Zn between patients with NSCLC

Table 3. Overall, locoregional progression-free, and progression-free survival

Variable	*No. of patients*	*Mean OS (95% CI)*	*p-value*	*Mean LRPFS (95% CI)*	*p-value*	*Mean PFS (95% CI)*	*p-value*
Gender			0.990		0.709		0.758
Male	64	16.2 (13.4-19.1)		11.3 (7.9-14.6)		11.1 (8.2-14.0)	
Female	6	15.3 (8.0-22.7)		8.3 (3.1 -13.5)		8.9 (2.8-15.0)	
Age (years)			0.450		0.721		0.365
<58	25	18.0 (12.5-23.5)		10.8 (5.6-16.1)		9.7 (5.7-13.7)	
≥58	45	15.4 (12.5-18.3)		10.6 (8.0-13.2)		11.6 (8.6-14.6)	
Histology			0.138		0.571		0.512
SCC	47	14.1 (11.9-16.4)		9.1 (7.1-11.2)		9.6 (7.2-11.9)	
AC	15	20.8 (13.1-28.6)		14.2 (6.7-21.7)		14.3 (7.0-21.6)	
Other	8	18.4(13.1-27.4)		10.0 (6.2-13.8)		9.7 (6.1-13.4)	
ECOG PS			0.551		0.989		0.361
0	28	17.7 (12.6-22.9)		11.9 (6.8-17.0)		14.7 (8.1-21.4)	
1	42	15.5 (12.3-18.6)		10.3 (7.6-13.0)		9.7 (7.2-12.2)	
T stage			0.286		0.061		0.089
T 1-2	14	19.6 (12.4-26.8)		17.5 (7.8-27.3)		16.4 (7.9-24.9)	
T 3-4	56	15.3 (12.6-18.0)		9.3 (7.2-11.4)		9.7 (7.4-12.0)	
N stage			0.734		0.874		0.717
N 0-1	15	17.3 (10.4-24.2)		12.8 (4.8-20.8)		12.8 (5.1-20.6)	
N 2-3	55	15.9 (13.1-18.6)		10.1 (7.9-12.3)		10.5 (8.1-12.9)	
TNM stage			0.498		0.214		0.382
3A	26	17.5 (12.6-22.4)		14.6 (8.1-21.1)		13.3 (7.9-18.8)	
3B	44	15.6 (12.4-18.7)		9.4 (7.0-11.8)		10.3 (7.4-13.1)	
Response to treatment			0.350		0.942		0.929
Complete	7	21.8 (13.7-29.9)		10.2 (6.3-14.1)		10.2(6.7-13.7)	
Partial	16	20.2 (11.7-28.7)		12.6 84.8-20.5)		12.6 (4.8-20.5)	
Stable	18	15.1 (10.2-20.0)		10.3 (7.6-13.2)		10.3 (6.6-13.9)	
Progression	29	14.0 (10.7-17.2)		10.2 (7.5-13.2)		10.3 (7.2-13.3)	
Response to treatment			0.095		0.793		0.936
Yes	23	20.4 (14.3-26.6)		12.1 (6.1-18.1)		14.0 (7.1-21.0)	
No	47	14.5 (11.7-17.3)		10.3 (7.8-12.8)		10.3 (7.9-12.7)	

AC: adenocarcinoma, SCC : squamous cell carcinoma, CI :confidence interval; ECOG PS: Eastern Cooperative Oncology Group performance status, LRPFS: local regional progression-free survival, OS : overall survival, PFS : progression-free survival

and controls were noticed. In patients with cancer, Zn and Se levels, among basic elements required, were decreased, whereas the levels of Al, As, Be, Ca, Cr, Hg, Li, Ni, Rh, Sb and Ti, among toxic elements, were increased. It is speculated that this is due to changes in the body induced by environmental exposure and cancer.

In the previous three decades, hair analysis has been widely used for diagnosis of diseases due to environmental or occupational exposure [8,9]. Trace element analysis in hair can reveal interesting results that may be translated to beneficial outcomes for human body and environmental health in the short-term. Thus, we preferred hair as tissue sample in our study.

We previously investigated the value of trace elements in the diagnosis of patients with stage III breast cancer and in those with stage IIIB NSCLC [5,7]. We emphasized that heavy metals accumulated in the body might represent high risk for development of breast and lung cancer, where healthy controls were compared with those with breast or lung cancer. The results of the present study were similar to previous studies. In a study on 224 cases with lung cancer, Adachi et al. reported that only Cu levels were high, while levels of elements such as calcium, magnesium, zinc and cobalt were low in lung tissue [10]. Hart et al. found that Cu level was markedly high in lung cancer tissue when compared to healthy tissue [11]. In a study on lung cancer and various cancer types, Poukkula et al. found that serum Cu level was increased while serum Zn level was decreased [12]. Several authors [5,8,12] observed that Zn levels were decreased in tissue harboring small cell lung cancer compared to other cell types when they classified these tissues by histopathology. In our study, it was seen that Zn levels were decreased, while Cu levels were increased in NSCLC patients.

In this study, the mean OS was 16.3 months, while the mean local control and PFS were 11.4 and 11.1 months, respectively. OS rates are low in these patients since local recurrence and dis-

Table 4. Univariate analysis of risk factors for overall and progression-free survival

Risk factors	*Univariate analysis of overall Survival*		*Progression-free survival Univariate analyis*	
	OR (95% CI)	*p-value*	*OR (95% CI)*	*p-value*
Gender (male or female)	1.0 (0.4-2.5)	0.990	0.7 (0.3-2.2)	0.760
Age (<58 or ≥58 years)	0.8 (0.5-1.5)	0.459	1.3 (0.7-2.3)	0.368
Histology (SCC or AC)	1.7 (0.7-4.1)	0.215	1.2 (0.5-3.0)	0.651
ECOG (0 or 1)	0.8 (0.5-1.5)	0.559	0.8 (0.4-1.3)	0.365
T stage (T1-2 or T3-4)	0.7 (0.3-1.4)	0.301	0.5 (0.2-1.1)	0.095
N stage (N0-1 or N2-3)	0.9 (0.5-1.7)	0.739	0.9 (0.4-1.8)	0.719
TNM stage (3A or 3B)	0.8 (0.4-1.4)	0.506	0.7 (0.4-1.4)	0.385
Response to treatment (presence or absence)*	0.6 (0.3-1.1)	0.104	0.9 (0.5-1.8)	0.936
Ag	0.6 (0.3-1.2)	0.109	1.0 (0.6-1.7)	0.794
Al	1.0 (0.9-1.0)	0.708	0.9 (0.9-1.0)	0.782
As	0.9 (0.8-1.1)	0.510	0.8 (0.7-1.0)	0.374
Au	1.0 (0.9-1.1)	0.961	1.0 (0.9-1.1)	0.017
B	0.9 (0.8-1.1)	0.651	0.9 (0.7-1.1)	0.822
Ba	0.9 (0.8-1.1)	0.658	0.9 (0.7-1.0)	0.323
Be	0.6 (0.1-2.1)	0.411	0.1 (0.0-0.0)	0.430
Bi	1.0 (0.7-1.5)	0.878	1.1 (0.8-1.7)	0.679
Ca	0.7 (0.4-1.5)	0.371	1.0 (0.9-1.0)	0.329
Cd	0.6 (0.3-1.3)	0.239	0.9 (0.4-1.5)	0.479
Ce	0.9 (0.9-1.1)	0.861	1.0 (0.8-1.1)	0.974
Co	0.4 (0.4-1.0)	0.098	0.6 (0.3-1.0)	0.070
Cr	0.9 (0.8-1.1)	0.758	1.1 (0.9-1.3)	0.599
Cs	0.6 (0.2-1.5)	0.250	0.6 (0.2-1.6)	0.344
Cu	1.0 (0.9-1.0)	0.534	1.0 (1.0-1.0)	0.020
Fe	0.9 (0.1.0)	0.388	0.9 (0.9-1.0)	0.314
Ga	0.6 (0.3-1.3)	0.191	1.1 (0.5-2.0)	0.924
Hg	1.0 (0.8-1.2)	0.958	1.0 (0.8-1.2)	0.533
K	1.0 (0.9-1.0)	0.266	1.0 (0.9-1.0)	0.151
Li	0.6 (0.4-9.8)	0.079	0.7 (0.4-1.1)	0.075
Mg	1.0 (0.9-1.0)	0.597	0.9 (0.9-1.0)	0.313
Mn	0.9 (0.7-1.2)	0.602	0.9 (0.7-1.1)	0.569
Na	0.9 (0.9-1.0)	0.255	0.9 (0.9-1.0)	0.708
Ni	0.8 (0.5-1.2)	0.400	1.0 (0.7-1.6)	0.778
Pb	1.0 (0.9-1.1)	0.285	1.0 (0.9-1.0)	0.659
Pd	1.6 (0.4-5.7)	0.432	0.9 (0.2-3.4)	0.824
Rb	0.6 (0.2-1.3)	0.161	0.8 (0.3-1.8)	0.530
Rh	0.8 (0.5-1.3)	0.335	0.7 (0.4-1.2)	0.352
Sb	0.9 (0.7-1.2)	0.502	0.7 (0.5-1.0)	0.140
Sc	1.2 (0.8-2.0)	0.307	0.9 (0.6-1.5)	0.752
Se	1.0 (0.9-1.0)	0.581	0.9 (0.9-1.0)	0.622
Sn	0.9 (0.9-1.0)	0.256	0.9 (0.9-1.0)	0.420
Sr	0.9 (0.8-1.0)	0.330	0.8 (0.7-1.0)	0.405
Ti	1.0 (0.9-1.0)	0.650	0.9 (0.9-1.0)	0.362
V	0.8 (0.6-1.0)	0.145	1.0 (0.8-1.3)	0.461
Zn	1.0 (0.9-1.0)	0.123	1.0 (0.9-1.0)	0.300

OR: odds ratio; CI: confidence interval; *Presence: complete response or disease regression/Absence: stable disease or progression after chemoradiotherapy

Table 5. Multivariate analysis of risk factors for progression-free survival

Risk factors	*Progression-free survival*	
	OR (95% CI)	*p-value*
Cu	1.0 (1.0-1.0)	0.013
Au	1.0 (1.0-1.1)	0.008

OR: odds ratio, CI: confidence interval

tant metastasis is frequently seen in unresectable stage III NSCLC. When survival is assessed in patients with stage III lung cancer, the range of OS is 14-23 months in stage IIIA and 10-16 months in stage IIIB [2-4]. In our study, prognosis was better in patients with stage IIIA, T1-2 and N0-1 disease, male gender, with good performance status, those with adenocarcinoma and those with CR. Our results regarding survival and prognostic factors were in agreement with the relevant literature.

When 37 trace elements were analysed by univariate and multivariate analysis, it was seen that no element significantly affected OS, local recurrence and PFS, while Cu and Au had a significant impact on PFS (p=0.008 and p=0.013). Copper is a co-factor required for the normal function of many enzymes involved in physiological processes such as respiration, immune response and wound repair in humans [13] and is required for cell proliferation and tumor angio¬genesis. A high level of copper ions has been previously detected in tumor tissues [14]. Previous studies showed that Cu intake in high doses and accumulation in organs is harmful for human health. It has been reported that serum Cu levels were progressively increased by advancing stage of disease in cancers of the breast, lung and gastrointestinal system, acute lymphocytic leukemia, Hodgkin and non-Hodgkin lymphoma [5,14,15].

It is thought that Cu increase is associated with albumin. In 2008, Cavusoglu et al. [16] reported that Cu is transported to the liver by binding to plasma albumin where it is stored. It was suggested that decreased albumin that would occur due to cancer will lead to decreased Cu transport; therefore, copper is stored in plasma and in tissues such as hair.

Conclusion

The present study has shown that heavy metal levels were increased in patients with NSCLC when compared to healthy individuals. It was also found that Cu and Au impacted PFS, while other trace elements evaluated had no significant impact on survival. There is a need for further randomized studies on this topic. We believe that the present study can contribute to future studies by providing information regarding trace elements.

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