Indian Journal of Experimental Biology
Vol. 60, May 2022, pp. 315-321

Effects of Se, Zn and Cu levels on chemoradiotherapy related toxicity in patients with locally advanced lung cancer

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Received 30 April 2020; *revised 24 March 2022*

Lung cancer, with 12% of overall new cancer cases globally, has considerable mortality rate. Chemotherapy and radiotherapy are the most common treatment modalities apart from surgery. Both, chemo- and radio- therapies, are known to have side affects. Trace elements are reported to influence the radiotherapy related adverse effects in the body. In this context, here, we investigated whether there is a difference in serum Se, Zn and Cu levels in patients receiving chemoradiotherapy (CRT) due to lung cancer when compared to healthy individuals and to evaluate effects of serum trace element levels measured before and after therapy on CRT related toxicity. This prospective study included 50 patients received CRT due to lung cancer and 50 healthy individuals. Serum selenium (Se), zinc (Zn), and copper (Cu) levels were measured before and after radiotherapy in patients with cancer, while a single measurement was performed in controls. When serum trace element levels were compared between patients with lung cancer and healthy controls, a significant difference was found in Zn level. A significant difference was detected between serum Cu and Se levels measured before and after CRT in patients with lung cancer (P <0.001 and P =0.019). In the assessment of acute toxicity during, a significant difference was detected when Cu and Zn levels measured before and after treatment were compared. Our study indicates significant decreases in plasma Zn and Cu levels after radiotherapy, suggesting paying attention to nutritional status regarding these micronutrients and other antioxidant agents. Thus, Zn and Cu supplementation may reduce adverse effects in patients receiving CRT.

Keywords: Chemoradiotherapy (CRT), Lung cancer, Trace elements

Lung cancer is one of the frequently seen cancers and is associated with high mortality[1,2]. It accounts from 12% of all new cancer cases worldwide[3]. Despite advances in treatment modalities, 5-years survival is about 22% now[3]. Surgery, chemotherapy and/or radiotherapy are employed in the management of lung cancer[4-6].

The thoracic radiotherapy, as an important part of treatment, aims to deliver most effective radiotherapy (RT) dose to tumor within the therapeutic range while delivering appropriate RT dose to adjacent normal tissues. Several adverse effects occur in the patients during delivery of effective radiotherapy dose. With the application of concomitant chemotherapy, an increase in these effects is seen more[7-10]. It is thought that incidence and severity of radiotherapy-related adverse effects results from alterations in trace elements in the body[11,12].

Trace elements are defined as the elements that must be present but in small amounts in living tissue. These elements involve in many metabolic and physiological processes. It has been reported that imbalance in the trace elements is associated with cardiac diseases, autoimmune disorders, cancer, renal failure and neurological disorders[12-16].

In the present study, we investigated whether changes in selenium (Se), zinc (Zn), and copper (Cu) levels measured before and after therapy enhance treatment-related toxicity in patients with lung cancer.

Material and Methods

This prospective study conducted on 50 patients received CRT due to stage IIIB non-small cell lung cancer (NSCLC) in Kayseri City Education and Research Hospital. The patients were included in the study in a consecutive manner. Further, 50 healthy individuals were included as controls. The study was approved by Local Ethics Committee. The study was conducted in accordance with the local ethics regulations and Helsinki Declaration. Each participant

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was informed about the purpose of the study, its implementation, the voluntariness of participating in the study and that they could leave at any time. Verbal and written consent was obtained by giving a voluntary consent form. All patients underwent physical examination, histopathology evaluation, posterio-anterior chest radiography, thorax and abdomen CT scans, complete blood count, biochemical tests, ECG, abdominal sonography or PET-CT scans before inclusion. Staging was performed using 2009 AJCC staging system[17].

Only male patients older than 18 years with Karnofsky performance status ≥70% who had no history of cooperation disorder, radiotherapy/ chemotherapy, severe diseases (severe heart, liver, respiratory and renal failure) and no severe infection such as sepsis were included to the study.

Treatment modalities

Radiotherapy

In all patients, radiotherapy field was planned by 2-dimensional (coronal and sagittal planes) treatment planning system using X-ray simulator. Gross tumor volume, clinical target volume and treatment volume were calculated using thorax CT scan. Radiotherapy was delivered at overall dose of 60-66 Gy with 1.8-2 Gy fractions per day (5 days in a week) for 6-7 weeks by Co-60 (gamma beam, 1.3 MeV) or linear accelerator device (Varian CDX 2300) using 6-18 MV photon energies. Initially, planning target volume (PTV) was estimated to involve primary tumor, involved lymph nodes and whole mediastinum with margins of 2 cm. In superior and middle lobe tumors, lymph nodes at the area extending to 5 cm below carina were included to the field if there is no subcarinal lymph node involvement. Ipsilateral supraclavicular fossa was included if tumor is localized at superior lobe only or if there is lymph node involvement in supraclavicular fossa. Medulla spinalis was excluded from the field at the dose of 46 Gy. Then, RT dose was completed from parallel regions at oblique plane to gross tumor volume at both sides and involved lymph nodes with margins of 1.5 cm.

Chemotherapy

Simultaneous chemotherapy was initiated on the first day of radiotherapy by paclitaxel (50 mg/m^2) and carboplatin (AUC 2) which were given weekly.

Toxicity assessment

The patients were assessed by a radiation oncologist according to National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0 at baseline and by one-week intervals during CRT[18].

Preparation of serum samples and analysis by ICP/MS method

Blood samples were drawn at morning after 12 h fasting. One blood sample was drawn from controls, while two blood samples were drawn from patients with lung cancer before and after radiotherapy. Blood (5-6 mL) was drawn to closed tubes. Sera were removed by centrifugation at 5000 rpm for 10 min. Serum samples were placed into Eppendorf tubes (2 mL) and stored at –20°C until assays. Serum samples were thawed according to Berghof system before readings at ICP-MS. One milliliter of sera was diluted by adding 9 mL of ultrapure water. Then, serum Cu, Se and Zn levels were measured using inductively coupled plasma-mass spectrometer (ICP-MS). Results were calculated in µg/dL[19,20].

Statistical analyses

Data were analyzed using SPSS for Windows version 15.0 (SPSS Inc., Chicago, Illinos, USA). Normality of numeric data was tested by using Kolmogrov-Smirnov method. Normally distributed numeric data were expressed as mean ± standard deviation, while those with skewed distribution were presented as median (min-max). In the comparison of data obtained before and after radiotherapy in the patient group, paired 't' test was used for normally distributed numeric data, while Wilcoxon 't' test was used for skewed numeric data. Independent-sample 't' test was used to compare patient and control groups. Qualitative variables were presented as percentage. As the radiotherapy-related toxicities were heterogeneously distributed, toxicities were classified into two groups according to presence or absence of thrombocytopenia, anemia, leucopenia, mucositis and nausea; and grade I-II *vs*. grade III-IV for dysphagia and weigh loss. In case of skewed distribution, a median value was provided. Paired 't' test was used to evaluate relationships between trace element levels obtained before and after radiotherapy, while Student's 't' test was used to evaluate relationships between trace element levels and radiotherapy-related toxicities. P <0.05 was considered as statistically significant.

Result and Discussion

Table 1 presented demographic characteristics of patients with lung cancer. The study included 4 women and 46 men with a median age of 59.5 years.

According to TN staging, T3N3 is the most common stage by 42%. According to histopathological evaluation, 74% of patients had epidermoid carcinoma. Median BMI was found as 21.5 (15-34). Of the cases, 13 patients had malnutrition (BMI<18.5 and 18 cases were normal weight (BMI: 18.5-22.9) while 12 cases were overweight (BMI: 23-25) and 7 cases were obese (BMI≥25).

Table 2 presented results of statistical analyses for trace element levels measured before and after CRT in patients with lung cancer and those measured in controls. A significant difference was detected in Zn levels between patients with lung cancer and healthy individuals (*P*=0.001). Se level was measured as higher while Cu level as lower in controls, but the differences didn't reach statistical significance. Significant differences were detected between Cu and Se levels measured before and after therapy in patients with lung cancer (*P* <0.001 and *P*=0.019).

Table 1 — Characteristics of patients with lung cancer (n=50)

Characteristics		Patients n (%)
Gender	male	46 (92)
	female	4 (8)
Age (years)	median	59.5
	<60	21 (42)
	=>60	29 (58)
Histology	SCC	37 (74)
	AC	13 (26)
ECOG	0	20 (40)
	1	30 (60)
T stage	2	5 (10)
	3	21 (42)
	4	24 (48)
N stage	2	24 (48)
	3	26 (52)
TN Stage	T2N3	5 (10)
	T3N3	21 (42)
	T4N2	19 (38)
	T4N3	5 (10)
Body mass index (BMI), kg/m^2	median (range)	21.5 (15-34)
Smoked cigarette		35 (70)
Consumed alcohol		10 (20)
Body mass index category	malnutrition	13 (26)
	normal	18(36)
	overweight	12 (24)
	obese	7 (14)

[AC, adenocarcinoma; ECOG, Eastern Cooperative Oncology Group; N, lymph node, SCC, squamous cell carcinoma; and T, tumor stage]

Acute toxicities during CRT were assessed according to NCI-CTC adverse events chart (Table 3). Grade 1 toxicities were most seen adverse events in our cases. There was grade 2 esophagitis in 12 cases, anemia in 5 cases, leucopenia in 11 cases, weight loss in 9 cases, nausea in 5 cases and vomiting in 6 cases.

Table 4 presented associations between trace element levels and CRT-related toxicities as evaluated by Student's t test. In the assessment of acute toxicity during CRT, significant differences was detected in Cu and Zn when levels before and after therapy were compared. When Cu levels measured before and after therapy were compared, it was seen that weight loss and thrombocytopenia were more common in patients with low Cu levels (*P*=0.047 and *P*=0.003). When Zn levels measured before and after CRT were compared, it was seen that leucopenia and thrombocytopenia were more common in patients with low Zn levels (Table 5, *P*=0.005 and *P*=0.007).

NSCLC comprises approximately 75-80% of all patients with lung cancer and 25-40% of the patients in this group are diagnosed at locally advanced stage[11]. Consecutive or simultaneous chemotherapy is given to the patients with locally advanced disease. CRT consists of at least 60 Gy radiotherapy combined to new-generation chemotherapeutics, such as cisplatin, paclitaxel, docetaxel, vinorelbine or gemcitabine. Survival is better but toxicity is higher in simultaneous CRT[7,10,12,21].

In lung cancer, radiotherapy is done to eliminate malignant cells by ionizing radiation but not harm

Table 3 — Radiotherapy-related toxicities developing during radiotherapy

Toxicity	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	Total n (%)
Esophagitis	20 (40)	12 (24)	2 (4)	0 (0)	34 (68)
Nausea	20 (40)	5 (10)	2 (4)	0 (0)	27 (54)
Vomiting	12 (24)	6 (12)	2 (4)	0 (0)	20 (40)
Weight loss	22 (44)	9 (18)	3 (6)	0 (0)	34 (68)
Leukopenia	18 (36)	11 (22)	5 (10)	1 (2)	35 (70)
Thrombocytopenia	7 (14)	0 (0)	0 (0)	0 (0)	7 (14)
Anemia	28 (56)	5 (10)	0 (0)	0 (0)	33 (66)

Table 2 — Lung cancer patients and healthy groups are given in form of mean±std deviation, *P* value (*P* <0.05)

Trace element (µg/dL)	Lung cancer group (mean±Std)	Healthy group (mean±Std)	*P* value (<0.05)	Lung cancer group (mean±Std) before treatment	Lung cancer group (mean±Std) after treatment	*P* value (<0.05)
Cu	3.58±1.34	3.47±1.40	0.689	3.58±1.34	1.61±1.28	<0.000
Zn	1.78±1.28	3.04±2.13	0.001	1.78±1.28	1.95±0.94	0.434
Se	3.40±1.24	3.82±1.48	0.134	3.40±1.24	2.93±1.14	0.019

Table 4 — Associations between trace element levels and radiotherapy-related toxicitie

Toxicity	Cu (µg/dL)			Zn (µg/dL)			Se (µg/dL)		
	Presence	Absence	*p* value	Presence	Absence	*p* value	Presence	Absence	*p* value
Esophagitis	3.39±1.23	4.09±1.54	0.098	1.75±1.36	4.09±1.54	0.778	3.42±1.38	3.37±0.8	0.917
Nausea	3.68±1.34	3.01±1.34	0.226	1.76±1.35	1.93±0.76	0.741	3.38±1.25	3.53±1.23	0.769
Vomiting	3.69±1.38	3.05±1.03	0.223	1.78±1.29	1.80±1.32	0.968	3.41±1.34	3.37±0.45	0.927
Weight loss	3.69±1.44	3.21±0.89	0.296	1.78±1.30	1.78±1.30	0.996	3.39±1.28	3.46±1.14	0.879
Leukopenia	3.42±1.35	3.57±1.45	0.718	2.47±1.41	1.43±1.04	0.005	3.28±1.62	3.52±1.01	0.509
Thrombocytopenia	3.58±1.24	3.63±1.97	0.918	1.91±1.32	0.99±0.59	0.007	3.31±1.16	3.99±1.61	0.182
Anemia	3.63±1.40	3.21±0.51	0.521	1.76±1.27	2.01±1.44	0.686	3.40±1.25	3.45±1.27	0.933

Table 5 — Associations between the level of Cu, Se and Zn before and after radiotherapy and radiotherapy-related toxicities

Toxicity	Treatments *P* value (<0.05)					
	Cu		Zn		Se	
	Before	After	Before	After	Before	After
Esophagitis	0.098	0.084	0.778	0.551	0.917	0.416
Nausea	0.226	0.288	0.741	0.806	0.769	0.416
Vomiting	0.223	0.328	0.968	0.490	0.927	0.389
Weight loss	0.296	0.047	0.996	0.638	0.879	0.186
Leukopenia	0.718	0.383	0.005	0.964	0.509	0.148
Thrombocytopenia	0.918	0.003	0.007	0.203	0.182	0.982
Anemia	0.521	0.264	0.686	0.507	0.933	0.144

normal tissues during therapy. However, exposure of normal tissues adjacent to malignant cells to high doses of radiation is mostly inevitable. Some adverse events are observed due to morphological, biochemical and metabolic changes occurring in healthy tissues. Acute adverse events, such as weight loss, nausea, vomiting, esophagitis and myelo-suppression are frequently seen complications in patients receiving radiotherapy. Simultaneously given chemotherapy causes increase in incidence and severity of adverse events[4,7,8,10,18,22].

Ionizing radiation increases production of reactive oxygen species (ROS) in the tissues. These products enhance lipid peroxidation and protein oxidation products by causing oxidation of macromolecules such as protein, DNA and lipids. Resultant products mediate harmful effects of ionizing radiation on biological systems. It is known that trace elements have a role in ROS production and oxidant/anti-oxidant system. It is also known that changes in trace element levels cause enhanced negative effects of free oxygen radicals on cellular integrity by decreasing activity of antioxidant defense mechanism[11,12]. In the literature, there are very few data indicating relationship of all trace elements with toxicity in patients receiving radiotherapy for lung cancer[23,24]. In the present study, we evaluate role of trace elements in acute toxicity caused by radiotherapy.

Our results here showed a significant difference in Zn levels between patients with lung cancer and healthy individuals. Se level was lower while Cu level was higher in patients with lung cancer, but the difference didn't reach statistical significance. In the studies, it is shown that intake of essential elements such as Zn, Cu, Fe and Mn at high doses and accumulation of these elements in organs are harmful for human health. It is known that Zn has both carcinogenic and anti-carcinogenic roles. Zn inhibits carcinogenesis by protecting against free radical injury; on the other hand, it is essential for tumor growth due to its role in gene transcription and cell proliferation[5,7,9]. Cihan *et al*. [12] evaluated Zn, Se and Cu levels in scalp hair samples of patients with stage IIIB NSCLC and found that Se and Zn levels were high while Cu levels were low. Authors reported that high levels of Se and Zn at scalp hair increased cancer risk[14,16,23,25,26]. In a study evaluating serum trace elements levels in patients with lung cancer, Çobanoğlu *et al*.[26] reported that serum Cu, Mg and Zn levels were decreased while serum Pb, Cd, Mn, Fe and Co levels were increased. Klarod *et al*.[23] reported that blood Zn and Se levels were significantly lower in patients with NSCLC when compared to healthy individuals. Trace elements Cu, Se and Zn are associated with the risk of lung cancer. To date, there is limited number of epidemiological studies regarding trace metals and risk for lung cancer in the literature[23,24]. Our results, which indicated that higher serum Zn level is associated with a significant protective trend while higher serum Cu level is associated with increased risk for lung cancer, are in agreement with the results of earlier studies.

In this study, the serum Cu and Se levels were found significantly increased after CRT when compared to levels measured before CRT. The decreased Cu levels after CRT were attributed to negative differentiation in serum trace element levels caused by radiation. It was thought that the reason of this decrease is use of SOD enzyme, where Cu is the cofactor, for elimination of negative effects of radiotherapy[24]. Mali *et al*.[25] investigated changes in serum Cu levels in 140 patients with several types of cancer who were exposed to 40-70 Gy radiation for 4-7 weeks and 50 healthy controls. They found that mean Cu levels were higher in cancer patients than controls, but decreased after radiotherapy; however, mean Cu levels were still higher than controls despite this decrease. However, although there are publications indicating increased serum Cu levels after radiotherapy, it is unclear whether this increase is a result or a cause and how this increase occurs.

Further, we observed serum Se level to decrease after CRT (*P*=0.019). In recent studies, Se is considered as an important and beneficial element for biological systems, which was known for toxic and carcinogenic features previously. It is cofactor of many enzymes in human body and it is primarily known for its antioxidant function. By recognition of anti-carcinogenic properties of selenium, potential role in carcinogenesis in human body has taken great interest and studies on this field have accelerated[26,27]. Levander[28] had already demonstrated that selenium deficiency triggers tumor development and that this deficiency occurs due to damage or deficiency in glutathione oxidase enzyme in red blood cells. In addition, studies showed that selenium protects cells against cancer and free radical formation and that there is an increase in the risk for cancer development and free radical formation in case of selenium deficiency. Dubova *et al*.[29] investigated selenium levels before and after radiotherapy in cases with locally advanced lung cancer, head and neck cancer and cervix carcinoma and relationship between selenium level and treatment response. Also, they found that mean selenium levels before radiotherapy were significantly lower in cancer patients when compared to healthy individuals. In addition they had observed that selenium levels were decreased in some patients while increased in other after radiotherapy and interpreted decreased selenium levels after radiotherapy as poor treatment response while increased levels as better treatment response[29].

In the present study, a significant difference was found with Cu and Zn in the assessment of acute toxicity during CRT. Cu, Zn and Se are essential trace elements, which are involved in critical enzyme systems and play role in the maintenance of DNA integrity through prevention of oxidative DNA damage or influence on gene mutations. Zn and Cu play role in various enzymes as a cofactor including Cu/Zn superoxide dismutase (SODs) that degrades superoxide radicals to H_2O_2 and are involved in defense against oxidant stress in lung[30]. In the literature, it has been suggested that both radiotherapy and chemotherapy can further aggravate Se, Zn and Cu deficiency, increasing the likelihood of radiation-induced adverse effects during and after radiotherapy[31]. Zeng *et al*.[32] evaluated Se levels before and after radiotherapy in 95 NSCLC patients with brain metastasis and found Se level as 90.4 µg/L before and 56.3 µg/L after radiotherapy. In the multivariate analysis, authors found that there were significant differences in plasma selenium levels measured before and after radiotherapy when adjusted according to age (*P* <0.001), BMI (*P* <0.001), smoking (*P* <0.001), alcoholism (*P* <0.001), prior chemotherapy (*P* <0.001) and pathological type (*P* <0.001)[32]. German study group data published by Micke *et al*.[33] indicated positive effects of selenium in the management of interstitial edema results from irradiation. In cervix and uterine cancer patients with selenium deficiency, it is reported that selenium supplementation is effective in correcting selenium status in blood which reduces number and severity of radiation-induced diarrhea episodes[31].

Conclusion

In patients with lung cancer, the pre-treatment Zn levels were low compared to healthy individuals. Significant decreases were observed in Cu and Zn levels measured before and after CRT. This study demonstrated that CRT has negative effect on trace element balance. Thus, during radiotherapy and chemotherapy, radiation-related adverse events may be minimized not only by adding appropriate nutrients regarding trace and macro elements into patient's diet or restricting nutrients considered to be risk factor but also using commercial supplements such as vitamins and minerals.

Conflict of interest

Authors declare no competing interests.

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