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KLİNİK ÇALIŞMA
RESEARCH ARTICLE

The effect of surgical specimen-derived phosphorus and lead concentrations in non-small cell lung cancer patients on disease course

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SUMMARY

The effect of surgical specimen-derived phosphorus and lead concentrations in non-small cell lung cancer patients on disease course

Introduction: *Lung cancer is one of the leading causes of cancer-related mortality. There are many exogenic and endogenic factors associated with the development of lung cancer. One of these factors is trace elements. Under- or overabundance of trace elements can disrupt cellular functions and lead to the formation of cancer. In this study we conducted elemental analysis of lung cancer tissue and normal lung tissue to investigate the role of tissue trace element concentrations in lung cancer.*

Materials and Methods: *Elemental analysis was performed on 30 lung cancer tissue samples and a control group of 15 normal lung tissue samples, all taken from patients diagnosed, treated and followed at our hospital between 2005 and 2010. The solubilized tissue samples were analyzed for the presence of 19 elements using inductively coupled plasma-optical emission spectroscopy (ICP-OES). Total element amounts in the tissue were calculated.*

Results: *Concentrations of magnesium, potassium, zinc, manganese, lead, boron, chromium and phosphorus were significantly higher in the patient group compared to the control group. Deceased patients had significantly lower phosphorus concentrations and significantly higher lead concentrations than the other patients.*

Conclusion: *Elevated levels of magnesium, potassium, zinc, manganese, lead, boron, chromium and phosphorus in lung cancer tissue*

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indicate that these elements may play a role in the development of lung cancer. The results of our evaluation of the association between trace elements and lung cancer suggest that, together with other factors, low phosphorus concentration and high lead concentration in tumor tissue may influence disease course.

Key words: *Lung cancer; phosphorus; lead; inductively coupled plasma-optical emission spectroscopy (ICP-OES)*

ÖZET

Küçük hücreli dışı akciğer kanseri hastalarında, cerrahi materyalden tespit edilen fosfor ve kurşun düzeylerinin hastalığın seyrine etkisi

Giriş: *Akciğer kanseri, kansere bağlı ölümlerin en sık sebeplerinden biridir. Akciğer kanserinin oluşumu ve gelişmesi ile ilişkili pek çok ekzojen ve endojen faktör vardır. Bu faktörlerden biri olan eser elementler, vücut dokularında düşük konsantrasyonlarda bulunmalarına rağmen önemli biyolojik fonksiyonlarda görev yaparlar ve bunların yetersiz ya da aşırı alınımı, hücresel fonksiyonları bozarak, kanser oluşumuna sebep olurlar. Bu çalışmada, akciğer kanserli dokular ile normal akciğer dokularının elementel analizi yapılarak, dokulardaki element konsantrasyonlarının akciğer kanserindeki rolü araştırıldı.*

Materyal ve Metod: *Çalışmada 30 akciğer kanserli doku ile kontrol grubu olarak 15 normal akciğer dokusunun element analizi yapıldı. Parafin bloklara gömülü olan doku örneklerinin numune hazırlama sürecinden sonra Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) cihazı ile 19 elementin analizi yapıldı. Olgular 2005-2010 yılları arasında tanı konulan, tedavi ve takibi hastanemizde yapılan hastalardı.*

Bulgular: *Çalışmamızda magnezyum, potasyum, çinko, manganez, kurşun, bor, krom ve fosfor konsantrasyonları hasta grubunda kontrol grubuna göre anlamlı şekilde yüksek bulundu. Ölen hastalarda diğer hastalar karşılaştırıldığında anlamlı oranda fosfor miktarı düşük ve kurşun konsantrasyonu ise yüksek bulundu.*

Sonuç: *Akciğer kanserinin oluşumu ve gelişiminde elementlerin rolünü değerlendirdiğimiz bu çalışmada elde ettiğimiz sonuçlar göz önüne alındığında; kanser dokusundaki fosfor konsantrasyonu düşüklüğü ve kurşun konsantrasyonu yüksekliği diğer faktörler ile birlikte hastalığın seyrinde etkili olabileceği düşünülebilir.*

Anahtar kelimeler: *Akciğer kanseri; fosfor; kurşun; inductively coupled plasma-optical emission spectroscopy (ICP-OES)*

INTRODUCTION

Lung cancer is among the leading causes of cancer-related deaths worldwide. It is the most common cancer in men and the third most common in women. Lung cancer has a high mortality rate, and both surgical and medical therapies are employed in its treatment. The primary treatment for stage I and II cancer is surgery. The 5-year survival rate is 60-80% for stage I patients and 40-60% for stage II patients (1,2). Despite all curative procedures, the mean 5-year survival rate of stage IA lung cancer patients is 73%. In other words, lung cancer recurs in 19% of stage IA patients and 30% of stage IB patients within 5 years after surgery (3). Lung cancer has poor prognosis and high likelihood of recurrence, and despite the elucidation of many aspects of its etiology, much remains unknown. One of these unknowns is trace elements.

Trace elements perform important biological functions despite being present at low concentrations in the tissues of the body. The basic roles of trace elements are not fully known; close associations with enzyme systems are considered to be their main biological function. Trace elements act as enzyme components in biological systems or catalyzers of intracellular chemical reactions. For this reason, insufficient or excessive intake of many trace elements is known to lead to many diseases, including various forms of cancer. The main elements known to cause cancer are beryllium, chromium, cobalt, nickel, arsenic, cadmium, antimony, lead, silver and platinum, whereas the effects of manganese, iron, copper, zinc, selenium and strontium on cancer development have not been conclusively proven (4).

The aims of this study were to compare trace element concentrations in tissue samples from lung cancer patients and healthy controls; to determine whether tumoral tissue element concentrations vary by cell subtype (squamous cell carcinoma and adenocarcinoma) or in living versus deceased patients; and to investigate the relationship between trace element concentrations and survival of lung cancer patients.

MATERIALS and METHODS

Elemental analysis was performed on tissue samples from the patient and control groups. Tumor tissue samples from 35 patients with early stage (stage I and II) lung cancer of different histological subtypes and

healthy lung tissue samples from 15 controls were analyzed. The control samples were obtained from areas of healthy lung in the surgical samples taken from patients who underwent surgery for various diseases other than tumors. Five patients who died of causes other than lung cancer were excluded from the study. Patients' employment histories revealed no profession involving definitive substance exposure. All patients in the study were diagnosed, followed and treated in our hospital between the years 2005 and 2010. Patients who died during the study period were referred to as 'deceased'; patients who were still alive at the conclusion of the study were 'surviving'. Postoperative survival of the patients was calculated in months.

Tissue Sample Preparation

For this study, lung cancer tissue samples of different histological subtype and grade as well as normal lung tissue samples were obtained from the Medical School Department of Pathology. Specimens obtained during surgery were embedded in paraffin blocks, tissue samples were dried in an incubator at 80°C for 24 hours. The dried samples weighed approximately 0.5 g according to a precision scale. Microwave containers were cleaned and prepared for measurement by adding 5 ml of HNO_3 and heating in a microwave oven. The dried tissue samples were placed into the pressurized microwave containers and 3 mL of 30% H_2O_2 and 2 mL of 65% HNO_3 were added sequentially.

Following solubilization of the samples in the microwave oven, the containers were cooled at room temperature for 30 minutes. The solutions were filtered through 125 mm diameter Whatman Grade 42 filter paper into 25 mL volumetric flasks. Distilled/deionized water was added to the flasks to bring the solutions to a total volume of 25 mL; this volume was then divided evenly into two 14 mL tubes.

Elemental analysis was performed on the solubilized tissue samples using an inductively coupled plasma-optical emission spectroscopy (ICP-OES) instrument (Optima 2100 DV ICP/OES, Perkin-Elmer, Shelton, CT, USA).

ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) Analyzer

The working principle of the device is that high-temperature plasma is used to atomize the elements present in solubilized samples; the light emissions of the elements are then measured by a detector to determine the elements' concentrations in the solution. Advantages of ICP-OES instruments are that they provide highly accurate, precise and sensitive analytical results, allow the assessment of low concentrations, and are easy to use (5).

Elemental Analysis

ICP-OES elemental analysis of the cancerous and normal tissues revealed a total of 19 elements: Ca (calcium), Mg (magnesium), Na (sodium), K (potassium), Fe (iron), Cu (copper), Zn (zinc), Mn (manganese), Al (aluminum), B (boron), Ba (barium), Cd (cadmium), Cr (chromium), Mo (molybdenum), Ni (nickel), P (phosphorus), Pb (lead), S (sulfur), and Se (selenium). Total element amounts were calculated after the analysis.

Calculation of the ICP-OES Results

Concentrations expressed in mg/L by the ICP-OES instrument were converted to mg/kg (ppm) using the following formula:

$$\frac{V \times C}{m} = \text{ppm (mg kg}^{-1}\text{)}$$

V: Sample volume (mL)

C: Sample concentration measured by the instrument (mg/L)

m: Initial mass of the solid sample (g)

This study was planned according to the ethics guidelines of the Helsinki Declaration, and the study protocol was approved by the local ethics committee.

Statistical Analysis

The data were analyzed using SPSS version 18 statistical software (SPSS Inc., Chicago, IL, USA). The Mann-Whitney U test was used to compare element concentrations between normal and pathologic tissue and between deceased and surviving patients. The chi-square test was used to compare cell type, disease grade, surgical intervention and comorbid diseases of deceased and surviving patients. The association between element concentrations and postoperative survival was evaluated using Pearson correlation analysis. p values < 0.05 were considered statistically significant.

RESULTS

The cases were separated into patient (n= 30) and control (n= 15) groups. Demographic and clinical data

Table 1. Characteristics of patients and control group

	Patient group	Control group
Number of cases	30	15
Mean age	61	54
Women	6 (20%)	4 (26.6%)
Men	24 (80%)	11 (73.4%)
Cigarette use (pack-years)	45.2 ± 12.1	42.8 ± 2.6
Adenocarcinoma	10 (33.33%)	-
Squamous cell carcinoma	20 (66.67%)	-
Stage I	20 (66.67%)	-
Stage II	10 (33.33%)	-

Table 2. Characteristics of deceased and surviving lung cancer patients

	Surviving (n= 17)	Deceased (n= 13)
Cell type		
Adenocarcinoma	5 (29.4%)	5 (38.5%)
Squamous cell carcinoma	12 (70.6%)	8 (61.5%)
Stage		
Stage I	11 (64.7%)	9 (69.2%)
Stage II	6 (35.3%)	4 (30.8%)
Surgical intervention		
Lobectomy	11 (64.7%)	10 (76.9%)
Pneumectomy	6 (35.3%)	3 (23.1%)
Presence of comorbid diseases	2 (11.8%)	4 (30.8%)
Mean age (years)	59.5 ± 7.3	63.3 ± 6.7
Cigarette use (pack-years)	42 ± 19.1	49.2 ± 19.7
Postoperative survival (months)	---	33 ± 19.6

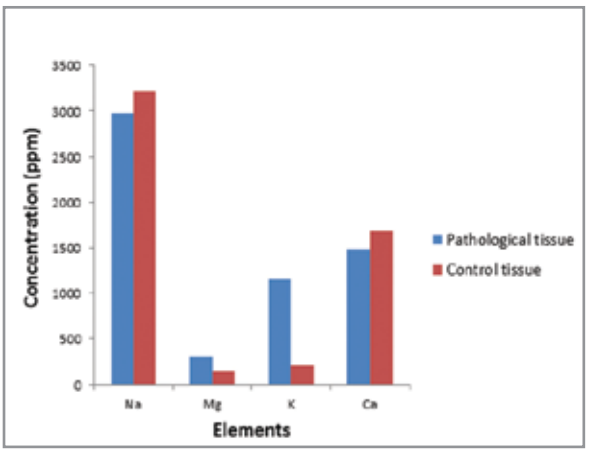

Figure 1. Comparison of macro element concentrations in cancerous and control tissue samples (ppm: parts per million).

for the patient and control groups are shown in Table 1. Patients were also separated into groups based on

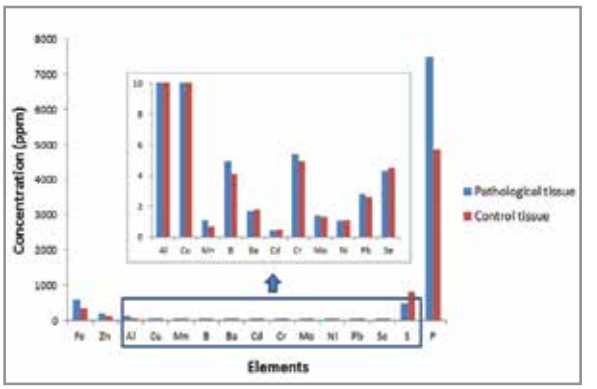

Figure 2. Comparison of micro element concentrations in cancerous and control tissue samples (ppm: parts per million).

histological subtype (adenocarcinoma, n= 10; epithelial cell carcinoma, n= 20) and survival (deceased, n= 13; surviving, n= 17). A comparison of the characteristics of the deceased and surviving patients is shown in Table 2. The individuals in both the patient and control groups were from the same geographical area, were of the same race, and had similar dietary habits. Neither group included anyone with an occupational history associated with elemental exposure. Concentrations of macro- and microelements in the tumoral and normal tissue samples are shown in Figures 1 and 2, respectively.

Of the 19 elements analyzed, concentrations of Mg, K, Zn, Mn, Pb, B, Cr and P were significantly higher in the patient group when compared with the control group (respectively $p< 0.0001$, $p< 0.0001$, $p= 0.001$, $p= 0.001$, $p= 0.001$, $p= 0.005$, $p= 0.014$ and $p= 0.002$). In the patient group, no significant differences in element concentrations were detected between the histological subtypes ($p> 0.05$). In the patient and control groups, tissue samples from both genders were analyzed. Assessment of the relation between gender and element concentrations in the patient group revealed significant differences in two elements ($p< 0.05$). S ($p= 0.001$) was found at higher concentrations in female patients, while Al ($p= 0.001$) was found at higher concentrations in male patients. The presence of age-related variations in element concentration was evaluated; no statistically significant association emerged between age and element concentrations ($p> 0.05$).

Comparison of deceased and surviving patients revealed significant differences in P and Pb concentrations ($p= 0.01$ and $p= 0.004$). The deceased and surviving patients had mean P concentrations of 6.417 ±

1.943 and 8.308 ± 3.000 parts per million (ppm) and mean Pb concentrations of 3 ± 0.75 and 2.4 ± 0.74 ppm, respectively. There were no statistically significant differences in cell type, disease grade, surgical intervention or accompanying diseases between the deceased and surviving patients ($p> 0.05$). No significant association was found between element concentrations and survival time among the deceased patients ($p> 0.05$).

DISCUSSION

Elements are known to act in lung and other cancers by interfering with the immune system and antioxidant defenses. In this study we investigated the effect of some of these elements on lung cancer development and prognosis. Our results indicate that lung cancer patients have higher concentrations of Mg, K, Zn, Mn, Pb, B, Cr and P compared to controls. Our study, which is the first study to compare element concentration between deceased and surviving patients, revealed that deceased lung cancer patients had significantly lower P concentrations and significantly higher Pb concentrations.

In addition to amino acids, glucose, fatty acids and vitamins, minerals are also required for the proliferation and differentiation of cells. Certain inorganic substances, such as iron, zinc, copper, selenium, molybdenum, manganese, chromium, cobalt and iodine, are essential and should be consumed in their daily recommended amounts to maintain human health. These elements are known as trace elements. Without these essential elements, organisms are unable to complete their life cycle or develop normally. Essential elements are necessary for proper bone and blood composition, the maintenance of normal cellular functions, cognitive and physical development, muscle and nerve function, fluid and electrolyte balance, and the normal function of enzymes, hormones and vitamins. Furthermore, these elements also act in many other important biological functions such as oxygen delivery and free radical deactivation (6,7).

Despite their low concentrations, trace elements have various important roles in the body's biochemical processes. In general, excessive intake of vital elements is toxic, while deficiency leads to many diseases and disorders. Therefore, the amounts of these elements in the body are important. Although all elements are important for human health, chrome, iron, phosphorus, cobalt, copper, zinc, selenium, molybdenum and iodine are considered beneficial trace elements; manganese, silicon, nickel, boron, vanadium and tin are possibly beneficial trace elements; and fluorine, arsenic, cadmium, lead, aluminum and mercury are known as potentially toxic elements (6,8,9). In the current study, of 19 elements analyzed in lung tumor tissue and normal lung tissue, Mg, K, Zn, Mn, Pb, B, Cr and P were found at significantly higher concentrations in the patient group, suggesting that in excess amounts, these elements may play various roles in cancer.

One of the elements analyzed in this study was phosphorus, which is the second most abundant chemical element in the human body after calcium. In its inorganic form, phosphorus functions as a part of the structural framework of important biological molecules like RNA and DNA (10). In previous studies evaluating the various functions of phosphorus, mice were injected with human cancer cells as a model, and phosphorus was found to have anti-cancer activity (11-13). Low-dose phosphorus injections were found to significantly inhibit tumor growth in a murine xenograft model (14). Another study demonstrated that ATP with phosphorus in its structure had good tumor penetration, low immunogenicity and anti-cancer potential due to its pharmacokinetic properties (15). A recent study of the anti-cancer activity of phosphorus demonstrated that aqueous P is incorporated into nascent DNA. Through phosphorilization of the H2-AX histone, P disrupts the double helix, resulting in cytotoxicity which in turn increases apoptosis (10). In our study, phosphorus was found at higher concentrations in lung cancer tissue than in normal lung tissues, but at lower concentrations in deceased lung cancer patients than surviving patients. These results suggest that the bodies of lung cancer patients attempted to increase their tissue P concentrations in defense; we hypothesize that deceased patients were unable to raise P concentrations sufficiently to achieve the cytotoxic effect and were therefore unable to eliminate the tumor tissue.

Trace elements act as enzyme components in biological systems or catalyzers of intracellular chemical reactions. For this reason, insufficient or excessive intake of many trace elements is known to lead to many diseases, including many forms of cancer. Beryllium, chromium, cobalt, nickel, arsenic, cadmium, antimony, lead, silver and platinum are elements known as the primary elements that contribute to cancer formation (4). In our study, Pb was found in high amounts in all cancerous tissue and deceased patients

had higher Pb levels compared to surviving patients. The association between Pb and disease could be due to its destructive effect on macrophages, which are present in most tissues and responsible for defense against various pathogens. In addition to diminishing the innate immune activity of macrophages, Pb exposure skews the immune system toward Th2 dominance, which can increase allergic and antibody-mediated autoimmune reactions. Furthermore, Pb exposure damages DNA through the generation of reactive oxygen and nitrogen species. DNA damage increases the incidence of cancer (16,17). We believe our finding of elevated Pb in all lung cancer tissue and especially in the tissues of deceased patients demonstrates that together with many other factors, lead has a negative effect on the immune system and its defense against tumors.

Our study is novel in that it is the first investigation of 19 elements in lung cancer tissue and normal tissue using the ICP-OES technique and is the first to compare deceased and surviving patients in this context.

One limitation of the study is that due to the small number of cases, it was not possible to statistically eliminate the effect of other factors on prognosis. Another limitation of this study was that the characteristics of the patient and control groups were not adequately matched.

There are many factors in the development and disease course of lung cancer, and chemical elements are one of these factors. The low P levels and high Pb levels observed in this study suggest that they may also effect the development and course of lung cancer. Larger studies are necessary to further elucidate the importance of these elements in the etiology of lung cancer.

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The authors declare that they have no conflict of interest. Informed consent was obtained from all individual participants included in the study.

REFERENCES

1. *Naidoo R, Windsor MN, Goldstraw P. Surgery in 2013 and beyond. J Thorac Dis 2013;5:S593.*
2. *Wright G, Manser RL, Byrnes G, Hart D, Campbell DA. Surgery for non-small cell lung cancer: systematic review and meta-analysis of randomised controlled trials. Thorax 2006;61:597-603.*
3. *Lim EGP. Principles of the surgical treatment of lung cancer. Krakow, Poland: Medycyna Praktyczna, 2014.*
4. *Carvalho M, Magalhaes T, Becker M, Von Bohlen A. Trace elements in human cancerous and healthy tissues: a comparative study by EDXRF, TXRF, synchrotron radiation and PIXE. Spectrochimica Acta Part B: Atomic Spectroscopy 2007;62:1004-11.*
5. *Carpenter RC. The analysis of some evidential materials by inductively coupled plasma-optical emission spectrometry. Forensic Sci Int 1985;27:157-63.*
6. *Patriarca M, Menditto A, Di Felice G, Petrucci F, Caroli S, Merli M, et al. Recent developments in trace element analysis in the prevention, diagnosis, and treatment of diseases. Microchem J 1998;59:194-202.*
7. *Zheng Y, Li X-K, Wang Y, Cai L. The role of zinc, copper and iron in the pathogenesis of diabetes and diabetic complications: therapeutic effects by chelators. Hemoglobin 2008;32:135-45.*
8. *Navarro Silvera SA, Rohan TE. Trace elements and cancer risk: a review of the epidemiologic evidence. Cancer Causes Control 2007;18:7-27.*
9. *Drake EN, Sky-Peck HH. Discriminant analysis of trace element distribution in normal and malignant human tissues. Cancer Res 1989;49:4210-15.*
10. *Cheng Y, Kiess AP, Herman JM, Pomper MG, Meltzer SJ, Abraham JM. Phosphorus-32, a clinically available drug, inhibits cancer growth by inducing DNA double-strand breakage. PloS One 2015;10:e0128152.*
11. *Richmond A, Su Y. Mouse xenograft models vs GEM models for human cancer therapeutics. Dis Model Mech 2008;1:78-82.*
12. *Morton CL, Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. Nat Protoc 2007;2:247-50.*
13. *Wang T, Weigt SS, Belperio JA, Lynch JP. Immunosuppressive and cytotoxic therapy: pharmacology, toxicities, and monitoring. Semin Respir Crit Care Med 2011;32:346-70.*
14. *Cheng Y, Yang J, Agarwal R, Green GM, Mease RC, Pomper MG, et al. Strong inhibition of xenografted tumor growth by low-level doses of [(32)P]ATP. Oncotarget 2011;2:461-6.*
15. *Patrick MR, Chester KA, Pietersz GA. In vitro characterization of a recombinant 32P-phosphorylated anti-(carcinoembryonic antigen) single-chain antibody. Cancer Immunol Immunother 1998;46:229-37.*
16. *Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology 2011;283:65-87.*
17. *Kasten-Jolly J, Lawrence DA. Lead modulation of macrophages causes multiorgan detrimental health effects. J Biochem Mol Toxicol 2014;28:355-72.*