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Research Article / Araştırma Makalesi

Analysis of Serum Nickel, Silicium, Arsenic and Boron in Smoking Individuals

Sigara İçenlerde Serum Nikel, Silisyum, Arsenik ve Bor Analizi

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Abstract

Smoking induces inflammation and oxidative stress via radical production from chemicals resulting in cardiocerebrovascular and respiratory diseases, cancers, stroke and sudden death. Elements found in tobacco plant and tobacco smoke are absorbed into blood circulation and transferred into blood and peripheral tissues. The aim of the present study is to evaluate the alterations of serum nickel (Ni), silicium (Si), arsenic (As) and boron (B) levels in smokers. The study groups were categorized as individuals who quitted smoking (Group 1; n: 35; 15 female/20 male), who were smoking (Group 2; n:35; 13 female, 22 male) and who never-smoked (Group 3; n; 40; 20 female/20 male). Biochemical parameters were analyzed in Biochemistry Laboratory of Haseki Traning and Research Hospital. Serum element levels were measured using Inductively Coupled Plasma Atomic Emission Spectroscopy in Trace Element Laboratory of Biophysics Department of Cerrahpasa Medical Faculty at Istanbul University-Cerrahpasa. ANOVA test and Pearson's correlation tests were used for statistical analysis and $p < 0.05$ was evaluated as statistically significant. Serum Ni levels of Group 2 were higher than the other study groups with no significance. Group 2 had statistically higher serum Si and As levels than Group 1 and Group 3 ($p < 0.01$, $p < 0.05$, respectively). There was no statistical significance by means of serum B levels among study groups. Increased serum levels of Si and As in smokers might induce atherosclerosis via inflammation, dyslipidemia and burden oxidative stress. Besides, higher serum Ni levels of smokers might reflect its toxic effects. However, serum B was lower in smokers probably related with its consumption in biological defence mechanisms. Monitorization of serum nickel, silicium, arsenic and boron levels should be considered as biomarkers for smokers.

Keywords: Nickel; silicium; arsenic; boron; smoking

Özet

Sigara içimiyle oluşan radikaller, inflamasyon ile oksidatif stresi uyararak kardiyoserebrovasküler ve solunum sistemi hastalıklar, kanserler, felç ve ani ölüme sebep olmaktadır. Tütün bitkisinde ve sigara dumanında bulunan elementler kan dolaşımı aracılığıyla perifer dokulara geçmektedir. Bu çalışmanın amacı, kronik obstrüktif akciğer hastalığı (KOAH) tanısı olmayan sigara içenlerde serum nikel (Ni), silisyum (Si), arsenik (As) ve bor (B) düzeylerindeki değişiklikleri değerlendirmektir.Gereç ve Yöntemler: Çalışma grupları sigarayı bırakanlar (Grup 1; n: 35; 15 kadın / 20 erkek), sigara içenler (Grup 2; n: 35; 13 kadın, 22 erkek) ve sigara içmeyenlerden (Grup 3; n; 40; 20 kadın / 20 erkek) oluşturuldu. Biyokimyasal parametreler Haseki Eğitim ve Araştırma Hastanesi Biyokimya Laboratuvarında ölçüldü. Serum element düzeyleri İstanbul Üniversitesi-Cerrahpaşa, Cerrahpaşa Tıp Fakültesi Biyofizik Anabilim Dalı Eser Element Laboratuvarı'nda İndüktif Eşleşmiş Plazma Atomik Emisyon Spektroskopisi kullanılarak ölçüldü. İstatistiksel analiz için ANOVA testi ve Pearson's korelasyon testleri kullanıldı. $p < 0.05$ istatistiksel olarak anlamlı olarak değerlendirildi. Grup 2'nin serum Ni düzeyleri diğer çalışma gruplarına daha yüksek olmasına rağmen, istatistiksel anlamlılık yoktu. Grup 2'nin serum Si ve As düzeyleri, Grup 1 ve Grup 3'ten istatistiksel olarak daha yüksekti ($p < 0.01$, $p < 0.05$, sırasıyla). Çalışma grupları arasında serum B düzeyleri açısından istatistiksel anlamlılık yoktu. Sigara içenlerde artmış serum Si ve As düzeyleri, inflamasyon, dislipidemi ve oksidatif stres aracılığıyla ateroskleroza neden olabilir. Ayrıca, sigara içenlerde serum Ni düzeylerinin yüksek olması, Ni'in toksik etkilerini yansıtabilir. Bununla birlikte, sigara içenlerde serum B düzeyinin daha düşük olması, muhtemelen biyolojik savunma mekanizmalarında tüketilmesinden kaynaklanmaktadır. Serum nikel, silisyum, arsenik ve bor düzeylerinin takibi sigara içenler için biyolojik belirteçler olarak kullanılabilir.

Anahtar Kelimeler: Nikel; silisyum; arsenik; bor; sigara içimi

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1. Introduction

Smoking-related diseases have increased the number of deaths in developing countries, reported by The World Health Organization (1). Smoking is not only hazardous for smokers, it also induces other healthy individuals in many ways. Smoking acts as a pathologic initiative factor that produces oxidant status including reactive oxygen species (ROS), damage for biomolecules like DNA, RNA, proteins and enzymes, and inflammation (2,3). Smoking not only establishes a risk factor for cardiocerebrovascular diseases (CCVD), but also induces respiratory diseases and many cancers especially lung cancer, stroke and sudden death (4).

Cigarette includes many toxic elements and metabolites like carbon monoxide and cyanide. Toxic elements inhaled from the tobacco smoke are transferred to blood circulation and are accumulated in special organs like lungs, liver, kidney. Smoking and exposure to smoking disturb blood circulation and peripheral tissue oxygenation both directly or indirectly (5,6). Smoking is also known to have a close relationship with trace elements, which are essential inorganic compunds for biological organism. Their levels in the body and body fluids are effected by nutritional, environmental and habitual factors like smoking and alcohol abuse (7).

In the present study, we focused on serum nickel (Ni), silicium (Si) and arsenic (As) toxic elements and boron (B) as a protective element for human health in smokers. Ni is not an essential element for nutrition in human metabolism. Its functional metabolism could not be explained for humans and animals. Humans are exposed to Ni by orally, by inhalation and by cutaneously producing inflammation and oxidative status within the organism. One of the exposure of human body to Ni is by smoking. Most of Ni in cigarette smoke is volatile and its chemical composition has not been explained yet (8,9). Silicium (Si) that is one of the most abundant elements in the world can be inhaled into the respiratory system in different forms of silicium oxide. Silica particles are transported into the lungs via tobacco smoke inhalation (10,11). Choux et al., reported that alveolar macrophages of a patient diagnosed with pulmonary fibrosis associated with tobacco was stated to contain many silica particles (12). Heckman and Lehman presented that rats had inclusions of Si in their lung epithelial cells after being exposed to chronic tobacco smoke (10). Individuals who do not have smoking habit may just be exposed to Si occupationally. Few data related with Si might be due to the difficulties of its analysis (13). Arsenic (As) is an element frequently found especially in water, soil and air within the environment. As toxicity can effect millions of people causing pathologic conditions in many body systems such as gastrointestinal, cardiovascular, respiratory, hematopetic and cutaneous region (14,15). The biological and positive effects of boron (B) have been discussed in several researches related humans and animals. B has important roles for mineral status, hormone, lipid and energy metabolisms, bone structure, cell wall metabolism and enzymatic reactions, anti-oxidant defence by elimination of reactive oxygen species and immune system (16-18).

Smoking is an initiative factor for pathological conditions like chronic obstructive pulmonary disease (COPD) inducing oxidative stress via ROS production from chemicals and additives. Despite the fact that Ni, Si and Ar are known to be inhaled by respiratory system, their specifity of being toxic even with very minor concentrations and their negative effects on biological organism could not have been elucidated yet. The aim of the present study was to evaluate the effects of smoking on alterations of serum nickel, silicium, arsenic and boron levels in smoking individuals.

Materıals and Methods

Study groups

The individuals who admitted to Outpatient Clinic of Department of Respiratory Medicine at Haseki Traning and Research Hospital, accepted to join the present study with their written consent included the study. The ethical approval was taken from Istanbul University-Cerrahpasa/Cerrahpasa Medical Faculty Ethics Committee. Serum levels of Ni, Si, Ar and B were analyzed for the all

individuals included in the study (n: 110). The study groups were establihed as individuals who quitted smoking (Group 1; n: 35; 15 female/20 male), who were smoking (Group 2; n:35; 13 female, 22 male) and who never-smoked (Group 3; n; 40; 20 female/20 male) (19).

Demographic variables of the individuals included in the study such as age, gender and body mass index, and systolic/diastolic blood pressures were recorded.

Individuals who were diagnosed with COPD (n: 2), diabetes mellitus (n: 4), metabolic diseases (n: 1), cancer (n: 2) and autoimmune diseases (n: 1), and who had pathologic values for blood parameters (n: 2) were excluded from the study. The blood samples of the individuals were collected in the morning after a night fasting interval into plastic blood test tubes without any preservative in order to prevent any metal or element interaction. Whole blood samples were centrifugated utilizing a Hettich Universal centrifuge at 3000 rpm for 20 minutes to obtain serum for biochemical and element analysis. Biochemical analysis were held in Biochemistry Laboratory of Haseki Traning and Research Hospital. All blood samples with hemolysis were excluded from the study. Centrifugated seum samples were collected in Eppendorf tubes at -80 ºC until the analysis of serum elements.

Analytical method

Element analysis for serum levels of Ni, Si, Ar and B was conducted using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) (iCAP 6000-Thermo) in Trace Element Laboratory of Biophysics Department of Cerrahpasa Medical Faculty at Istanbul University-Cerrahpasa. Each serum element analysis was measured triplicate and their average was evaluated. Standard solutions were prepared for each analyzed element (Ni, Si, Ar, B) from each of their 1000 µg/dL stock solutions. Calibration graphs were drawn for every element using these standard solutions and deionized water as a blank solution. The analysis of serum samples were done on the same day using the same calibration method in order to prevent any interaction from medium features like temperature and moisture. Element analysis was performed in both axial and radial mode for each element. Serum levels of analyzed elements were expressed in micrograms per milliliter (µg/mL) (2). The technical data related with ICP-OES system are summarized in Table 1.

Table 1. Technical and Analytical Parameters of ICP-OES

Parameters	Ni	B	Si	As
Analysis of replicate number	3	3	3	3
Wavelength (nm)	206.200	249.775	251.611	189.040
Power (kW)	1.3	1.3	1.3	1.3
Plasma gas flow (L/min)	15	15	15	15
Auxiliary gas flow rate (L/min)	15	15	15	15
Nebuliser gas flow (L/min)	0.7	0.7	0.7	0.7
Sample flow (L/min)	1.51	1.51	1.51	1.51
Pump speed (rpm)	100	100	100	100
Mist chamber	Stumar master	Stumar master	Stumar master	Stumar master
Nebuliser	V-groove	V-groove	V-groove	V-groove
Max curve order	1	1	1	1
Calibration curve limit	0.999	0.999	0.999	0.999

ICP-OES: inductively coupled plasma atomic emission spectroscopy, Ni: nickel, B: boron, Si: silicium, As: arsenic

Statistical analysis

Data obtained from analysis were evaluated utilizing SPSS 21.0. Serum element levels were expressed as means ± standard deviation (SD). ANOVA parametric test was used for variables with normal distribution to evaluate the mean within the three study groups. Non-parametric tests as Kolmogrov and Tamhane tests were used for variables without normal distribution to evaluate the median within the three study groups. The mean and median values were evaluated within 95% confidence interval. Pearson correlation analysis was used for the evaluation of parameters among the study groups and $p < 0.05$ was evaluated as statistically significant.

2. Results

Demographic data and biochemical parameters of study groups were given in Table 2. There was no statistical significance among study groups by means of age, body mass index, systolic blood pressure and diastolic blood pressure (Table 2).

Table 2. Demographic Data and Biochemical Parameters of Study Groups

Elements	Group 1 (n:35) (Ex-smokers)	Group 2 (n:35) (Smokers)	Group 3 (n:40) (Healthy controls)
Age	49±7	40±9	46±6
Gender (F/M)	15/20	13/22	20/10
BMI (kg/m2)	25.36±4.37	24.97±4.23	26.82±4.15
SBP (mmHg)	116.28±16.46	126.82±14.91	124.74±14.81
DBP (mmHg)	72.20±8.15	71.65±7.12	74.45±8.45
WBC (10^3/mL)	7.16±1.02	7.45±1.62	6.85±1.42
Hgb (g/dL)	14.20±1.12	13.68±2.23	14.56±1.42
Hematocrit (%)	42.70±3.77	43.27±4.45	42.85±3.73
Glucose (mg/dL)	95.06±9.01	92.16±4.45	96.87±8.02
FT_3 (ng/dL)	3.24±0.47	3.38±0.56	3.20±0.38
FT_4 (ng/dL)	1.21±0.53	1.19±0.37	1.20±0.32
TSH (mIU/L)	1.67±0.40	1.80±0.87	1.73±0.57
ALT (U/L)	21.83±8.92	25.70±7.60	17.90±5.97 [a**, b**]
AST (U/L)	23.20±6.83	24.35±8.42	19.56±6.62 [a**, b**]
Uric acid (mg/dL)	4.80±0.97	4.70±1.10	4.38±1.43
Creatinin (mg/dL)	0.83±0.12	0.94±0.14	0.70±0.13 [a*, b*]
TC (mg/dL)	173.12±30.30	175.00±27.30	184.26±27.53
TG (mg/dL)	103.65±37.82	121.84±60.45	89.00±25.52 [b*]
LDL (mg/dL)	98.75±34.69	104.45±35.53	92.82±30.20 [b*]
HDL (mg/dL)	46.29±11.15	41.10±13.78	53.12±13.67 [b*]

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, WBC: white blood cell, Hgb: hemoglobin, FT_3: free triiodothyronine, FT_4: free thyroxine, TSH: tyhroid stimulating hormone, ALT: alanine aminotransferase, AST: aspartate aminotransferase, TC: total cholesterol, TG: triglyceride, LDL: low density lipoprotein, HDL: high density lipoprotein. Data are shown as the means±SD. [a]Statistical comparison of Group 1 with Group 2 and 3, [b]Statistical comparison of Group 2 with Group 3. ∗p < 0.05, ∗∗p < 0.01

No statistical significancy was found among study groups in terms of white blood cells (WBC), hemoglobin (Hgb), hematocrit, and levels of serum glucose, free triiodothyronine, free thyroxine levels and thyroid stimulating hormone. Group 1 and Group 2 had significantly higher serum alanine aminotransferase and aspartate aminotransferase levels compared with Group 3 ($p < 0.01$). Serum creatinine levels were statistically higher in Group 1 and Group 2 than Group 3 ($p < 0.05$). Group 1 and Group 2 had higher serum uric acid levels compared with Group 3, however there was no statistical

significancy. Serum levels of total cholesterol (TC) were higher in Group 2 compared with other study groups with no statistical significancy. Serum triglyceride (TG) and low density lipoprotein (LDL) levels were statistically higher in Group 2 than Group 3 ($p < 0.05$) and Group 1 with no statistical significancy. However, serum high density lipoprotein levels of Group 2 were significantly lower than Group 3 ($p < 0.05$) and Group 1 with no statistical significancy (Table 2).

Serum Ni levels were higher in Group 2 compared with Group 1 and 3, however there was no statistical significance. Serum Ar levels were measured to be statistically lower in Group 2 compared with Group 3, however no statistical significancy was detected between Group 1 and Group 3. Serum Si levels were statistically higher in Group 2 than in Group 3 ($p < 0.01$). Group 1 also had lower serum Si levels compared with Group 3, but with no statistical significance. When serum B levels were compared, Group 1 (0.04 ± 0.02 µg/mL) and Group 2 (0.03 ± 0.01 µg/mL) had lower values of serum B levels than Group 3 with no statistical significance (Table 3).

The correlations between changes in variables in Group 1 and Group 2 were given in Table 4 and Table 5, respectively.

Table 3. Serum Levels of Ni, Si, As and B in Study Groups

Elements	Group 1 (n:35) (Ex-smokers)	Group 2 (n:35) (Smokers)	Group 3 (n:40) (Healthy controls)
Ni (µg/mL)	0.55 ± 0.18	0.61±0.20	0.51±0.10
Si (µg/mL)	9.66±1.53	12.75±3.06	9.26±2.40 [b**]
As (µg/mL)	0.08±0.02	0.12±0.03	0.07±0.02 [b*]
B (µg/mL)	0.04±0.02	0.03±0.01	0.05±0.02

*Ni: nickel, Si: silicium, As: arsenic, B: boron. Data are shown as the means±SD. [a]Statistical comparison of Group 1 with Group 2 and 3, [b]Statistical comparison of Group 2 with Group 3. *p < 0.05, **p < 0.01.*

Table 4. Correlations Between Changes in Variables in Group 2

Parameters	r
Ni - TC	0.520**
Si - As	0.542**
Si - B	0.534**
As - B	0.385*
As - TG	0.354*
B - TC	-0.410*
B - LDL	-0.320*

*r: correlation coefficient, Group 2: smokers, Ni: nickel, Si: silicium, As: arsenic, B: boron, TG: triglyceride, TC: total cholesterol, LDL: low density lipoprotein. *p<0.05, **p < 0.01.*

Table 5. Correlations Between Changes in Variables İn Group 1

Parameters	r
Ni - TC	0.530**
Si - WBC	0.626**
Si - Hgb	0.608**
As - Hgb	0.475*
As - Creatinine	-0.433*

*r: correlation coefficient, Group 1: ex-smokers, Ni: nickel, Si: silicium, As: arsenic, TC: total cholesterol, WBC: white blood cell, Hgb: hemoglobin. *p<0.05, **p < 0.01.*

3. Discussion

There is strong evidence related with smoking and its pathological consequences in biological organism invluding CCVD, many cancer types especially lung cancer and COPD (2,13). It has been accepted that serum levels of elements such as Ni, Si and Ar are higher in smokers and individuals who are exposed to smoking than non-smokers, whereas the underlying mechanisms related with these isssue have not explained in detail yet (9,13,15). Many studies related with B reported that B was a protective element functioning in decreasing the risk of CCVD by inhibiting atherosclerosis, inflammation and stroke, and supporting the immune system by scavenging ROS (16,20). Our study groups presented some alterations in their demographical data and biochemical parameters within reference ranges, but these results did not reflect to their clinical situation. In this study, we aimed to investigate particular effects of serum Ni, Si, Ar and B elements in smokers.

Ni and its compounds are toxic for biological organism. The effects of Ni on biological organisms depend on its chemical and physical structure, concentration and exposure time. Ni is a potentially toxic element that has hazardous effects on lungs, kidneys, liver and hematopetic system (8,9,13). There have been also many studies related with healthy individuals exposed to Ni. Gil et al. (21) reported that whole blood Ni levels were higher in smokers compared with non-smokers with no statistical significance. Khlifi et al (22) revealed out that there was a strong correlation between smoking and whole blood Ni levels. In a thesis related with heavy metals, it was stated that smokers had higher whole blood Ni compared with non-smokers (8). Besides, urine Ni levels were also higher in children who had seconhand exposure smoking than un-exposed children (23). Nickel levels of the lung tissue of smokers were reported to be higher than non-smokers (13). Consistent with the previos studies, the present study revealed out that serum Ni levels of smokers and individuals who quitted smoking were higher than healthy controls with no statistical significancy. There was a positive correlation between serum Ni levels and TC both in Group 1 and Group 2 that might indicate the inflammation process in blood vessels triggered by dyslipidemia.

Si and its chemical compounds are reported to present on both the surface section and interior components of tobacco leaves (11). Thus, Si analyzed in cigarettes and its smoke have a strong correlation with the concentration of these Si compounds. Si is distributed from inhalation into blood flow leading to atherosclerosis via inflammation in vital organs like lung, brain, liver and kidneys. It has been widely accepted that Si exposure induced inclusion particles formation in macrophages found in bronchiolar, alveolar and interstitial space of lungs in smokers (10,11,24). The difference of the present study was that serum Si levels were analyzed in study groups and our results were consistent with the previous studies by means of Si accumulation in biological tissues. The higher serum Si levels in smokers compared with healthy controls might report the bioaccumulation of Si in blood tissue. The positive correlation of serum Si with WBC and Hgb in Group 1 indicated that defence mechanisms to compensate burden of ROS and to improve oxygen status in biological organism have just started.

Exposure to As for humans is not only by water sources, also As is taken into the organism via smoking and is excreted by urine with a mean half-life of three or four hours (25). While As is transfered through alveolocapillary membrane, it is readily distibuted to the whole body by blood flow. Kucukkurt et al. (26) used As diluted drinking water in rats to induce oxidative stress in blood and vital tissues such as kidney, liver, brain and heart. According to the results of this work, Group 2 had significantly higher serum As levels than Group 3 indicating the accumulation of Si in blood tissue. Even the half-life of As is about three to four hours and its analytical determination is difficult, As levels of the smokers in our study could be detectable due to the fact that smokers had the habit of smoking regularly. Besides, there was a positive correlation between Si and As in

smokers supporting their synergistic hazardous effects for organism. The higher serum levels of TG and the positive correlation between As and TG in Group 2 might indicate the deteriorating effect of As on lipid profile. The higher value of creatinine and the negative correlation between serum As levels and creatinine in Group 1 might present that the filtration functions of kidneys started to improve after quitting smoking. Serum As levels were positively corrrelated with Hgb values in Group 1 that might be a consequence of re-distribution of As from organs like kidney, liver and brain. It is known that As is excreted from blood tissue quickly after smoking cessation by its short half-life, so that its re-distribution would start from the organs.

B is accepted as a protective element regulating vital mechanisms for human health, taking an active role in anti-oxidant defence systems and immune system (16-18). Protano et al. (23) reported that children who had seconhand exposure had statistically higher urine B Levels compared with un-exposed children. Kucukkurt et al. (26) reported in As induced oxidative stress in female and male rats that B significantly decreased oxidant status in liver, kidney, heart and brain tissues. Although there is no significancy among study groups by means of serum B levels, the positive correlation of B with Si and As in Group 2 might postulate the defence system of B over the harmful effects of these elements. B has a decreasing effect on lipid molecules by diminishing their clustering and preventing atherosclerosis (20). Consistently, there was a negative correlation of B with TC and LDL in Group 2 that might emphasize the consuption of B due to dyslipidemia.

As a result, the present study postulated the inflammation, dyslipidemia and burden of ROS leading to atherosclerosis associated with disturbed blood flow in smokers. Alterations of serum trace element levels have not reflected to the clinical onset of smokers. Statistically higher serum levels of Si and As in smokers might introduce their synergistically accumulation in blood tissue. Despite there was no statistical significance in terms of serum Ni levels, higher Ni levels in smokers might be a reflection of its toxic effects. The positive correlation of serum B levels with Si and As might be clarified with the protective effect of B via balancing dyslipidemia. The overlooked effect of elements on smoking may be achieved by conducting detailed research covering their underlying mechanisms. In conclusion, we consider that serum nickel, silicium, arsenic and boron levels should be evaluated as biomarkers for smokers.

- Nickel and boron elements were presented at Turkish Physical Society 36th International Physics Congress as an oral presentation.
- Karis D., Ates Alkan F., Cakmak G., Ercan A.M., “Effects of Smoking on Serum Nickel and Boron Levels”, Turkish Physical Society 36th International Physics Congress, Kadir Has University, Istanbul, Turkey, 01 - 05 September 2020, (Abstract Book, 08OP3, pp 134).

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