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Cerrahi Endarterektomi Plaklarının Elemental Yapısı

Elemental Composition Of Surgically Removed Carotid Plaques

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ÖZ

GİRİŞ ve AMAÇ: *Epidemiyolojik çalışmalar diyet, sigara ve çevresel faktörler ile alınan metallerin artmış kardiyovasküler risk oluşturduğunu göstermektedir. Aterosklerotik karotis plaklarının element yapısı ile ilgili sınırlı çalışma mevcuttur.*

YÖNTEM ve GEREÇLER: *Çalışmada cerrahi karotis endarterektomi materyallerinin element yapısı ve hastaların klinik özellikleri incelendi. Karotis endarterektomi yapılan 19 hastadan alınan plaklarda indüktif-eşli plazma emisyon spektrometresi (ICP-OES) yöntemi ile element yapısı incelendi.*

BULGULAR: *Hastalardan sadece iki tanesi kadındı. Ortalama yaş 71.9±7.2 yıl olarak bulundu. Hastaların %47.4'ünde diyabet ve %52.6'sında hipertansiyon saptandı. Sadece 1 hasta hiç sigara içmemişti. Plaklarda sodyum (Na), magnezyum (Mg), potasyum (K), kalsiyum (Ca), fosfor (P), demir (Fe), bakır (Cu), bor (B), manganez (Mn), çinko (Zn), alüminyum (Al), sülfür (S), krom (Cr), nikel (Ni), platin (Pt), antimon (Sb), selenyum (Se) elementleri farklı konsantrasyonda saptandı fakat arsenik (As), bizmut (Bi), kadmiyum (Cd), kobalt (Co), molibden (Mo), kurşun (Pb) seviyeleri ICP-OES sisteminin belirleme limitinin altında saptandı. Hastalarda varyasyon olmakla birlikte en yoğun olarak Ca ve P saptandı.*

TARTIŞMA ve SONUÇ: *Saptanan elementlerin aterojenik sürece katkıları detaylı incelenmelidir. Ca ve P'nin sık bulunan Ca(NO3)2, CaCl2, Na2HPO4, (NH4)2HPO4 gibi tuzları kanda ve suda yüksek çözünürlüğe sahiptir fakat Ca10 (PO4)6(OH)2'nin (kemikteki hidroksi apatit) çözünürlüğü çok düşüktür. Kan akımı ile aterosklerotik plak arasında bir hücre tabakası olduğu için muhtemelen Ca ve P elementleri plak içine ayrı olarak gelip suda çözünmeyen kompleksler oluşturarak birikmektedir. Karotis plaklarında element birikmesi, bu elementleri kemik ve diyetten gelen kısımlarının detaylı araştırılması ile yeni önleyici ve tedavi edici yaklaşımlar geliştirilebilir.*

Anahtar Kelimeler: *endarterektomi, element, ateroskleroz*

ABSTRACT

INTRODUCTION: *Epidemiological studies indicate that metal exposure through diet, environment and smoking is associated with increased risk of cardiovascular disease. There are few data on the elemental composition of atherosclerotic carotid plaques.*

METHODS: *We examined clinical characteristics and elemental composition of surgical carotid endarterectomy plaques. Carotid endarterectomy plaques from 19 patients were collected and elemental compositions were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) method.*

RESULTS: *Two of the 19 patients were female. Average age of the patients was 71.9±7.2 years. 47.4% of the patients were diabetic and 52.6% were hypertensive. Only one of the patients never smoked. Sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), phosphorus (P), iron (Fe), copper (Cu), boron (B), manganese (Mn), zinc (Zn), aluminum (Al), sulphur (S), chromium (Cr), nickel (Ni), platinum (Pt), antimony (Sb), selenium (Se) were found in different concentrations but concentrations of arsenic (As), bismuth (Bi), cadmium (Cd), cobalt (Co), molybdenum (Mo), lead (Pb) in the carotid endarterectomy samples were below the detection limit of ICP-OES. Despite of fluctuations of metal concentrations among the patients, concentrations of Ca and P were significantly high with averages of 68613 ppm and 36669 ppm respectively.*

DISCUSSION AND CONCLUSION: *Results of those element levels should be carefully evaluated for their role in proatherogenic role. Common salts of Ca and P such as Ca(NO3)2, CaCl2, Na2HPO4, (NH4)2HPO4 are highly soluble in water and in blood but complexes of Ca with P such as Ca10(PO4)6(OH)2 (hydroxyapatite in bones) has very low solubility. Since there is a cell layer between the atherosclerotic plaques and the blood flow, probably soluble salts of Ca and P ions come to the accumulation site separately and they form insoluble complexes, which cannot pass the cell layer and accumulate. Also, reasons of the elemental accumulation and sources of the elements from bones, diet or other sources should be investigated in detail because they lead to development of new preventive and treatment approaches for carotid atherosclerotic plaques.*

Keywords: *endarterectomy, elements, atherosclerosis*

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Cardiovascular diseases are still the pathology most widespread all over the world and many factors have effects on the development of cardiovascular diseases. There are several theories that account for a progressing atherosclerotic process in heart vessels. Atherosclerotic plaques increase with age but most of them are clinically silent (1). Requirements for plaque rupture are formation of a vulnerable plaque and later plaque rupture, thrombus formation and clinical events of stroke or myocardial infarction. Vulnerable plaque has a thin fibrous cap, neovascularization and a large lipid core (2). Many atherosclerosis studies have focused on changes in lipid metabolism, atherothrombosis, inflammatory processes, and cellular plaque composition, however there are few studies on the elemental composition of atherosclerotic carotid plaques. In previous autopsy studies high cadmium levels were detected in aortic wall and carotid endarterectomy materials (3-8). Elucidating the content and ratio of chemical elements in the atherosclerotic plaque of heart vessels may add the missing links of atherogenesis (9). The goal of this study was to determine and estimate the complex chemical elements in surgical endarterectomy specimens.

METHODS

In the study we used endarterectomy samples from the carotid plaques of symptomatic or asymptomatic patients with carotid stenosis. The endarterectomy samples in the present study (n = 19) were put into ice bags and transported for analysis. Clinical data were obtained through patient questionnaires and medical records.

Sample preparation and inductively coupled plasma-mass spectrometry:

Using the Berghof microwave digestion system, tissue samples were digested in an acid solution – first in a microwave inside a closed Teflon vessel for 1 h 45 min, and then in a microwave oven (Berghof Speedwave, Germany). Prior to analysis, samples were isolated from metallic materials and dust to avoid contamination. A 250 mg sample was placed in the digestion vessel and the mixture was carefully shaken after adding 5 mL of nitric acid (65%). After waiting for at least 20 min, the vessels were closed, and samples were heated in the microwave oven in accordance with the digestion program (10). Digestion solutions were diluted and analyzed on an Agilent 7500a series ICP/MS. For quality control, the concentration of internal standards was 200 ppb (9Be,45Sc,103Rh,208Bi); reference materials were run with the samples, and tuning parameters were controlled before the analyses. The axis cut point of the calibration line was used to obtain the detection limit for each element. It was also important to assure linear rank of the methodology by analyzing different standards with lower and higher known concentrations of each element (0, 1, 5, 10, 20, 30, 40 and 50 ppb). At least five different reference materials were used to encompass all the elements used. Duplicate samples were also used to determine the precision of the analysis. For each element, at least three standards were used to take account of the instrument's analytical working range. Ultrapure water was used to prepare calibration standards and blanks, and three replicate determinations were performed for each sample. A reagent blank was subtracted from all sample results, and detection limits were calculated as three times the standard deviation for reagent blanks (11). The study was carried out in accordance with the Declaration of Helsinki and protocol was reviewed and approved by the Ethics Committee of İstanbul Aydin University (2018- ?). All participants gave written informed consent to participate.

Statistical analysis

Each variable was reported as mean and standard error (SE). Shapiro–Wilk test was used to determine the normality of continuous variables. Data were evaluated using SPSS for Windows (version 15.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Two of the 19 patients were female. Average age of the patients was 71.9±7.2 years. 47.4% of the patients were diabetic and 52.6% were hypertensive. Only one of the patients never smoked (**Table** 1).

Na, Mg, K, Ca, P, Fe, Cu, B, Mn, Zn, Al, S, Cr, Ni, Pt, Sb and Se were found in different concentrations but concentrations of As, Bi, Cd, Co, Mo and Pb in the carotid endarterectomy samples were below the detection limit of ICP-OES (**Table** 2).

Table 1. Demographics of carotid endarterectomy patients	
Gender Female/Male	2 (10.5%) /17 (89.5%)
Age	71.9± 7.2
DM Type II	9 (47.4%)
Hypertension	10 (52.6%)
Stroke	2 (10.6%)
TIA	16 (84.2%)
Asymptomatic	1 (5.3%)
Coronary artery disease	9 (47.4%)
Never smoked	1 (5.3%)
Quitted smoking	13 (68.4%)
Active smoker	5 (26.3%)

Despite of fluctuations of metal concentrations among the patients, concentrations of Ca and P were significantly high with averages of 68613 ppm and 36669 ppm respectively.

Table 2. Elemental composition of carotid endarterectomy material

Element type	**Concentration (ppm;mean±standart deviation)**
Na	6425.6±2317.35
Mg	099.5±1049.6
K	981.5±739.3
Ca	68613±65357
P	36668.7±25479.2
Fe	271.54±339.1
Cu	9.5±15.4
B	4.42±6.39
Mn	0.47±0.41
Zn	67.36±35.37
Al	1.98±2.88
S	8069.6 ±11671.4
As	0
Bi	0
Cd	0
Co	0
Cr	1.51 ±1.54
Mo	0
Ni	0.45±0.41
Pb	0
Pt	0.78±0.5
Sb	0.88±1.93
Se	1.82±3.83
Sn	6.43±6.05
Tl	0
W	0
Hg	5.559 ±3.16

DISCUSSION

With the advent of newer technologies like inductively coupled plasma mass spectrometry (ICP-MS) several of the medically interesting elements can now be quantitated precisely and accurately simultaneously. The measurement of trace elements is increasingly attracting interest from physicians because deviations in trace element uptake and/or metabolism are known to be related to certain dysfunctions. Some trace elements form part of enzymes [e.g., Se in glutathionperoxidase (12) and copper in tyrosinase, ascorbic acid oxidase, cytochrome oxidase, uricase, and superoxide dismutase]. Others are involved in the synthesis of hormones [e.g., Se in the formation of triiodothyronine (12,13)]. Some trace elements are known to be associated with certain diseases if they are present in the body in abnormally low concentrations: Hemolytic anemias can develop in Zn deficiency and some form of coronary heart diseases are associated with Cu and Zn deficiency (14). Molybdenum is the coenzyme of different enzymes (xanthine oxidase, aldehyde oxidase, and sulfite oxidase) involved in the detoxification of xenobiotic compounds (15). Selenium and zinc are potent antioxidants involved in cellular defense against free radicals (16). Several trace elements have been documented as being involved in blood pressure control (hypertension: Cd, Cr deficiency, Mg, etc.) (17). Some trace elements may lead to intoxications in humans and animals if ingested in high concentrations, such as cadmium, thallium, lead, zinc, selenium, and copper.

The reported chemical elements in endarterectomy samples may allow an early diagnosis of atherosclerotic plaque formation at the various stages of its development in large-scale population studies.

Results of those element levels should be carefully evaluated for their role in proatherogenic role. Common salts of Ca and P such as Ca(NO3)2, CaCl2, Na2HPO4, (NH4)2HPO4 are highly soluble in water and in blood but complexes of Ca with P such as Ca10(PO4)6(OH)2 (hydroxyapatite in bones) has very low solubility. Therefore, when aqueous solutions of Ca and P are mixed, the reaction products (calcium phosphate derivatives) are insoluble in water and they precipitate in water

(18). Since there is a cell layer between the atherosclerotic plaques and the blood flow, probably soluble salts of Ca and P ions come to the accumulation site separately and they form insoluble complexes, which cannot pass the cell layer and accumulate. Also, reasons of the elemental accumulation and sources of the elements from bones, diet or other sources should be investigated in detail because they lead to development of new preventive and treatment approaches for carotid atherosclerotic plaques. Approaches to define Ca and P deposition mechanisms and innovative therapies to dissolve those complexes are urgently needed.

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