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Smoking effect on the cadmium and zinc concentration in smokers and nonsmokers

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Abstract: Studies have shown that cigarette smoking affects the accumulation of some heavy metals in certain tissues and metabolism of essential elements. The aim of the study was to determine the differences in the concentrations of cadmium in the blood and urine and zinc in the urine of smokers and ex-smokers in relation to non-smokers, and to determine the possible influence of cadmium concentration on zinc excretion as an essential element. The study included 106 subjects. Subjects were regular smokers (n=51), ex-smokers (n=38) and non-smokers (n=17). Atomic absorption spectrophotometry (AAS) with an electrothermal atomizer was used to determine cadmium. Zinc was determined by AAS with a flame atomizer. There was a significant difference in the values of cadmium in the blood between the groups: smokers and non-smokers ($p<0.001$), smokers and ex-smokers ($p <0.001$), and between ex-smokers and non-smokers ($p = 0.045$). There is a significant positive and strong correlation in the level of zinc and cadmium in urine per gram of creatinine, and as the level of cadmium increases, the level of zinc also increases (rho=0.781; p=0.001). The data indicate that cigarette smoking has been shown to be a factor that can increase cadmium levels to an extent that will significantly increase zinc excretion, or its increased loss.

INTRODUCTION

Cigarette smoking, which produces chemical compounds that have a very detrimental effect on the health of smokers and people in their vicinity, is a widespread bad habit among people of all ages. Harmful effects on the human body are based on the fact that cigarette smoke, in addition to nicotine, contains more than 5300 other ingredients, of which more than 70 are carcinogenic (Talhout, Schulz, Florek, *et al.* 2011). Heavy metals, such as cadmium, mercury, chromium, lead, antimony, nickel, and zinc, are important categories of carcinogenic ingredients in tobacco smoke. After entering the body, toxic metals have a long biological lifespan, which affects the accumulation of some heavy metals in certain tissues and the metabolism of essential elements, with changes in their values in biological material, primarily blood, urine, hair, and nails (Alrobaian, Arida, 2019).

The role of these metals in the pathophysiology of diseases caused by cigarette smoking is still poorly understood. New data suggest that changing the homeostasis of these metals in the human body by cigarettes smoking plays a crucial role in the development of several diseases, primarily respiratory and cardiovascular, and malignant cell alteration (Bernhard, Rossmann, Wick, 2005). Cadmium as an element has no physiological or biological role in the human body. The most common pathways when cadmium enters the body are inhalation and intake of food or water that contains large amounts of cadmium. Cadmium poisoning rarely causes death and leads to cancer and toxicity of organs such as skeletal, urinary, reproductive, cardiovascular, nervous, and respiratory systems. Most commonly it causes nausea and vomiting. The rest of the cadmium accumulates well in the body, and if metallothionein does not remove it from the body

in a short period, it can remain in humans for up to several decades. The decay time of cadmium in the kidneys is 6 to 38 years, while in the liver, it is 9 to 18 years, and the reason is that cadmium has no function in the body, and the body does not consume it (Prashanth, Kattapagari, Chitturi, *et al.* 2015).

The effect of cadmium on the body is to increase the activity of proinflammatory cytokines and causes diseases in many tissues, including the lungs and kidneys (Alkan, Cakmak, Karis, 2014). In addition, cadmium negatively affects the process of DNA replication and reparation and increases the possibility of error, *i.e.*, it acts as a mutagen. With chronic exposure to cadmium, this mutagenic effect multiplies and becomes carcinogenic, which often cannot be corrected by a subsequent increase in the presence of zinc (Lützen, Liberti, Rasmussen, 2004). Previous studies have shown that smokers have higher cadmium concentrations in urine, blood, hair, and tissues than non-smokers. While urinary cadmium concentrations correlate with chronic exposure to tobacco smoke, blood cadmium concentrations provide information on recent cadmium exposure (Richter, Faroon, Pappas, 2017).

Zinc is an essential element necessary for the proper growth, development, and functioning of the human body. This mineral is found in almost every phase of activation of antioxidant enzymes and is necessary for participation in important biological functions. It has a high antioxidant potential and is an integral part of 300 enzymes, as cofactor it participates in the synthesis and breakdown of carbohydrates, lipids, proteins, and nucleic acids. The human body contains 2-3 grams of zinc, and it is after iron the second trace element in the human body (Prashanth, Kattapagari, Chitturi, 2015). Zinc is essential for the creation, development, and maintenance of the immune system involved in the healing wounds, injuries, and burns. It needs the conversion of vitamin A in its active form to play an important role in the production of the hormone testosterone and the balancing insulin in the human body. With its antioxidant properties, zinc participates in the body's defense against free and harmful radicals. It is also needed for a normal sense of taste and smell and normal growth and development during pregnancy, childhood, and adolescence (Osredkar, Sustar, 2011). Less than 0.5% of the total body amount of zinc is found in plasma, while most is stored mainly in muscles and bones (approximately 90%) and the liver, teeth, hair, skin, leukocytes, testes, and others. Most of the plasma zinc binds up to proteins, about 50% to albumins (Al-Assaf, 2010). It is known that even the slightest change in zinc concentration can lead to the development and progression of many mild and severe acute and chronic diseases. Zinc deficiency can negatively affect the processes of genetic mutation and carcinogenesis and increase oxidative stress (Proudfoot, McPherson, Kolb, 2011; Dhawan, Chadha, 2010).

Data on the concentration of zinc in tobacco smoke are sparse and inconsistent. The content of cadmium in the topsoil (0-10 cm) varied from 0.01 to 16.9 μg/g. The concentrations of cadmium in cigarettes range from 0.5 to 3.5 μg/g, with a mean level of 1.7 μg/g. These are very high levels compared to those in food that are normally below 0.05 μg/g. Zinc concentration, analysed in 12 American cigarette brands, range from 16.8 to 30.5 μg/g (Chiba, Masironi, 1992). During combustion, less zinc is generated from tobacco into smoke than cadmium, indicating a negligible zinc concentration compared to cadmium in common cigarette smoke particles (Fresquez, Pappas, Watson, 2013).

The high positive correlation or interdependence between the values of cadmium and zinc in urine can be explained by the metabolic antagonism of cadmium and zinc, which arises on the principle of competition for the same carrier. In this case, it is metallothionein, a low molecular weight cytoplasmic protein for which cadmium and zinc compete for cystine sites (Goyer, 1997). This competition goes to the account of zinc if the value of cadmium is high, which explains this strong, cross-correlation and which causes an increase in the loss of zinc. Cadmium bound to metallothionein in renal and liver, epithelial cells, is not toxic, but cadmium bound to plasma metallothionein is toxic to the renal tubules as it is excreted in the urine, as confirmed by Chan and Cherian (1993) in an animal model.

The aim of the study was to determine the difference in the values of cadmium and zinc in the biological material of smokers in relation to ex-smokers and non-smokers and to examine the interdependence of cadmium and zinc levels in smokers, ex-smokers, and non-smokers.

EXPERIMENTAL

Subjects

The research included 108 participants living in Sarajevo Canton., According to the inclusion criteria, the participants were classified into three groups. The first group consists of regular smokers (n = 51), the second group consists of ex-smokers (n = 38), and the third group of participants who have never consumed tobacco (n = 17). During the study, two subjects were excluded due to extremely high levels of cadmium from the first and second group. The smoking experience of ex-smokers was 30.94 ± 18.46 pack-year, while for current smokers, this experience was 32.78 ± 17.70 pack-year.

All participants had a detailed medical interview. Individual questionnaires included data on gender, age, smoking status, height and weight, and body mass index (BMI), eating habits (consumption of fish, meat, alcohol, and coffee), existence and a number of amalgam dental fillings, type of occupation (office or more physical work), education, the presence of an acute illness or a chronic illness. For smokers, data on the length of smoking experience with average daily smoked cigarettes were taken, and for the group of former smokers, data on the length of the smoking experience, average daily smoked cigarettes, and the length of non-tobacco consumption.

Criteria for inclusion in the study were: participants who are not occupationally exposed to heavy metals, participants who do not take trace element supplements as a dietary supplement, voluntary consent to participate in research, participants who do not take other intoxicants, permanent residence in Canton Sarajevo for at least 20 years, and consumption at least10 cigarettes

daily for smoking participants. Exclusion criteria were: consumption of less than 10 cigarettes per day in a group of smokers, consumption of less than 10 cigarettes by ex-smokers during smoking, occupational exposure to heavy metals, data on drug use, taking zinc supplements as dietary ones, existence of metal implants, residence in Sarajevo Canton for less than 20 years. The study was conducted with the approval of the Ethics Committee of the Faculty of Medicine, University of Sarajevo (1324-AS/11) in accordance with the recommendations contained in the Declaration of Helsinki on Biomedical Research Involving Human Subjects as revised in 2013.

Sample analysis

After a medical interview and physical examination, the subjects had their blood taken for laboratory tests by puncturing the cubital vein. Serum was extracted from blood samples after coagulation and centrifugation for 10 minutes at 4000 rpm and stored until the required results were obtained. A blood sample (10 cm^3) was taken between 8.00 and 9.30 am using a vacutainer system. The vacutainer system consists of a plastic cylinder, a disposable needle, and a vacuum tube. Contact with the needle, vacutainer walls, and stopper was avoided during blood collection. The blood vacutainer is clogged with care to avoid contamination. The blood sample was analyzed immediately or left for up to 2 days at + 4ºC. If the analysis was performed over several days, then the samples were stored at -20ºC. Before analysis, the blood was stirred for half an hour on a ROLLER. EU-certified blood (serum) BCR194L, BCR194N, BCR 194H was used as a control. For the first sample, 20 cm^3 of urine was taken from the patient once in a plastic container made of chemically inert material. The sample was labeled and left at -20°C.

Determination of heavy metal concentration in a sample

Determination of heavy metal content was performed in the Laboratory for the Toxicology of the Public Institution Institute for Occupational Medicine of Sarajevo Canton and the Institute for Public Health of Sarajevo Canton by atomic absorption spectrophotometry (AAS). This is the most commonly used analytical technique for determining metals in samples. The metals being analyzed absorb radiation of a certain wavelength, whereby excitation occurs at the level of electrons and their transition to higher energy levels. Radiation from an external source of energy corresponds to the difference between the ground and excited states of atoms or ions. For the same electron transition, the energy of the emitted photon is equivalent to the energy of the absorbed, that is, the wavelength of the emitted is equal to the wavelength of the absorbed radiation. The advantage of AAS is the speed of analysis, simplicity, relatively low cost of instruments, and favorable sensitivity and selectivity for many elements. Limitations are the difficulty in determining multiple elements at the same time because each element requires a corresponding hollow cathode lamp (Jignesh, Vineeta, Abhay, 2012).

Determination of cadmium levels in blood and urine

Cadmium levels were determined by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS). The main instruments and devices used in the measurement were:

- Atomic absorption spectrophotometer Perkin Elmer Model, USA, AAnalyst 600. THGA technique (thermally heated graphite cuvette),
- Autosampler model AS-800;
- WinLab 32 software.

Other instruments used:

- Nahita centrifuge, model 2690
- Centrifuge EBA 20 (HettichzentrifugenTuttlingen, Germany)
- Mixer Vortex - 2 - Genie, Scientific Industries BOHEMIA N.Y. 11716 USA Laboratory Equipment Model No. G-560E
- Demineralized water machine Sartorius Arium 63316, Sartorius Arium 611UV, Sartorius Tank 613 APV31 30 L, 10 bar, 90°C.

Preparation of blood and urine for the cadmium level measurement

Due to its low affinity for the measured heavy metals, K2-EDTA (Ethylenediaminetetraacetic Acid) was used as a blood anticoagulant. Before determining the cadmium levels in the sample, it was necessary to create a calibration curve. A pool of blood and a pool of urine were added to all solutions for the calibration curve when determining cadmium in whole blood and urine. All solutions were prepared in deionized water of very high purity with 0.2% HNO_3. Calibration is performed by a four-point instrument from two standard solutions 1 µg/L and 5 µg/L. Calibration points (standards) were: 0.2 µg/L, 0.5 µg/L, 1 µg/L and 5 g/L. Each point was read three times, which was the basis for the mean value calculation. The minimum number of points on the calibration curve was four, and the correlation coefficient must be $\geq$ 0.995. Standards have been developed whose values cover the possible range of cadmium levels in the samples. The sample amount is 10 µl. HNO_3 precipitated blood proteins. An aliquot of the sample was injected into an AAS graphite cuvette, and the cadmium absorbance was measured at 228.8 nm. Cadmium pyrolysis (thermal decomposition) takes place at 700°C and atomization at 1550°C. The recommended atomization time was 3 seconds (max. 10 sec.). The cadmium level is calculated using a calibration curve made by the standard addition of cadmium to the blood. They were read in three measurements from which the mean value was taken.

Method for determining zinc in urine

The level of zinc in the urine was determined by atomic absorption spectrophotometry with a flame atomizer. The measurement was performed on a Varian device, model: SpectrAA 110 Atomic Absorption Spectrometer. The zinc standard used in the analysis was 1 mg/ml, manufactured by Panreac. The principle of this method is to determine the concentrations of zinc in different samples by measuring the absorbance of the sample.

When the sample burns, an atomization process occurs, and the resulting zinc metal atoms are exposed to line radiation of a specific wavelength (213.9 nm). The photodetector detects the change in radiation intensity, and the instrument software translates the radiation intensity into the metal concentration in the sample. Prior to each use, the instrument should be calibrated with a pre-prepared concentration series of standard zinc solutions. After calibration of the instrument, it is possible to determine the zinc in the samples. The samples are homogenized and aspirated using a plastic tube in such a way that the tube is immersed in the sample solution where the device automatically sucks up the solution continuously. Since the device allows multiple consecutive measurements and analysis of the same homogeneous sample quickly, based on a pre-set sample, three measurements are performed, and the average value is calculated.

Adjustment of measurements and standards in determining the value of zinc:

- Wavelength: 213.9 nm.
- Separation wavelength: 1.0 nm
- Lamp current: 5.0 mA
- Number of replica samples and standards: 3
- Time measurement: 5s
- Flame type: air / acetylene
- Air flow rate: 3.5 L / min.
- Acetylene flow rate: 1.5 L / min.

The sample is prepared for measurement by taking a 400 μL urine sample in a glass tube and adding 1600 μL of purified water and stirring. The blank is made of ultrapure water that does not contain a sample. The flame atomizer needs to be purified with 2% HNO_3, and between individual measurements, ultrapure water is used for rinsing. After preparation and homogenization, the samples are measured. The device allows multiple consecutive measurements of the same sample quickly, which contributes to greater measurement accuracy. Zinc concentrations in urine samples were calculated using a calibration curve and expressed in mg/L.

Other analyzes

Each participant was determined for a complete blood count and the concentration of creatinine in the urine. The concentration of creatinine in urine is the best indicator of urine concentration, and its determination was necessary to express the concentration of heavy metals so that the concentration of heavy metal is expressed in micrograms per gram of creatinine in urine (μg/g creatinine in urine - μg/g Ucr). Thus, the concentrations of heavy metals can be compared with each other regardless of the concentration of urine, which is an excellent advantage over the volume expression of heavy metals in μg/L of urine. This is a convenient method when the metal concentration is not determined in 24-hour urine.

Statistical analysis

For statistical data processing *Microsoft Excel 2013* and *IBM SPSS Statistics 20* were used. The results are presented as the median, with the first and third quartile values (Q1 and Q3) or as an arithmetic mean with the corresponding standard deviation, depending on the data distribution. The values were compared using Student t-test or non-parametric test (Man Whitney test). The Kruskall-Wallis test or analysis of variance (ANOVA) was used to compare the values of more than two groups, depending on the data distribution. The Spearman's coefficient correlation analysis was used to correlate data. A significance level of 5% was used to determine statistical differences.

RESULTS AND DISCUSSION

The basic characteristics of respondents, smokers, non-smokers and ex-smokers are shown in Table 1. These data represent characteristics of age, body mass index (BMI), gender, type of job (intellectual, or physical), and the presence of a chronic disease.

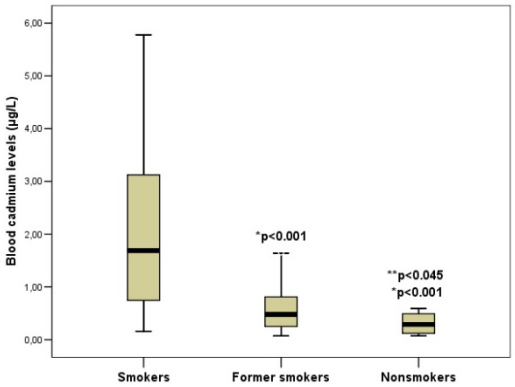

Figure 1: Concentration of cadmium in the blood *smokers in comparison to non-smokers and ex-smokers **ex-smokers in comparison to non-smokers

The analysis of cadmium in the respondent's blood (Figure 1) showed the highest level in smokers with a median of 1.74 μg/L (0.74 μg/L; 3.19 μg/L), followed by ex-smokers 0.44 μg/L, (0.22 μg/L; 0.64 μg/L), while in non-smokers this value was 0.29 μg/L (0.10 μg/L; 0.50 μg/L).

There was a difference in the values of cadmium in the blood between the groups: smokers and non-smokers ($p<0.001$), smokers and ex-smokers ($p <0.001$), and between ex-smokers and non-smokers ($p = 0.045$).

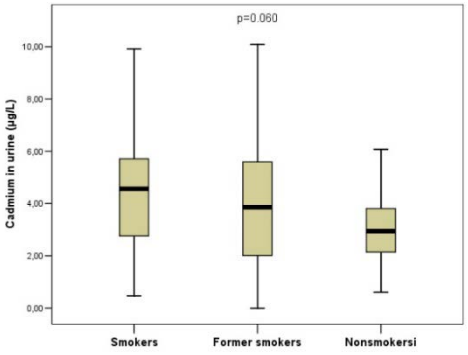

Figure 2: Cadmium volume values in urine

The volume values of cadmium in the urine of the respondents (Figure 2) were found to be at the highest levels in smokers with a median of 4.56 μg/L (2.68 μg/L; 5.80 μg/L), followed by ex-smokers 4.22 μg/L, (2.01 μg/L; 5.79 μg/L), while in non-smokers this value was 2.94 μg/L (1.77 μg/L; 3.90 μg/L).

Table 1: Patients characteristics

		Smokers	**Former smokers**	**Non-smokers**
	Age (years)	47.80 ± 5.68	50,57 ± 6.16	48,88 ± 5.60
	BMI (kg/m^2)	27.2 ± 4.6	28.6 ± 4.3	27.2 ± 4.6
Gender	Male (%)	47.1	55.3	41.2
	Female (%)	52.9	44.7	58.8
Occupation	Intellectual work (%)	51.0	63.0	59.0
	Physical work (%)	49.0	37.0	41.0
Health status	No concomitant disease (%)	49.0	47.4	94.1
	Chronic diseases (%)	45.1	44.7	5.9
Smoking experience (pack-year)		32.78 ± 17.70	30.94 ± 18.46	-

*For age, BMI and smoking experience period data expressed in mean±SD

The differences between the tested groups are indicative, but not significant (p=0.060).

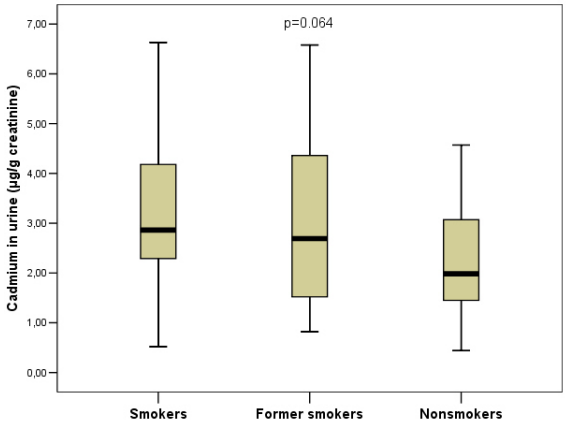

Figure 3: Cadmium levels in urine per gram of creatinine

Cadmium values per gram of creatinine in urine (Figure 3) indicate that the highest level was found in smokers with a median of 2.86 µg/L (2.22 µg/L; 4.24 µg/L), followed by ex-smokers 2.56 µg/L, (1.42 µg/L; 4.36 µg/L), while in non-smokers this value was 1.98 µg/L (1.43 µg/L; 3.24 µg/L).

Differences between the examined groups did exist, but were not significant (p=0.064).

Volume zinc concentrations and zinc concentrations per gram of creatinine in urine did not show significant differences between the tested groups (p=0.909; p=0.877) (Table 2).

In the group of smokers, a significant positive correlation was found between the concentration of cadmium in urine per gram of creatinine (µg/g creatinine) and the duration of smoking (rho=0.323; p<0.05). In the same group, a significant positive correlation was found between the concentration of cadmium in the blood with the number of pack-year (rho=0.293; p<0.05) as well as the concentration of cadmium in urine per gram of creatinine with the number of pack-year (rho=0.300; p<0, 05) (Table 3) A strong positive correlation was also found between the concentration of cadmium in the blood and the number of cigarettes smoked per day (rho=0.485; p<0.01) (Table 3).

Table 2: Median values and 25 and 75 percentiles for level of cadmium in blood and urine and zinc in urine

Metal/sample type	Groups	Percentiles		
		25th	Median	75th
Cadmium/Blood (µg/L)	Smokers	0.75	1.74	3.20
	Ex-smokers	0.22	0.44	0.64
	Non-smokers	0.11	0.29	0.50
Cadmium/Urine (µg/L)	Smokers	2.68	4.56	5.81
	Ex-smokers	2.01	4.22	5.79
	Non-smokers	1.77	2.94	3.91
Cadmium/Urine Creatinine (µg/g)	Smokers	2.23	2.86	4.24
	Ex-smokers	1.42	2.56	4.36
	Non-smokers	1.44	1.98	3.25
Zn/Urine (µg/L)	Smokers	189.8	375.5	579.3
	Ex-smokers	238.0	353.5	525.5
	Non-smokers	254.0	333.0	532.5
Zn/Urine Creatinine (µg/g)	Smokers	196.6	249.5	370.3
	Ex-smokers	171.6	254.2	389.3
	Non-smokers	222.8	295.9	347.1

Correlations between cadmium levels in blood with smoking duration, cadmium levels in urine with smoking duration, number of pack-year, number of cigarettes per

day, cadmium per gram of creatinine values in urine with number of cigarettes per day, zinc levels in urine with smoking duration, number of pack-year, number of cigarettes per day, zinc per gram of creatinine values in urine with smoking duration, number of pack-year, number of cigarettes per day did not show statistical significance (Table 3).

Table 3: Correlations between the concentration of cadmium in blood and urine, zinc in urine with years of smoking duration, number of pack-year and daily smoked cigarettes in a group of smokers

Variable	Smoking duration	Number of pack- year	No. of cigarettes per day
Cadmium Blood	Rho= 0.022	**Rho= 0.293***	**Rho= 0.485****
Cadmium Urine	Rho= 0.072	Rho= 0.144	Rho= 0.093
Cadmium Urine/Urine Creatinine	**Rho= 0.323***	**Rho= 0.300***	Rho= 0.159
Zinc Urine	Rho= -0.172	Rho= -0.074	Rho= 0.014
Zinc Urine/Urine Creatinine	Rho= -0.051	Rho= 0.015	Rho= 0.090

Level of significance:
*p<0.05
**p<0.01

There is a statistically significant positive and strong correlation in the level of zinc and cadmium in urine per gram of creatinine, and as the level of cadmium increases, the level of zinc also increases (rho=0.781; p=0.001)

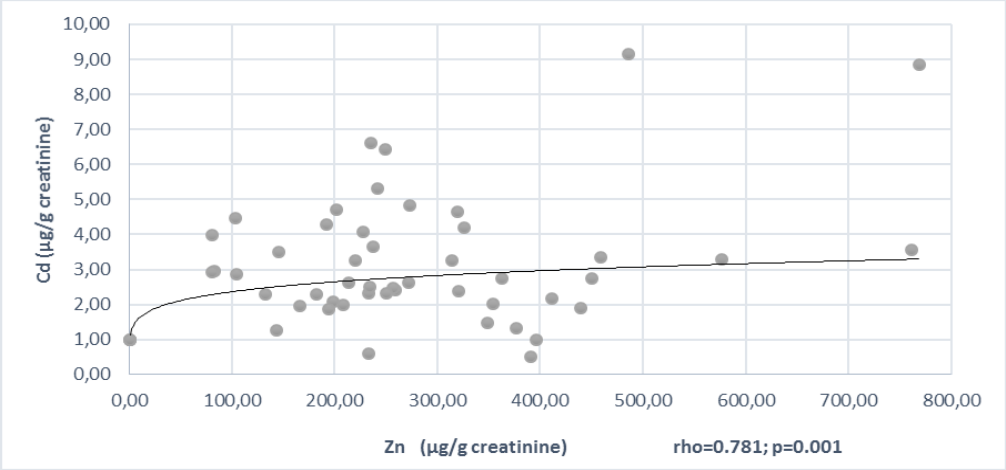

Figure 4: Correlation of zinc and cadmium levels in the urine of smokers per gram of creatinine

Previous studies have shown that cadmium absorption is most effective by the respiratory system (Nordberg, Kjellstrom, Nordberg, 1985). So perennial smoking represents a chronic exposure to low values of cadmium. Olsson, Bensryd, Lundh*et al.* (2002) conducted a study on 105 participants, of whom 26 participants were ex-smokers and 79 who had never consumed tobacco. They determined the level of cadmium in blood and urine of all subjects. They found higher blood cadmium values in ex-smokers, compared to non-smokers. Our study also found a statistically significant difference in cadmium values between ex-smokers and non-smokers (p=0.045). Since the half-life of cadmium in the blood is 2-3 months, we expected that the values will not be higher in ex-smokers compared to non-smokers, because according to some studies, the level of cadmium in the blood is a measure of short-term exposure (Birgisdottir, Knutsen, Haugen, *et al*., 2013). It is likely that the level of cadmium in the blood is a reflection not only of the short-term load of the body with cadmium, but also a consequence of long-term accumulation in the kidneys and liver (50-75% of the total cadmium content in the body), from where cadmium partially re-enters the blood, as shown in study conducted by Hoffmann, Krause, and Seifert (2001). Therefore, this increase in the levels of cadmium in blood of ex-smokers compared to non-smokers, and after several years of smoking cessation, could be taken into account in the biomonitoring cadmium exposure.

Our study found a statistically significant difference cadmium levels in blood between smokers and non-smokers (p<0.001). These results are consistent with the results of Anetor, Ajose, Anetor, *et al.* (2008) who conducted a study on 55 healthy smokers and 41 healthy non-smokers. They determined the level of cadmium and zinc in the serum, and assessed the health risks of the examined groups, primarily the risk of prostate cancer. The level of cadmium, in the aforementioned study by Anetor, *et al.* (2008), in the group of smokers was significantly higher compared to non-smokers. The same authors found a positive correlation between smoking history and blood cadmium concentration (p<0.05). Such large differences in the values of cadmium in the blood of smokers and non-smokers support the importance of respiratory absorption, as the most efficient form of intake of this heavy metal, which was confirmed by the studies by Elinder, Kjellstrom, Lind, *et al.* (1983).

Our study, in accordance with the described study, found a statistically significant difference between the measured values of cadmium in the blood between

smokers and ex-smokers ($p<0.001$). In addition, we found a positive correlation between the concentration of cadmium in the blood and smoking duration of in the group of smokers, so that long-term smokers had a higher concentration of cadmium in the blood. Smoking duration is expressed by the number of packs-year of cigarettes. We also found a positive correlation between the concentration of cadmium in the blood of smokers and the number of cigarettes smoked per day, which is consistent with previous study conducted by Anetor, Ajose, Anetor*et al.* (2008).

The results of the study we conducted are in accordance with the results of the study conducted by Massadeh, Gharibeh, Omari, *et al.* (2010) on a sample of 79 participants. These authors also found a positive correlation between blood cadmium levels and smoking duration as well as the number of cigarettes consumed per day. However, these authors, like the previous Anetor, *et al.*, did not include ex-smokers in their study. The results of the presented study on the level of cadmium in the blood are also consistent with the results of research by Birgisdottir, Knutsen, Haugen, et al. (2013) and Afridi, Kazi, Kazi, *et al.* (2011). Blood cadmium values were higher in subjects from all three groups (smokers, ex-smokers, non-smokers) with the chronic disease (chronic obstructive pulmonary disease-COPD, cardiovascular disease-CVD and malignancies; $p<0.05$). These results are confirmed by the studies of Anetor*et al.* (2008), and Afridi*et al.* (2010).

In our study, in addition to the volume value of cadmium levels in urine, we also determined its level expressed per gram of creatinine in urine (μg/g Ucr). The concentration of creatinine in urine is the best indicator of urine concentration and its determination is necessary in order to express the levels of heavy metals in this medium. This enables a more objective comparison of the values of heavy metals regardless of the concentration of urine, which is an advantage over the volume expression of the values of heavy metals, or in μg/L of urine. The values of cadmium in urine, expressed by this method, in our study were the highest in smokers, but without statistical significance ($p=0.06$). We also found a positive correlation between urine cadmium levels per gram of creatinine and smoking duration (number of pack-year), as well as the number of cigarettes consumed per day ($p<0.05$), which is in accordance with the studies of Bamgbose, Opeolu, Bamgbose (2007), and Olsson *et al.* (2002).

The results of our study are consistent with the results of a study by Benemann, Bromen, Lehmann, *et al.* (2004) (n=4551) conducted as part of heavy metal biomonitoring in the general population. This study found that a group of smokers with a smoking volume of 25 pack-year had an increase in urine cadmium levels of 45.5% expressed in μg/g Ucr. One of the possible causes of the increase in the amount of cadmium in urine among ex-smokers compared to non-smokers, and if it does not reach statistical significance in our study ($p=0.06$) is the biokinetics of cadmium, because this heavy metal is excreted by the kidneys upon release from its depots in the body. If we take into account that the half-life of elimination of cadmium from the body is long (20-30 years) and that the main route of elimination is through the kidneys and urine, it probably takes a longer period of time than the cessation of exposure, for the difference in concentrations between the group of ex-smokers and non-smokers to be insignificant, which we found in the study of Olson *et al.* (2002). A study conducted by Mutti, Corradi, Goldoni, *et al.* (2006) showed that cadmium and lead were still in measurable values in exhaled air condensation, years after smoking cessation.

In our study, the values of zinc in urine were determined. The volume values of zinc in urine and values of zinc in urine per gram of creatinine did not differ statistically significantly between the examined groups ($p=0.909$; $p=0.877$). However, there is a significant positive correlation between urine cadmium and zinc values expressed per gram of creatinine. As the level of cadmium increases, the level of zinc also increases ($rho= 0.781$; $p=0.001$). Such a high degree of correlation indicates that the increase in the content of cadmium in the body, or in its depots, which in turn increases the intensity of zinc loss by increased excretion, suggests increased loss of this essential element rather than its increased bioavailability as shown by studies by Afridi, Kazi, Kazi*et al.* (2010), and Lin, Caffrey, Chang *et al.* (2010).

Considering that zinc acts in the process of DNA replication, its stabilization, and in the processes of DNA reparation, as an integral and essential part of enzyme systems involved in these processes (Proudfoot *et al*, 2011), the effect of cadmium on its metabolism is even more significant.

In addition, zinc reduces oxidative stress and exhibits antioxidant activity. Zinc deficiency can adversely affect the processes of genetic mutation and carcinogenesis, and increase oxidative stress, as shown in studies conducted by Proudfoot, McPherson, Kolb*et al.* (2011), Dhawan, Chadha (2010) and Bertini, Decaria, Rosato, (2010). On the other hand, cadmium, as a zinc antagonist, negatively affects the process of DNA replication and reparation and acts as a mutagen. In chronic exposure to cadmium, this mutagenic effect multiplies and becomes carcinogenic, which often cannot be partially canceled by a subsequent increase in the presence of zinc [Clark and Kunkel (*2004*), Jin, Clark, Slebos*et al. (2003*), Waisberg, Joseph, Hale*et al. (2003*), Lützen, Liberti, Rasmussen (2004)].

CONCLUSION

The presence of a large number of harmful chemical elements in tobacco smoke such as cadmium has consequences on biological indicators, the so-called biomarkers of exposure. Biomarkers of cadmium exposure in blood and urine show significant shifts, especially in the blood of active smokers. This study also found a significant difference in cadmium values between ex-smokers and non-smokers. Therefore, this

increase in the level of cadmium in the blood of ex-smokers compared to non-smokers, and after several years of smoking cessation, could be taken into account in the biomonitoring of cadmium exposure. There is a significant positive correlation between cadmium and zinc values in urine expressed per gram of creatinine. Such a high degree of correlation indicates that the increase in the content of cadmium in the body, or in its depots, which in turn increases the intensity of zinc loss by increased excretion, suggests increased loss of this essential element rather than its increased bioavailability.

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Summary/Sažetak

Istraživanja su pokazala da pušenje cigareta utiče na akumulaciju nekih teških metala u pojedinim tkivima i na metabolizam esencijalnih elemenata. Cilj istraživanja je bio utvrditi razlike koncentracija kadmijuma u krvi i urinu te cinka u urinu pušača i bivših pušača u odnosu na nepušače i utvrditi mogući uticaj koncentracije kadmijuma na ekskreciju esencijalnog elementa cinka. Istraživanjem je obuhvaćeno 106 osoba. Ispitanici su bili stalni pušači (n=51), bivši pušači (n=38) i nepušači (n=17). Za određivanje kadmijuma korištena je metoda atomske apsorpcione spektrofotometrije (AAS) sa elektrotermalnim atomizerom. Cink je određen metodom ASS sa plamenim atomizerom. Utvrđena je značajna razlika u vrijednostima kadmijuma u krvi između skupina: pušači i nepušači ($p<0.001$), pušači i bivši pušači ($p<0.001$), te između bivših pušača i nepušača ($p=0,045$). Postoji značajna pozitivna i jaka korelacija u nivou cinka i kadmijuma u urinu na gram kreatinina, te povećanjem nivoa kadmijuma povećava se i nivo cinka ($rho= 0.781$; $p= 0.001$). Rezultati naše studije pokazuju da je pušenje faktor koji može povećati nivo kadmijuma u mjeri koja će znatno povećati ekskreciju cinka, tj. njegov povećan gubitak.