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EFFECT OF SMOKING ON MERCURY AND COPPER CONCENTRATION IN SMOKERS AND EX-SMOKERS

Pepic Esad*[1], Secic Damir[2], Kurtagic-Pepic Emina[3] Musanovic Jasmin[4], Naser Nabil[5], Metovic Azra[4], Lujinovic Almira[6] and Dzubur Alen[7]

[1]Associate Professor, Department of Pathophysiology, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina.
[2]Full professor, Internist, Department of Pathophysiology, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina.
[3]Epidemiologist, Institute for Public Health of Canton Sarajevo, Sarajevo, Bosnia and Herzegovina.
[4]Associate professor, Department of Medical Biology with Human Genetics, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina.
[5] Associate professor, cardiologist, Polyclinic „Dr. Nabil", Sarajevo, Bosnia and Herzegovina.
[6]Associate Professor, Department of Anatomy, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina.
[7]Associate Professor, Cardiologist, Clinical Center of Sarajevo University, Sarajevo, Bosnia and Herzegovina.

***Corresponding Author: Pepic Esad**
Associate Professor, Department of Pathophysiology, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina.

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ABSTRACT

Introduction: Smoking can cause accumulation of some harmful chemical elements in tissues such as heavy metals cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), cesium (Cs), etc. Smoking can also cause disturbances in the metabolism of trace elements such as copper, zinc and selenium. This is manifested by a change in their levels in biological material, primarily in blood, urine, hair and nails. **The aim** of the study was to determine the differences in the concentrations of mercury in urine and copper in blood serum of smokers and ex-smokers in relation to non-smokers. **Methods:** The study included 108 respondents divided into three groups. The first group of respondents consists of regular smokers (n=51), the second group of ex-smokers (n=38) and the third group of respondents who have never consumed tobacco (n=17). Determination of the level of mercury in urine was performed by the method of atomic absorption spectrophotometry with an electrothermal atomizer. Level of copper in blood serum was performed by spectrophotometric method without deproteinization. **Results:** The analysis showed that there is no statistically significant difference in the level of mercury in the urine of smokers compared to non-smokers and ex-smokers (p=0.09). There is a statistically significant difference in copper values in the blood serum compared to the examined groups (p=0.039). **Conclusion:** Smoking did not prove to be a significant factor for increased concentration of mercury in the urine, but it significantly affected the level of copper in the blood serum.

KEYWORD: Mercury concentration, Copper concentration, Smoking, Ex-smokers, Smokers.

I. INTRODUCTION

Tobacco smoke contains thousands of different chemical compounds. Among them there is also a large number of harmful chemical elements, especially some heavy metals and metalloids such as cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), cesium (Cs), etc. When a cigarette is smoked, the temperature at the mouth end is around 30˚C, and at the burning part up to 900˚C. At such a high temperature, some tobacco ingredients are subject to decomposition, and floating substances are directly distilled into smoke. Some of these ingredients act locally (directly on the mucosa), while others are absorbed into the blood or dissolve in saliva and are swallowed.[1,2]

Studies has shown that smoking affects the accumulation of some heavy metals in certain tissues and the metabolism of trace elements, which is manifested by changes in their values in biological material, primarily in blood, urine, hair and nails.[3,4,5] Increased levels of cadmium, lead and mercury in biological material, regardless of the cause, are associated with increased exposure and levels in target tissues, i.e., depots, which can be linked to more potential health risks.[2,3,4,5]

Elemental mercury vapor is almost 100% absorbed in the lungs. Absorption of elemental mercury through the digestive tract and skin is negligible. On the other hand, the absorption of organic compounds of mercury in the

digestive tract is about 90%, and through the skin at a maximum of 5%.[6,7,8,9] Elemental mercury, as well as inorganic and organic mercury compounds, are mostly eliminated in the urine. A much smaller part is eliminated through the gastrointestinal tract, bile, lungs, mammary, sweat, sebaceous and salivary glands.[10,11,12]

Copper is considered one of the essential trace elements. There are between 75 and 150 mg of copper in the body of an adult. More than half of the copper in the human body enters the composition of muscles and bones. Its concentration is highest in the liver, brain, kidneys and heart.[13,14] Copper is an important component of many oxidoreductase enzymes that play an important role in the generation of energy in the cell. The activity of these enzymes is highest in the heart, brain, liver and kidneys.[14,15] Copper is a component of the enzyme copper-zinc superoxide dismutase (Cu/Zn SOD), which serves as an antioxidant important for protecting cells from damage caused by free radicals.[16] Maintaining an adequate ratio of copper and zinc is very important for the normal functioning of enzyme systems. Copper is also important in the immune response. In the course of the inflammatory process or infection, two compounds that include copper ions in their structure are mobilized: superoxide dismutase and ceruloplasmin. Copper is also essential for T lymphocyte maturation and function.[17,18]

Some studies[19] have determined altered values of trace elements such as copper (Cu), zinc (Zn) and selenium (Se) in body fluids of smokers compared to non-smokers. Changes in copper concentration are often associated with inflammatory processes[20,21], and in smokers it is induced by processes such as chronic inflammation of the respiratory mucosa.[22] Smoking increases Cu concentration by influencing the secretion of corticosteroids and catecholamines that have an effect on Cu metabolism.[23,24,25]

The study's objectives are to determine the difference in Hg concentrations in urine and Cu in blood serum of smokers to former smokers and non-smokers. Then, to determine the difference in the Hg and Cu concentrations in the biological material of former smokers in relation to non-smokers and the influence of smoking experience on the concentration of these metals.

II. MATERIALS AND METHODS

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.

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.

Determination of Hg levels in urine: Determination of the level of Hg in urine was performed in the laboratory for the toxicology of the Public Institution for Occupational Medicine of the Sarajevo Canton by the method of atomic absorption spectrophotometry with an electrothermal atomizer**.**

Hg 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.

Disposable urine is taken from the patient for the first sample. Urine is taken in a plastic container made of chemically inert material. Therefore, it is necessary to take about 20 cm3 of urine. This sample is labeled and stored at -20° C.

The urine sample is destroyed by wet digestion with concentrated sulfuric acid (H_2SO_4) and 6% potassium permanganate ($KMnO_4$) solution at room temperature without heating. By adding a 20% solution of Hydroxylamine hydrochloride ($HONH_2$ HCl), ionic mercury is reduced to elemental form. The resulting mercury vapors are separated from the solution by an air current and passed through the absorption cell of a circular closed air system. Cold digestion takes at least 12 hours. The mercury value is calculated using a calibration curve made by the method of standard addition of known concentrations of ionic mercury to the pool.

Reagents

1. Solution of 6% $KmNO_4$ (6.0g $KMnO_4$ and up to 100ml H_2O)
2. Solution of 20% $HONH_3Cl$ – Hydroxylamine hydrochloride (10.0g $HONH_2$ x HCl and up to 50ml of H_2O)
3. 0.1mol/l HNO_3 solution
4. Solution of 3 mol/l H_2SO_4 (163.44ml 96% H_2SO_4 and up to 1000 ml H_2O)

5. 3% HCl solution (81.08ml of 37% HCl and up to 1000ml of H_2O)
6. Solution of 0.2% Na BH_4 (Sodium borohydride) in 0.05 % NaOH (2.0 g $NaBH_4$ + 0.5 g NaOH and up to 1000 ml H_2O)
7. Standard for Hg (original packaging 1000µg/ml – 1g/l Hg)
8. Urine pool

All standards are made from the original standard 1000 µg/ml-1g/l Hg, standards are diluted with 0.1 mol/l HNO_3 and the basic standard is made in it. They are stable in plastic containers

Determination of copper (Cu) in blood serum by spectrophotometric method without deproteinization with 3,5-DiBr-PAESA-(3,5-Bromo-2-pyridylazo)-N-ethyl-N-(3-sulfopropyl) aniline, monosodium salt, monohydrate: Determination of copper in blood serum was done using a spectrophotometric method without deproteinization on a *Shimadzu* brand machine, model *uv spectrophotometer UV-1800*. Copper is separated from the protein (ceruloplasmin) in blood serum by acidification and oxidizes cupro to the cupric form. The cupric form of copper reacts with 3,5-DiBr-PAESA to form a red complex. The intensity of the resulting color is proportional to the concentration of copper in the blood serum.

Reagents
R_1 - sodium acetate 100mmol/l
- sodium sulfate 96mmol/l
- Hydroxylamine sulfate 50mmol/l
R_2- 3,5-DiBr-PAESA- 0.001%
R_3- Standard for Cu - 200µg/dl- 31.46 µmol/l
Sample: non-hemolyzed blood serum (Cu is stable in the sample for 8 days at 2-8°C)

Conditions for determination
Wavelength: 580nm (570-800nm)
Operating temperature: 15-25°C

Table 1: Work procedure for copper.

Reagents	BLIND TRIAL	STANDARD	SAMPLE
	1500µl	1500µl	1500µl
Distilled water	100µl	----------	----------
Standard for Cu	--------	100µl	----------
Sample - blood serum	--------	---------	100µl

Mix and incubate for 10 minutes at 15-25 °C
Read against the blank at 580 nm

Other analyses: Complete blood count and urine creatinine concentration were determined for each of the subjects. Urine creatinine concentration is the best indicator of urine concentration, and its determination was necessary to express the concentration of heavy metals.

Statistical analysis: For statistical data processing Microsoft Excel 2013 and IBM SPSS Statistics 20 were used. Analysis of variance (ANOVA) was used to compare the values of more than two groups, depending on the data distribution. A significance level of 5% was used to determine statistical differences.

III. RESULTS AND DISCUSSION

The basic characteristics of respondents, smokers, non-smokers and ex-smokers are shown in Table 1. These data represent characteristics of smoking status, smoking experience, age, body mass index (BMI) and gender.

Table 2: Baseline characteristics of participants.

		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
Smoking experience (pack-year)		32.78 ± 17.70	30.94 ± 18.46	-

Urine mercury level: The level of mercury in urine expressed by volume (µg/L) in non-smokers was 0.67±0.58 µg/L, in ex-smokers 0.75±0.58 µg/L and in smokers 0.67±0.21 µg /L. The analysis of these values showed that there is no statistically significant difference in the level of mercury in the urine (p=0.09) (Table 3).

Table 3: Average concentrations of mercury in the urine.

	X	Median	95% CI		Minimum	Maximum
			Lower Bound	Upper Bound		
Smokers	0.79	0.58	0.41	1.17	0.12	3.14
Ex-smokers	0.75	0.58	0.33	1.17	0.02	5.27
Non-smokers	0.67	0.21	0.23	0.71	0.01	3.85
χ^2=9.081; df=2; p=0.09						

Non-smokers had the same mean value of mercury expressed per gram of creatinine in urine (μg/g Ucr) 0.65±0.54 μg/g Ucr and ex-smokers 0.65±0.70 μg/g Ucr. The level of Hg expressed in μg/g Ucr in smokers was 0.52±0.99. The analysis of these values showed that there is no statistically significant difference in the level of mercury in urine per gram of creatinine (p=0.087) (Table 4). The median for smokers was 0.99 μg/g Ucr, ex-smokers 0.70 μg/g Ucr and non-smokers 0.54 μg/g Ucr.

Table 4: Average levels of mercury in the urine of subjects per gram of creatinine.

	X	Median	95% CI		Minimum	Maximum
			Lower Bound	Upper Bound		
Smokers	0.52	0.99	0.17	0.86	0.01	5.47
Ex-smokers	0.65	0.70	0.36	0.94	0.03	3.01
Non-smokers	0.65	0.54	0.36	0.93	0.05	1.90

***Blood serum copper level*:** Table 5 shows the average values of copper in the blood serum of the subjects. In the group of smokers, the average value of copper in blood serum was 17.58±3.32 μmol/L, in the serum of ex-smokers the average value was 17.04±3.27 μmol/L, while in non-smokers it was 15.85± 3.72 μmol/L. The ANOVA showed that there is a statistically significant difference in the average values of copper in the blood serum compared to the examined groups (p=0.039).

Table 5: Average values of copper in the serum.

	X	SD	SEM	95% CI		Minimum	Maximum
				Lower Bound	Upper Bound		
Smokers	17.58	3.32	0.46	14.6454	16.5150	7.47	21.39
Ex-smokers	17.04	3.27	0.53	15.9639	18.1166	10.85	23.25
Non-smokers	15.85	3.72	0.90	13.9454	17.7722	10.19	22.63
F=1.271; df=2; p=0.039							

In the study by Mortada et al.[26] was determined Hg values in blood, urine, hair and nails in 35 smokers and 33 subjects who had never consumed tobacco. The mentioned researchers did not find a significant difference in Hg content between these two groups of subjects. Thus, the mean value of Hg in urine, in the group of smokers, was 0.44±0.23μg/g Ucr, and in the group of subjects who never consumed tobacco 0.52±0.24μg/g Ucr (p>0.05). The results of the mentioned study[26] are in line with the results of our results, where the mean value of Hg in the urine of smokers was 0.59 μg/g Ucr, in ex-smokers it was 0.65 μg/g Ucr and in non-smokers 0.65 μg/g Ucr. This means that we did not establish the existence of a statistically significant difference in the level of Hg in urine among the tested groups, smokers, non-smokers and ex-smokers (p=0.087). Similar results were obtained in study by Mannino et al. (n= 16,024)[27], Bamgbose et al. (n=200)[21], Afridi et al. (n= 475)[28], Mortada et al. (n=68).[26] We did not find any data from the literature that take into account the concentration of mercury in the urine of ex-smokers.

In contrast to this, a smaller number of studies such as one by Slojewski et al. (n=219)[29], determined the existence of a statistically significant difference in the level of Hg in urine between the investigated groups, smokers and non-smokers (p=0.045). This can be partly explained by the fact that mercury concentration can be affected by several other factors, such as mercury from pesticides, amalgam teeth fillings, eating fish and seafood. Mercury from amalgam fillings is in an inorganic form and is released in the form of vapor, and it enters the body by inhalation, as shown by the research of Berdouses et al.[30] and Berglund.[31] Otherwise, inorganic mercury that enters the body through ingestion is almost not resorbed (max. 15%), but inorganic mercury or so-called metallic mercury, which is inhaled in the form of vapor, is absorbed by 80%.[32] Chewing gum can significantly increase the intensity of elemental mercury vapor release from dental amalgam fillings and thus raise the urinary mercury level to more than 20 μg/g Ucr.[33] The consumption of fish is the ingestion of organically bound mercury in the form of methylmercury.

The results of our research show that the average concentration of copper (Cu) in blood serum in the group of smokers was 17.58±3.32 μmol/L, in the serum of ex-smokers the average value was 17.04±3.27 μmol/L, while in non-smokers it was 15.85±3.72 μmol/L. The difference is statistically significant in the group of smokers and ex-smokers compared to non-smokers ($p< 0.05$). These results are consistent with the study of Anator et al.[34], who in their research in 55 clinically healthy smokers and 41 clinically healthy non-smokers, among others (Cd and Zn), analyzed the concentration of Cu in the serum. The same researchers tried to assess the health risks of the examined groups, primarily the risk of prostate cancer. Smokers were defined as respondents who had smoked continuously for at least 3 years. The respondents lived in the same area and had a similar diet. They found statistically significantly lower values of Zn in smokers compared to non-smokers. On the other hand, Cd and Cu values were statistically significantly higher in smokers compared to non-smokers. Cu concentration in the blood serum of smokers was 111 ug/100ml vs. 98 ug/100ml in non-smokers ($p<0.05$). This study did not include ex-smokers in the observation.

Al-Numair's study[19] is in line with our results. This study examined the values of Zn, Se and Cu in the blood serum of 70 healthy male smokers and 70 healthy male non-smokers. The mean concentration of Cu in smokers was statistically significantly higher in smokers compared to non-smokers (123.71 mg/dL vs. 114.75 mg/dL; $p<0.05$).

The increased concentration of Cu in the serum may be a consequence of the antagonism of Zn and Cu. The first form of antagonism arises from competition for the same storage carrier, which is metallothionein, a low molecular weight cytoplasmic protein for which cystine sites compete, also Cu and Zn. Another site of antagonism is resorption from the gastrointestinal tract.[34] Considering that smoking lowers the bioavailability of Zn by the mechanism of competition with Cd, the concentration of Cu increases, because it is also an antagonist of Zn.

Smoking increases Cu concentration by influencing the secretion of corticosteroids and catecholamines, which have an effect on Cu metabolism, as shown by studies by Fuxe et al.[23], Pomerleau et al.[24], and Miller et al.[25] The synthesis of ceruloplasmin (the main copper carrier in plasma) is stimulated by glucocorticoids[35], while epinephrine can increase the concentration of Cu in mammalian plasma.[36] In this sense, tobacco smoke can act as an endocrine-disruptive mixture of highly toxic elements and compounds.

An increased concentration of Cu is often associated with inflammatory processes, as shown in study by Perrin-Nadif et al.[20] and Beshgetoor et al.[37], and in smokers it is induced by processes such as chronic inflammation of the respiratory mucosa.[19]

IV. CONCLUSION

Smoking was not found to be a significant factor in increasing the concentration of mercury in urine. This can be partly explained by the fact that mercury concentration in urine can be affected by several other factors, such as mercury from pesticides, dental amalgam fillings, consumption of fish and seafood, and tobacco smoke does not have such a significant effect. In contrast to this copper values are significantly higher in smokers compared to non-smokers. Considering that smoking reduces the bioavailability of zinc, it increases the concentration of copper, because zinc is its antagonist. Increased copper concentrations can also be explained by the fact that smoking affects the secretion of corticosteroids and catecholamines, which affect copper metabolism.The synthesis of ceruloplasmin is stimulated by glucocorticoids, while epinephrine can increase the concentration of Cu in mammalian plasma. In this sense, tobacco smoke can act as an endocrine-disrupting mixture of highly toxic elements and compounds.

Ethical Considerations: 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.

Conflict of Interest Statement: I didn't have any conflicts of interest to declare.

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