

Concentrations of some elements in the pubic hair of males with peripheral artery disease

Zawartość wybranych pierwiastków we włosach łonowych mężczyzn ze schorzeniami tętnic obwodowych

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ABSTRACT

INTRODUCTION

The present study analyzed the concentrations of calcium, magnesium, lead, cadmium and nickel in the pubic hair of males suffering from peripheral artery disease.

MATERIALS AND METHODS

The tests were carried out on pubic hair collected from 88 males (42 – experimental, and 46 – control group) at the age of 37–87 yrs living near Bytom (Silesia, southern Poland). The pubic hair was prepared under a procedure established by the International Atomic Energy Agency, followed by wet digestion in a microwave oven. The concentration of the metals in the pubic hair was assayed with the flame (Mg, Ca) and flameless (Pb, Cd, Ni) AAS.

RESULTS

The mean concentration of the metals in the pubic hair was similar to that assayed in head hair by other authors. The pubic hair of males suffering from peripheral artery disease revealed slightly higher median concentrations of lead and nickel than the control group. Calcium concentration was lower for the pubic hair of males with peripheral artery disease than in the control group. Magnesium and cadmium concentrations were at a similar level in experimental and control groups. Much higher concentrations of toxic elements (Pb, Ni) were found in the hair of smokers suffering from peripheral artery disease compared to non-smokers.

CONCLUSIONS

The research did not reveal a significant difference in the concentrations of selected elements in the pubic hair of males suffering from peripheral artery disease compared to the control group, which could be due to small numbers of the group. However, the much higher concentration of the toxic elements (Pb, Ni) in the hair of smoking people from the experimen-

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tal group compared to the non-smokers was a very interesting observation. Such a significant difference between smokers and non-smokers was not found in the control group.

KEY WORDS

pubic hair, elements, peripheral artery disease, males, smoking

STRESZCZENIE

WSTĘP

W pracy analizowano zawartość wapnia, magnezu, ołowiu, kadmu i niklu we włosach łonowych mężczyzn ze schorzeniami tętnic obwodowych.

MATERIAŁ I METODY

Materiał do badań stanowiły włosy łonowe pobrane od 88 mężczyzn w wieku od 37 do 87 lat, mieszkających w okolicy Bytomia (GOP, południowa Polska). Grupa badana liczyła 42 mężczyzn, kontrolna – 46 mężczyzn. Włosy łonowe zostały przygotowane do badań zgodnie z procedurą polecaną przez Międzynarodową Agencję Energii Atomowej (IAEA) i poddane mineralizacji mikrofalowej. Zawartość metali w próbkach włosów łonowych oznaczono metodą płomieniową (Mg, Ca) oraz bezpłomieniową (Pb, Cd, Ni) AAS.

WYNIKI

Średnia zawartość metali we włosach łonowych badanych mężczyzn mieściła się w zakresie wartości oznaczanych przez innych autorów we włosach z głowy. We włosach łonowych mężczyzn ze schorzeniami tętnic obwodowych stwierdzono nieznaczco większą zawartość ołowiu i niklu w porównaniu z grupą kontrolną. Zawartość wapnia była w grupie mężczyzn ze schorzeniami tętnic obwodowych nieistotnie mniejsza niż w grupie kontrolnej. Zawartość magnezu i kadmu występowała na podobnym poziomie. Stwierdzono większą zawartość ołowiu i niklu we włosach palaczy z grupy mężczyzn ze schorzeniami tętnic obwodowych w porównaniu z osobami niepalącymi.

WNIOSKI

Przeprowadzone badania wykazały brak istotnej różnicy w zawartości wybranych pierwiastków we włosach łonowych mężczyzn ze schorzeniami tętnic obwodowych w porównaniu z grupą kontrolną, co może wynikać ze zbyt małej liczebności grup. Znacznie większa zawartość pierwiastków toksycznych występowała we włosach palaczy z grupy badanej w porównaniu z osobami niepalącymi. Tak istotnego zróżnicowania między palącymi i niepalącymi nie obserwowano w grupie kontrolnej.

SŁOWA KLUCZOWE

włosy łonowe, pierwiastki, schorzenia tętnic obwodowych, mężczyźni, palenie tytoniu

INTRODUCTION

Over the last many years, hair analysis has been used as an alternative to blood and urine analysis and biopsy. Hair has a lot of important advantages as a diagnostic material: it is neutral, stable tissue which does not biodegrade,

sample collection is simple and non-invasive, it is easy to remove contaminants from it, which makes analytical results repeatable and reliable. Moreover, researchers have observed a correlation between the concentration of basic elements in hair and their concentration in the body, both healthy and sick [1,2,3].

Hair is commonly used in laboratory diagnostics to assess the mineral condition of the body. It enables the determination of deficiency or excess of elements indispensable to the human body or toxic ones [4]. Hair is also used as an analytical material in toxicology and environmental research. In the former, it acts as an indicator of metal poisoning while in the latter, it is successfully applied to assess the extent of environmental pollution [5,6,7]. The determination of metal concentration in hair also helps diagnose diseases and monitor element changes in pathological conditions [1,3,8,9,10,11,12].

A good example of that is research which demonstrates changes in the concentrations of elements in hair and their proportions carried out on people suffering from arterial hypertension [9], and a decrease in the concentration of essential elements in the hair of people with heart diseases [1]. There are also numerous reports indicating changes in the elemental composition of hair in neuropathy [11], malignant diseases [2,12] and diabetes [3].

This study was aimed at assessing the concentrations of selected elements in the pubic hair of males suffering from peripheral artery disease. Cardiovascular diseases (peripheral artery disease inclusive), being the most common cause of death, admission to hospital and permanent disability to work, are a very important aspect in the development of contemporary medicine and their detection and prevention are of great significance. The determination of selected elements in the pubic hair of people with peripheral artery diseases and the comparison of the results with those assayed in the hair of healthy people may be helpful while diagnosing the diseases.

MATERIAL AND METHODS

The study has been approved by the Bioethics Committee of the Medical University of Silesia in Katowice.

The experimental material were the pubic hair, as opposed to hair of the head are not subjected to so many beauty treatments (e.g. coloring), and are not exposed to environmental pollution in a direct way.

The males whose pubic hair was collected for the study were informed about the objective of the research and they agreed to participate in

the project. They were confidentially surveyed about their gender, age, date of birth, dwelling place, employment, smoking habits and had to meet the following criteria: one dwelling place for the last 20 years (near Bytom, Silesia, southern Poland), lack of occupational exposure to metals, not suffering from any chronic diseases. The experimental group (people with peripheral artery disease) consisted of 42 males at the age of 41–87, the average being 62.14 ± 8.8 . 16 subjects had abdominal aortic aneurysm, 6 suffered from thoracoabdominal aortic abdominal, the remaining 20 were afflicted by atherosclerosis of lower limbs. 14 people in the experimental group smoked cigarettes. The control group consisted of 46 males aged 37–79, the average being 59.89 ± 10.36 . 17 subjects smoked cigarettes.

The collected pubic hair was prepared following a procedure established by the International Atomic Energy Agency [13]. The hair was washed with deionized water and pure acetone. The hair samples were rinsed with acetone, then three times with water followed by acetone again. Each time, the contact time with the solutions was about 10 min.

After being dried at 105°C to constant mass, uniform weight (about 200 mg) of hair samples was digested in a microwave, using 2 mL of concentrated spectrally pure HNO_3 . After digestion, the acid was vaporized out of the samples, 0.5 mL of HNO_3 and 10 ml of water was added and then the content was transferred into 25 mL measuring flasks and filled to volume.

Apart from the pubic hair, digestion was simultaneously carried out on CRM 397 reference material (Trace elements in human hair), Community Bureau of Reference). The average results from six runs are given in table I.

Table I. Analytical results of CRM 397 reference material (Trace elements in human hair) [$\mu\text{g/g}$]

Tabela I. Wyniki analizy materiału referencyjnego CRM 397 (Trace elements in human hair) [$\mu\text{g/g}$]

Element	Certified value	Measured value
Ca	2900	2789 ± 98
Pb	33.0	31.5 ± 1.6
Cd	0.521	0.49 ± 0.03
Ni	0.83	0.80 ± 0.06

The concentrations of metals in the pubic hair and reference material samples were assayed

with the flame (Mg, Ca) and flameless (Pb, Cd, Ni) atomic absorption spectrometry, using Varian SpectrAA 880 and SpectrAA 880Z spectrophotometers. The concentrations of the elements in the pubic hair were determined following commonly acknowledged measurement procedures.

The results were statistically analyzed with Microsoft Excel and Statistica ver. 8pl.

The distribution of the data sets considerably differed from the normal one, therefore the median was used in the discussion. The testing of the statistical variability of the differences between the groups was based on the Mann-Whitney U test. The correlation of the metals in the pubic hair was analyzed, using the Spearman's correlation coefficient.

similar in the experimental group, irrespective of the type of peripheral artery diseases, the table gives total results for the experimental group.

Magnesium concentration in the pubic hair from the experimental group ranged from 15.0 µg/g to 147 µg/g and did not differ significantly from the concentration assayed in the control group (Mann-Whitney U test, $p = 0.51$). The median calcium concentration in the experimental group was 307 µg/g, being a slightly higher in the hair of the control group – 330 µg/g.

The median lead concentration in the male pubic hair from the experimental group reached 1.80 µg/g, while its concentration in the control group was 1.74 µg/g. The median cadmium concentration in the male pubic hair from the experimental group reached 0.10 µg/g, similar to that assayed in the control group – 0.11 µg/g. The median nickel concentration in the male pubic hair from the experimental group reached 0.40 µg/g and was higher than its concentration in the control group (0.33 µg/g).

RESULTS

The concentrations of the elements in the male pubic hair from the control and experimental groups are shown in table II. Since they were

Table II. The comparison of magnesium, calcium, lead, cadmium and nickel concentrations [µg/g] in the pubic hair of males from the experimental (E) and control (C) groups

Tabela II. Porównanie zawartości magnezu, wapnia, ołowiu, kadmu i niklu [µg/g] we włosach łonowych mężczyzn z grupy badanej (E) i kontrolnej (C)

	Mg		Ca		Pb		Cd		Ni	
	E	C	E	C	E	C	E	C	E	C
Mean	64.7	59.4	406	371	2.72	2.60	0.16	0.14	0.48	0.42
Standard deviation	33.7	29.6	309	156	2.95	2.60	0.18	0.12	0.27	0.22
Minimum	15.0	17.7	69.3	181	0.40	0.36	0.01	0.01	0.08	0.15
Maximum	147	122	1653	870	17.8	13.8	0.82	0.55	1.29	0.98
Median	53.5	50.4	307	330	1.80	1.74	0.10	0.11	0.40	0.33
Lower quartile	43.4	37.0	212	243	1.26	1.01	0.04	0.06	0.29	0.26
Upper quartile	83.1	85.7	526	448	3.26	3.23	0.19	0.18	0.59	0.61

Table III. Spearman correlation coefficient for elements in the pubic hair of males from the experimental and control groups

Tabela III. Współwystępowanie pierwiastków we włosach łonowych mężczyzn z grupy badanej i kontrolnej

	Experimental group					
	Mg	Ca	Pb	Cd	Ni	
Control group	Mg		0.70*	0.13	-0.19	0.31*
	Ca	0.84*		0.15	0.05	0.20
	Pb	0.11	0.01		0.32*	0.18
	Cd	-0.33*	-0.31*	0.51*		0.13
	Ni	0.14	0.03	0.15	-0.00	

* Significant correlation at $p \leq 0.05$

Table IV. Concentrations of selected metals in the male pubic hair of smokers and non-smokers from the experimental and control groups [$\mu\text{g/g}$]**Tabela IV.** Zawartość wybranych metali we włosach łonowych mężczyzn palących i niepalących z grupy badanej i kontrolnej [$\mu\text{g/g}$]

	Non-smokers				Smokers			
	Average	Median	Minimum	Maximum	Average	Median	Minimum	Maximum
Experimental group								
Mg	70.7	67.3	15.0	147	55.1	49.0	20.2	128
Ca	489	335	69.3	1653	307	278	104.4	579
Pb	2.17	1.38	0.61	6.12	3.98	2.30	0.71	17.8
Cd	0.15	0.07	0.01	0.82	0.18	0.11	0.03	0.65
Ni	0.44	0.35	0.08	1.29	0.54	0.51	0.26	1.07
Control group								
Mg	64.8	57.5	18.2	122	52.2	43.5	17.7	108
Ca	409	371	188	870	318	310	181	469
Pb	2.77	1.75	0.36	13.8	2.33	1.61	0.35	7.66
Cd	0.13	0.09	0.01	0.35	0.16	0.11	0.01	0.55
Ni	0.41	0.32	0.15	0.98	0.47	0.47	0.17	0.80

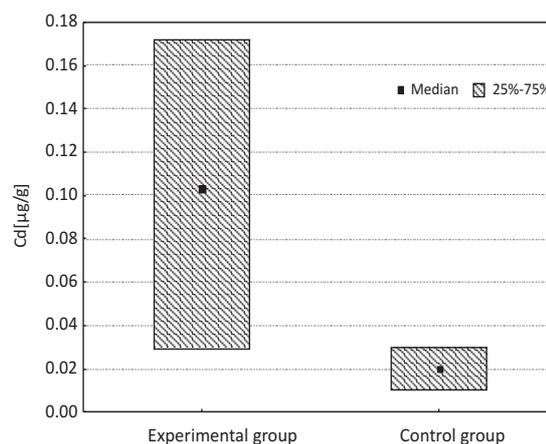
The antagonistic correlation between cadmium and calcium concentrations was found in the pubic hair from the control group, while for magnesium and cadmium in both groups – table III. A correlation between lead and cadmium concentrations in hair, which pointed to their mutual source of origin, was also significant.

The concentrations of the elements assayed in the pubic hair of smokers and non-smokers are given in table IV.

The pubic hair of male smokers demonstrated lower concentrations of macroelements, calcium and magnesium. They were lower in the pubic hair of smokers both from the experimental and control groups.

The experimental group revealed a median lead concentration in the pubic hair of smokers by around $1 \mu\text{g/g}$ higher compared to the non-smokers, the difference being statistically significant (Mann-Whitney U test, $p = 0.05$). What should be emphasized was that lead and nickel concentrations were higher in the pubic hair of smokers from the experimental group than the control one. Nickel concentration was also higher in the pubic hair of smoking males from the experimental group (Mann-Whitney U test, $p = 0.02$).

The median cadmium concentration in the hair of smoking males from the experimental and control groups was at a similar level, however, considering its concentrations in the pubic hair of people smoking 10 cigarettes

**Fig. 1.** Cadmium concentrations in the pubic hair of men smoking 10 cigarettes a day from the experimental and control groups.**Ryc. 1.** Zawartość kadmu we włosach łonowych mężczyzn z grupy badanej i kontrolnej wypalających 10 papierosów dziennie.

a day, it appeared that it was significantly higher for the experimental group (Mann-Whitney U test, $p = 0.04$) – fig. 1.

An impact of the quantity of cigarettes smoked a day on the concentrations of other metals in pubic hair was also observed. For instance, magnesium concentration in the male hair from the control group decreased proportionally to the quantity of cigarettes smoked daily. In the experimental group, the lowest magnesium concentration was assayed for males who smoked up to 10 cigarettes a day – fig. 2.

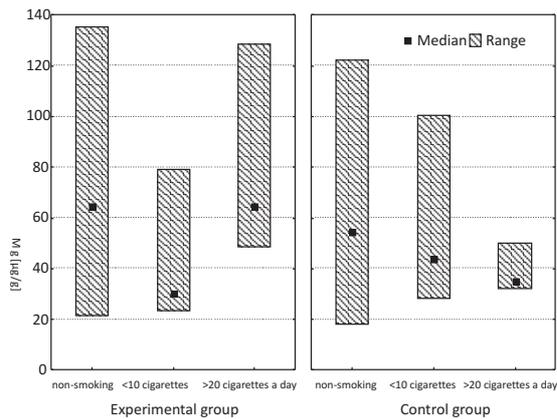


Fig. 2. Magnesium concentrations in the male pubic hair from the experimental and control groups against the number of cigarettes smoked daily.

Ryc. 2. Zawartość magnezu we włosach łonowych mężczyzn z grupy badanej i kontrolnej w zależności od liczby wypalanych dziennie papierosów.

DISCUSSION

In the literature there is no data on the metal concentration in the pubic hair of people with peripheral arterial disease. The obtain results were discussed based on the concentration of elements in the hair healthy person and with other cardiovascular disease, e.g. myocardial infarction.

Magnesium concentration in the pubic hair from the experimental group ranged from 15.0 µg/g to 147 µg/g. Similar concentrations were found by Chojnacka et al. [14] in healthy male head hair, but Bass et al. [15] assayed almost twice as much of the element in hair – 94 µg/g. Much lower concentrations of magnesium were found in the head hair tested by Kozielc and Sałacka [16], and Goch et al. [17].

The results of experimental and metabolic research indicate the important role of magnesium in the cardiovascular system. It protects the walls of blood vessels, fighting the excess of calcium and acting spasmolytically. It functions as a physiological antithrombotic agent. Moreover, it has been discovered that a low supply of magnesium in food and its low concentration in serum can be an etiopathogenetic factor for ischaemic heart disease, arterial hypertension, diabetes and atherosclerosis [17]. For instance, Kawano et al. [18] found a negative correlation between arterial hypertension and magnesium concentration in serum.

The median calcium concentration in the experimental group was 307 µg/g, being a little higher in the hair of the control group – 330 µg/g. The results are slightly higher compared to the ones reported in the literature [5]. Results similar to ours were found in the head hair of healthy people investigated by Forte et al. [11], but the highest results of around 500 µg/g were assayed in male head hair by Unkiewicz-Winiarczyk et al. [19], and DeAntonio et al. [20]. Furthermore, DeAntonio et al. [20] did not discover a marked difference between the mean concentrations of metals, such as Ca, Mg, Cu, Fe and Zn, in head hair and pubic hair.

The role of calcium in the development of arterial hypertension and cardiovascular disease has been widely emphasized. Investigations showed that an elevated content of calcium in food is connected to the more frequent occurrence of arterial hypertension [9]. There are also reports suggesting possible use of calcium analysis in hair to diagnose cardiovascular disease. MacPherson et al. [21] described a correlation between calcium concentration in hair and the risk of coronary heart disease.

At present, a lot more attention is paid to the connection between vascular changes and the presence of toxic elements in the body. The results of epidemiological, clinical and experimental studies indicate that there is a correlation between occupational and environmental exposure to lead, cadmium and artery hypertension [9].

Lead, due to its considerable toxicity and widespread occurrence in the natural environment, is one of the most common metals assayed in hair. Changes found in the cardiovascular system are extremely important, particularly when a long occupational exposure is the case. The atherogenic activity of lead, suggested in epidemiological and clinical studies, can take place as a result of its impact on lipid metabolism, inhibition of antioxidant enzymes and cytotoxicity to endothelium [22].

The median lead concentration in the male pubic hair from the experimental group reached 1.80 µg/g, in the control group was 1.74 µg/g. The fact that the people examined lived in industrial areas should be emphasized here. Comparison of the results mentioned above with the literature data shows that only Kle-vay et al. [23] assayed lower concentrations – 1.49 µg/g. In most cases, lead concentrations in head hair found by different authors were

higher than that assayed in the pubic hair from the experimental group. The average lead concentration in the works by Sukumar and Subramanian [24] was 10.8 $\mu\text{g/g}$ in the head hair of men living in industrial areas, while the head hair of males from rural areas contained 8.0 $\mu\text{g/g}$ on average. Higher lead concentrations were also reported by Bush et al. [4], Bass et al. [15], Gellein et al. [25] and Lekouch et al. [6]. Kozielc and Sałacka [16] as well as Kazi et al. [26] found even higher lead concentrations – 28.37 $\mu\text{g/g}$ and 17.8 $\mu\text{g/g}$ respectively. Wilhelm et al. [27] assayed 2.95 $\mu\text{g/g}$ of lead in pubic hair while its concentration in head hair reached 1.82 $\mu\text{g/g}$. The research by Goch and Goch [9] revealed statistically significant differences in lead concentrations in the head hair collected from both healthy people and those suffering from arterial hypertension, and the research by Afridi et al. [28] demonstrated higher concentrations of lead in the hair of males with myocardial infarction.

Miekeley et al. [29] revealed that the average cadmium concentration in human head hair should be lower than 1.0 $\mu\text{g/g}$. Its median concentration in the male pubic hair from the experimental group reached 0.10 $\mu\text{g/g}$, similar to that assayed in the control group – 0.11 $\mu\text{g/g}$. The values were higher than those assayed by Wilhelm et al. [27] in male pubic hair. A concentration similar to ours was found by Klevay et al. [23] in male head hair. Other authors assayed higher cadmium concentrations in hair [4,29]. Average concentrations over 1 $\mu\text{g/g}$ were found by Afridi et al. [28], and Lekouch et al. [6].

The results of numerous studies indicate the important role of cadmium in the pathogenesis of vascular diseases. Its activity in blood vessels can damage the synthesis and regeneration of intercellular matrix, notably glycosaminoglycans, and affect the structure and metabolic processes of endothelial cells. The damage of the structures and metabolic pathways mentioned above can result in premature atherosclerosis. If vascular endothelium is regarded as a target organ for the toxic effect of cadmium and keeping in mind the ability of metals to accumulate in endothelial cells, it is assumed that long-lasting effect of cadmium causes endothelium hypofunction resulting in an increase in vessel contractility and proliferation as well as intravascular clotting [30].

It should also be stated that cadmium interacts with other elements, mainly calcium and zinc

[14,30]. In our research, an antagonistic correlation between cadmium and calcium concentrations was found in the pubic hair from the control group, while for magnesium and cadmium in both groups (table 3).

Nickel can cause faster development of atherosclerosis and induce oxidative stress [9]. The median nickel concentration in the male pubic hair from the experimental group reached 0.40 $\mu\text{g/g}$ and was higher than its concentration in the control group (0.33 $\mu\text{g/g}$). A similar nickel concentration in hair was found by Rodushkin and Axelsson [31] – 0.43 $\mu\text{g/g}$. On the other hand, the men examined by Chojnacka et al. [14] had, on average, 0.83 $\mu\text{g/g}$ of nickel in their hair, and even higher concentrations were found by Sukumar and Subramanian [24]. The research carried out by Shah et al. [32] revealed much higher levels of nickel in the head hair of males from Pakistan. Its concentration ranged from 0.16 $\mu\text{g/g}$ to 8.36 $\mu\text{g/g}$, and its arithmetic mean was 2.45 $\mu\text{g/g}$. Shah et al. [32] investigated nickel concentrations in male head hair from Libya. Its mean concentration reached 0.53 $\mu\text{g/g}$ and was very close to our mean value. The highest mean nickel concentrations were found by Afridi et al. [33]; it was 7.53 $\mu\text{g/g}$ in the head hair of 16–30-year-old healthy males and 20.0 $\mu\text{g/g}$ in a group of people with dermatosis. As to the hair of males with myocardial infarction, nickel concentration was around 8 $\mu\text{g/g}$, while its value in the reference group reached 5.4 $\mu\text{g/g}$ [28].

Smoking is one of the main risk factors for cardiovascular diseases. It creates favorable conditions for atherosclerosis changes in coronary vessels in heart and peripheral arteries, of lower limbs in particular. Hypoxia which results from smoking increases the number of erythrocytes, reduces their ability to deform, increases blood viscosity and slows down its flow through vessels, which causes increased incidence of peripheral artery disease [34]. Most epidemiological studies investigating the problem discovered a strong correlation between smoking and atherosclerosis, and the relative risk was estimated to be 1.7–7.5. It has been found that smoking plays an important role in the formation of intravascular clots, notably in the coronary arteries and obliterated peripheral vessels. Moreover, smokers are diagnosed as having the disease ten years earlier than non-smokers [35]. Multiple and complex mechanisms that lead to pathological changes in the cardiovascular system caused by arte-

rial hypertension and atherosclerosis cover the dysfunction and morphological changes in vascular endothelium. They are brought about by a number of exogenous substances, including the components of cigarette smoke. Nicotine, carbon oxide, lead, cadmium or carbon disulfide are among those usually mentioned as etiological causes of those diseases [36].

The pubic hair of male smokers demonstrated lower concentrations of macroelements, calcium and magnesium, and higher median concentrations of lead and nickel than the control group.

Lead and nickel are elements which, apart from cadmium, exert substantial influence on the body of smokers. The findings of Mortada et al. [37] were similar to ours and showed that the average lead concentration for smokers was considerably higher compared to non-smokers.

It is well-known that cadmium can be responsible for sclerosis of peripheral arteries. Smokers revealed elevated cadmium concentrations in blood and increased incidence of atherosclerosis, of peripheral arteries in particular. Much higher cadmium concentrations are found in the arteries of smokers than non-smokers, and

its amount correlates with the quantity of cigarettes smoked [36].

In our experimental group, the concentration of cadmium in the pubic hair of smokers was twice as much as for non-smokers. That increase in cadmium concentrations in smokers was also reported by Sukumar and Subramanian [38] who found almost a two-fold increase for smokers compared to non-smokers.

CONCLUSIONS

The research did not reveal a significant difference in the concentrations of selected elements in the pubic hair of males suffering from peripheral artery disease compared to the control group. Slight, statistically insignificant differences found suggest that they may occur and the examination of a wider group of subjects can help assess them. However, the much higher concentration of the toxic elements (Pb, Ni) in the hair of smoking people from the experimental group compared to the non-smokers was a very interesting observation. Such a significant difference between smokers and non-smokers was not found in the control group.

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