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The effect of environmental factors on concentration of trace elements in hip joint bones of patients after hip replacement surgery

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Abstract

The aim of this study was to assess the impact of environmental factors: cigarette smoking, dental amalgam fillings, eating habits and osteoporosis, on the concentrations of copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd) and mercury (Hg) in the bone of hip joint in patients in the Orthopedics Clinic in Szczecin, Poland. Amalgam dental fillings had an effect on the Cu concentration in the cartilage with the adjacent compact bone, and on Hg concentration in the spongy bone. The highest concentrations of Cu and Hg were found in people with multiple amalgam fillings. Smoking appeared to influence Pb concentration in the cartilage with the adjacent compact bone, and Cd concentration in the spongy bone. Increased Pb was detected in smokers, and increased Cd in non-smokers. Diets rich in fish and seafood correlated with Cd concentration in the cartilage with adjacent compact bone. The greatest concentration was in people who ate fish or seafood at least once a month.

Key words

element environmental exposure, femur head, human, trace elements

INTRODUCTION

The natural environment contains metals essential for the proper functioning of the human body, such as copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), calcium (Ca) and magnesium (Mg), as well as highly toxic metals such as lead (Pb), cadmium (Cd) and mercury (Hg), which can adversely affect metabolism [1]. The levels of these metals in the human body depend on the dose, absorption pathway, excretion rate and diet. The concentrations in tissues vary, depending on environmental factors, health status and diet [2].

Significant amounts of metals in the environment are the result of human economic activity and pose a serious health risk to humans and animals [3]. Zinc and copper are essential microelements which are introduced into the environment during the production of crude oil and its products, electrical equipment and alloys (e.g. brass and bronze). These metals are also widely used in the production of tools, machinery, ships, lighting, and in the printing, electrical, metallurgical, ceramic, pharmaceutical and paint industries. Copper is often impregnated into wood and is an important component of insecticides and fungicides [4, 5]. Zinc is contained in approximately 200 enzymes. It is essential for the proper functioning and development of teeth and bones [6]. Too high doses of Zn and too small Zn content in the bone contribute to a gradual decrease in bone mass [7]. Excess zinc accumulates in the kidneys, liver and reproductive organs. People under prolonged exposure to zinc dust and zinc oxide experience respiratory irritation and symptoms of metal fume fever, disorders of the gastrointestinal and nervous systems, and anemia [8, 9]. Copper is involved in redox reactions [10] and is essential for the proper functioning of the connective tissue and brain [8]. Poisoning with copper salts, especially sulphates, occurs infrequently. Its main symptoms include impairment of the liver, kidney, capillaries, hemolytic jaundice and hemolytic anemia.

The group of bone-toxic substances also includes the toxic three metals: lead, cadmium and mercury. Lead and cadmium are introduced into the environment during the manufacture of cables, wires, bearings, dyes, nickel-cadmium batteries, ammunition, plastics and insecticides. The sources of anthropogenic pollution by these heavy metals also include chemical, metallurgical, tannery and carbon-based electricity production. It was estimated that in 2007 the atmospheric emissions of anthropogenic lead and cadmium from the Baltic countries amounted to 1,142 and 106 tonnes, respectively, of which Poland emitted into the atmosphere 49% and 38% of that total amount [11]. Lead toxicity is manifested by impaired activity of enzymes involved in the synthesis of heme. Chronic exposure results in anemia, damage to the nervous system and kidneys, and also has a toxic effect on bones [12, 13, 14]. Human uptake of cadmium takes place mainly through food. Chronic oral exposure to Cd concentration > 1 mg/kg causes microcytic iron-deficient anemia [3]. Exposure to significantly higher cadmium levels occurs when people smoke tobacco. Cadmium toxicity affects the nervous system, the liver and bones. It induces changes in sex hormone concentrations [15]. Chronic cadmium exposure has been shown to have a embryotoxic and teratogenic effect [16]. Maternal exposure to Cd is associated with low birth weight and an increase in spontaneous abortion [17, 18].

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Mercury is mainly used in the production of biocides, batteries, dental amalgam fillings, industrial electrolysis and in the extraction of gold, but most of this metal (in the form of Hg0 vapor) is emitted into the atmosphere during the production of electricity based on burning coal, lignite and crude oil [19]. It was estimated that in 2005 global atmospheric emissions of anthropogenic Hg equaled 1,930 tonnes, of which over 66% came from Asia, and about 8% each from Europe and North America [20]. Poland is one of the largest polluters in terms of mercury atmospheric emissions and Hg contamination of the Baltic Sea. In 2007, the total atmospheric mercury emissions in Poland were estimated at nearly 16 tonnes [21]. Mercury enters the body mainly with food products, generally fish and seafood, as well as amalgam dental fillings [22]. Mercury is a potent neurotoxin disrupting the functioning of the central and peripheral nervous systems, as well as having a nephrotoxic effect [23].

Bone, due to its characteristics and long recovery time, is one of tissues that reflects long-term exposure to metals and may serve as the basis of indirect assessment of environmental exposure [24, 25]. Importantly, there is no evidence whether the mobilization of bone stores could occur at a rate that could result in poisoning.

In our previous studies [24] it was shown that in the bones of the femur head, obtained from patients after hip replacement surgery, the concentrations of trace elements occurred in the following descending sequence Zn>Pb>Cu>Cd>Hg. In addition, it was found that the concentration of lead in the cartilage with adjacent compact bone was higher in men than in women [24]. In this study, the aim was to assess the effect of environmental factors: cigarette smoking, dental amalgam fillings, eating habits and osteoporosis, on the concentrations of Cu, Zn, Pb, Cd and Hg in the femur of the hip joint of patients in an Orthopedics Clinic in Szczecin, Poland.

MATERIALS AND METHODS

Patients. The study group comprised 37 patients at the Chair and Clinic of Orthopedics and Traumatology of the Pomeranian Medical University in Szczecin: 24 women (age range 69.9 ± 10.76) and 13 men (age range: 58.6 ±13.1) who were examined between November 2007 – December 2009. The study included patients who had undergone arthroplasty of the femoral head. In most cases, the indication for this treatment was a degeneration of the left and/or right hip joint (15 and 19 patients, respectively), or fracture of the left femur (3 patients). The study was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin (KB-0080/62/09).

Determination of zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb) and mercury (Hg). Preparation of bone tissue material for analysis and determination of Zn, Cu, Cd, Pb and Hg were carried out as described previously [24]. From the prepared bones 2 types of materials were obtained: cartilage with adjacent compact bone and spongy bone. Parts of the femoral head and neck were dried to a constant weight at 55°C and 105°C. Concentrations of Cu, Zn, Pb and Cd were determined by atomic absorption spectrophotometry (ICP-AES) in inductively coupled argon plasma using a Perkin-Elmer Optima 2000 DV. Determination of total mercury (THg) concentrations was performed by atomic absorption spectroscopy using an AMA 254 mercury analyzer.

Statistical analysis. Statistica 9.0. (StatSoft) software was used for the analysis. In order to determine compliance with the expected normal distribution of results, a Kolmogorov-Smirnov test with a Lilliefors correction ($p<0.05$) was used. In order to compare the impact of various environmental factors on the concentration of metals in the bone test, the Kruskal-Wallis test (KW) was used, and the Mann-Whitney U test (M-W U test) in the case of significant differences ($p<0.05$). In addition, the Spearman rank correlation coefficients between trace elements in the material and between the individual metals in two different parts of the hip joint (cartilage with compact bone, spongy bone) were determined. In order to determine a possible connection between the studied metals and the patients' age, the number of cigarettes smoked and the number of amalgam dental fillings, the Spearman rank correlation coefficient (rs) was calculated to determined its significance.

RESULTS

Analysis was performed to determine the possible effect of amalgam dental fillings, cigarette smoking, food preferences, as well as osteoporosis, on the concentration of metals in the femoral hip joint bones of patients. Statistical analysis included data obtained from surveys of patients from whom the bone samples were taken.

Amalgam dental fillings and Cu, Zn and Hg in the cartilage with adjacent compact bone and in the spongy bone. Analysis took into account only the concentrations of elements contained in dental amalgam fillings, i.e. Cu, Zn and Hg. Patients were divided into 3 groups, depending upon the number of dental amalgam fillings:

AM0 – patients with no amalgam fillings;
AM1 – patients having from one to three amalgam fillings;
AM2 – people with four to six amalgam fillings.

Table 1 provides information on the concentrations of these elements in bone material derived from the 3 groups of patients, and the significance of differences in metal concentrations in the corresponding materials, based on the Kruskal-Wallis test ($p<0.05$).

Copper. Comparison of concentrations in the 2 types of examined bone materials showed statistically confirmed differences in Cu concentration in the cartilage with adjacent compact bone between groups AM0 and AM2 (M-W U test =41.0; $p<0.04$) and between AM0 and AM1 (M-W U test =12.0; $p<0.01$). The lowest average concentration of this metal was detected in AM1 and the greatest in AM2. There were also clear differences in the concentration of Cu in the spongy bone (M-W U test =14.0; $p<0.01$). The lowest concentration of Cu was in group AM0; it was higher in AM1 and similar in AM2.

Zinc. The concentration of Zn in the cartilage with adjacent compact bone was 27% higher in AM2 compared to AM0, but these differences were not statistically significant.

Mercury. There was only a weak statistically significant difference in Hg concentration in the spongy bone (M-W U test =32.0; p<0.10) between AM0 and AM1, although in AM1 it was 100% greater. In AM0 and AM2 mercury concentration was similar (Tab. 1). The concentrations of this metal in the cartilage with adjacent compact bone increased across the groups: 0.0023 mg/kg dw in AM0, 0.0035 mg/kg dw in AM1; finally reaching 0.0040 mg/kg dw in AM2 group, although the differences were not statistically significant.

Table 1. Concentrations of copper (Cu), zinc (Zn) and mercury (Hg) (in mg/kg dry weight) in 3 groups of patients with a variable number of dental amalgam fillings.

Group of patients	Parameter	Cu	Zn	Pb
		CACB		
AM0 n=25	AM±SD	0.76±0.30	86.0±16.6	0.0028±0.0015
	Med	0.75	85.3	0.0023
	range	0.33-1.77	54.3-130.9	0.0010-0.0015
	CV	40.0	19.4	53.8
AM1 n=5	AM±SD	0.37±0.17	73.7±15.7	0.0029±0.0018
	Med	0.28	67.5	0.0035
	range	0.20-0.63	57.9-91.8	0.0010-0.0049
	CV	47.7	21.3	61.1
AM2 n=7	AM±SD	1.20±0.47	106.8±34.2	0.0046±0.0037
	Med	1.40	108.4	0.0040
	range	0.49-1.78	66.8-163.8	0.0010-0.0123
	CV	38.9	32.0	80.1
Total	AM±SD	0.79±0.40	88.29±22.52	0.527±0.204
	Med	0.74	85.68	0.496
	range	0.20-1.78	54.28-163.86	0.285-1.440
	CV	50.60	25.50	38.70
		SB		
AM0 n=25	AM±SD	0.36±0.14	85.5±14.0	0.0020±0.0013
	Med	0.38	80.0	0.0017
	range	0.18-0.50	55.5-114.3	0.0002-0.0057
	CV	37.6	16.3	65.4
AM1 n=5	AM±SD	0.74±0.37	82.3±45.3	0.0034±0.0018
	Med	0.68	72.8	0.0034
	range	0.36-1.90	49.5-160.5	0.0015-0.0060
	CV	50.2	55.1	53.9
AM2 n=7	AM±SD	0.64±0.30	75.2±23.0	0.0020±0.0006
	Med	0.64	76.7	0.0018
	range	0.23-1.07	44.2-100.3	0.0011-0.0030
	CV	46.5	30.5	28.9
Total	AM±SD	0.67±0.36	83.10±21.48	0.0022±0.0013
	Med	0.58	79.36	0.0018
	range	0.18-1.89	44.23-160.50	0.0002-0.0060
	CV	53.00	25.90	61.60
		Significance of K-W		
CACB		p<0.05	NS	NS
SB		p<0.05	NS	NS

AM – arithmetic mean; SD – standard deviation; CV – coefficient of variation in percent; AM0 – no amalgams; AM1 – 1–3 amalgam; AM2 – 4–6 amalgam; CACB – cartilage with adjacent compact bone; SB – spongy bone; KW – Kruskal-Wallis test; NS – difference non-significant.

Table 2. The concentrations of lead (Pb) and cadmium (Cd) (mg/kg dry weight) in smokers and non-smokers among examined patients.

Group of patients	Parameter	Pb	Cd
	CACB		
NONS n=15	AM±SD	0.467±0.123	0.024±0.035
	Med	0.431	0.024
	range	0.309-0.715	0.002-0.151
	CV	26.3	121.1
S<20 n= 15	AM±SD	0.555±0.284	0.022±0.012
	Med	0.496	0.027
	range	0.285-1.440	0.001-0.036
	CV	51.2	52.6
S>20 n=7	AM±SD	0.593±0.098	0.030±0.031
	Med	0.586	0.028
	range	0.446-0.736	0.002-0.093
	CV	16.5	102.8
TS n=22	AM±SD	0.567±0.239	0.025±0.020
	Med	0.512	0.027
	range	0.285-1.440	0.001-0.093
	CV	42.1	79.3
Total	AM±SD	0.527±0.204	0.031±0.204
	Med	0.496	0.021
	range	0.285-1.440	0.001-0.151
	CV	38.70	100.50
	SB		
NONS n=15	AM±SD	0.529±0.168	0.024±0.013
	Med	0.481	0.025
	range	0.287-0.789	0.002-0.052
	CV	31.8	52.7
S<20 n= 15	AM±SD	0.473±0.099	0.035±0.066
	Med	0.464	0.014
	range	0.298-0.719	0.001-0.269
	CV	21.0	190.6
S>20 n=7	AM±SD	0.498±0.167	0.019±0.014
	Med	0.475	0.012
	range	0.329-0.750	0.002-0.040
	CV	33.6	71.9
TS n=22	AM±SD	0.481±0.121	0.030±0.055
	Med	0.481	0.022
	range	0.298-0.750	0.001-0.269
	CV	25.2	184.9
Total	AM±SD	0.500±0.142	0.028±0.040
	Med	0.500	0.023
	range	0.287-0.789	0.001-0.269
	CV	28.40	155.50
	Significance of K-W		
CACB		p<0.05	NS
SB		NS	NS

AM – arithmetic mean; SD – standard deviation; CV – coefficient of variation in percent; NONS – non-smokers; S<20 and S>20 – less or more than 20 cigarettes per day; TS – total smokers; CACB – cartilage with adjacent compact bone; SB – spongy bone; KW – Kruskal-Wallis test; NS – difference non-significant

Cigarette smoking and the concentration of metals in the examined materials. Table 2 shows the concentration of highly toxic metals present in cigarette smoke (Pb and Cd) in the 2 examined bone materials obtained from non-smokers (NONS) and smokers (S). Three groups were established: NONS – non-smokers (n=15); S<20 – smokers of up to 20 cigarettes per day (n=15) and S>20, smoking more than 20 cigarettes per day (n=7), but comparisons were also performed between NONS and total S (n = 22).

Lead and cadmium. Statistically confirmed differences in Pb concentration in the cartilage with adjacent compact bone, as shown by the Kruskal-Wallis test (p<0.05), were observed between S>20 and NONS (M-W U test =21; p<0.03). A more detailed statistical analysis of the cartilage with adjacent compact bone which compared pairs of the groups of patients (M-W U test) revealed differences in Pb concentration. The lowest concentration of this metal was detected in NONS and S<20, and the highest in S>20 which had a 35% higher concentration of Pb than NONS.

The maximum Cd concentration was recorded in the spongy bone of S (Tab. 2). In addition, in 2 S patients Cd concentration was at least 0.100 mg/kg dw. In NONS there were no such cases. Comparison of element concentrations between materials derived from NONS and S patients showed statistically weak differences between the concentration of Pb in the cartilage with adjacent compact bone (M-W U test =236.0; $p<0.20$) and Cd in the spongy bone (M-W U test=123,0; $p<0.20$). The concentration of Pb in the cartilage with adjacent compact bone in S was about 16% higher than NONS, and in spongy bone Cd concentration of S was 11% lower than in NONS. There was no statistically significant correlation between the concentration of highly toxic elements in both types of bone material and the number of cigarettes smoked. Only the concentration of Pb in the cartilage with adjacent compact bone exhibited a weak relationship with the number of cigarettes smoked (rs=0.321; $p<0.10$).

Diet and Cd, Pb and Hg in the examined bone materials. The survey of patients from whom the bone samples were taken included questions about eating habits which took into account the main sources of Hg and Cd in the human diet: fish and seafood. The bone material was divided into 3 groups:

R0 – bones from people who did not consume fish and seafood;

R1 – those who consumed fish and seafood once a month;

R2 – from those who ate fish or seafood several times a month.

Statistically significant differences ($p<0.05$) only existed in the case of Cd measured in the cartilage with adjacent compact bone (Tab. 3). This difference was observed between R1 and R2 (M-W U test = 60.0; $p<0.01$). The lowest concentration of Cd was detected in R2 and the greatest in R1.

Table 3. Concentrations of cadmium (Cd), lead (Pb) and mercury (Hg) (in mg/kg dry weight) in the bone material derived from patients with diets differing in fish and seafood consumption.

Group of patients	Parameter	Cd	Pb	Hg
CACB				
R0 n=4	AM±SD Med range CV	0.021±0.013 0.026 0.002-0.030 60.2	0.561±0.192 0.573 0.363-0.74 34.2	0.0025±0.0010 0.0023 0.0014-0.0038 39.7
R1 n=15	AM±SD Med range CV	0.036±0.033 0,031 0.008-0.151 91.0	0.562±0.273 0.521 0.309-1.44 48.7	0.0026±0.0015 0.0022 0.0010-0.0065 59.0
R2 n=18	AM±SD Med range CV	0.020±0.022 0.019 0.001-0.093 108.1	0.490±0.0131 0.491 0.285-0.723 26.8	0.0040±0.0030 0.0040 0.0010-0.0120 69.7
SB				
R0 n=4	AM±SD Med range CV	0.021±0.013 0.025 0.002-0.032 62.9	0.574±0.152 0.530 0.449-0.789 26.4	0.0017±0.0014 0.0015 0.0004-0.0035 78.6
R1 n=15	AM±SD Med range CV	0.040±0.064 0.023 0.014-0.270 157.6	0.441±0.0134 0.439 0.287-0.750 29.2	0.0019±0.0012 0.0017 0.0002-0.0060 62.5
R2 n=18	AM±SD Med range CV	0.018±0.016 0.021 0.001-0.052 86.5	0.520±0.0143 0.490 0.342-0.750 27.6	0.0025±0.0014 0.0018 0.0005-0.0060 58.5
Significance of K-W test				
CACB		p<0.05	NS	NS
SB		NS	NS	NS

AM – arithmetic mean; SD – standard deviation; CV – coefficient of variation in percent;
R0 – no fish and seafood in diet; R1 – once a month; R2 – several times a month;
CACB – cartilage with adjacent compact bone; SB – spongy bone; KW – Kruskal-Wallis test;
p – level of significance; NS – difference non-significant

In the cartilage with adjacent compact bone, Pb concentrations were arranged in a descending manner in successive groups:

0.573 mg/kg dw in R0;

0.521 mg/kg in R1;

0.491 mg/kg dw in R2, although the differences were not statistically significant.

Hg concentrations in the cartilage with adjacent compact bone were arranged in an ascending manner – the smallest and similar values were observed in R0 and R1, and nearly twice as much in R2, although again the differences were not statistically significant. A similar trend, but even weaker, was observed in spongy bone.

Zn, Cu, Pb, Cd and Hg in bone material of patients with osteoporosis. The samples were divided into 2 groups of patients: O0 – patients without osteoporosis, and O1 – patients with documented osteoporosis. Mann-Whitney U tests showed no statistically confirmed differences in metal concentrations between groups O0 and O1. Both in patients with and without osteoporosis, the highest concentration among the essential elements for life was Zn in the cartilage with adjacent compact bone (Tab. 4). The concentration of this metal was about 12% lower in patients with osteoporosis, but this group had only 5 people, which could have strongly affected the outcome of the comparisons. Copper in the cartilage with adjacent compact bone had a similar concentration in both groups, but its concentration in spongy bone in patients without osteoporosis (O0) was about 25% lower than in group O1. Among the toxic elements found in the material derived from both groups (O0 and O1), similar levels of Pb were observed in cartilage with adjacent compact bone and in spongy bone. Cadmium concentration was also similar in both types of samples obtained from patients from groups O0 and O1, and average values ranged from 0.020 – 0.026 mg/kg dw. The concentration of Hg in the cartilage with adjacent compact bone in group O0 was about 60% higher than in group O1, and in spongy bone Hg concentration was similar in both groups.

DISCUSSION

Determination of trace elements in living organisms, including bone tissue, is one of the main ways of using bio-indicators for indirect assessment of environmental pollution.

Dental amalgams and mercury concentration in the bones. In the presented study, there was a statistically weak difference in the concentrations of mercury in the spongy bone between people with and without amalgam fillings. As is apparent from available scientific literature, the average absorption of mercury from amalgam fillings is 1–2 µg per day, which represents 10% of the daily intake of Hg with food, water and air [26, 27]. Studies on the relationship between the number of amalgam fillings, their age and the concentration of Hg in urine showed significant relationships. In addition, there is evidence that during the process of filling cavities with amalgam, Hg may penetrate soft tissues and bones of patients [27]. There are reasons to believe that the presence and installation of amalgam fillings may contribute to

Table 4. Concentrations of zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd) and mercury (Hg) (in mg/kg dry weight) in bone material derived from patients with and without osteoporosis.

Material	Parameter	Zn	Cu	Pb	Cd	Hg
			O0, n=32			
CACB	AM±SD	89.7±23.5	0.80±0.42	0.530±0.21	0.022±0.016	0.0026±0.0022
	Med	86.5	0.74	0.490	0.026	0.0030
	range	54.3-163.9	0.21-1.78	0.280-1.440	0.001-0.038	0.0010-0.0123
	CV	26.2	52.4	40.1	52.4	71.4
SB	AM±SD	83.9±21.8	0.68±0.36	0.500±0.143	0.030±0.050	0.0021±0.0013
	Med	79.6	0.57	0.481	0.023	0.0017
	range	44.2-160.5	0.23-1.89	0.290-0.799	0.001-0.269	0.0002-0.0060
	CV	25.9	53.7	28.6	161.1	63.7
			O1, n=5			
CACB	AM±SD	79.2±12.6	0.71±0.20	0.506±0,153	0.056±0.064	0.0037±0.0019
	Med	77.1	0.76	0.496	0.025	0.0019
	range	62.2-159.6	0.45-0.90	0.310-0.689	0.002-0.151	0.0019-0.0065
	CV	15.9	28.5	30.1	115.6	53.3
SB	AM±SD	78.2±21.3	0.62±0.32	0.510±0.150	0.021±0.012	0.0022±0.0011
	Med	76.3	0.72	0.470	0.0020	0.0018
	range	49.5-107.7	0.18-0.99	0.346-0.746	0.002-0.034	0.0011-0.0042
	CV	27.2	51.5	29.6	55.9	53.2
			O0 vs O1			
CACB	U p	NS	NS	NS	NS	NS
SB	U P	NS	NS	NS	NS	NS

O0 – patients without osteoporosis; O1 – patients with documented osteoporosis; n – number patients; AM – arithmetic mean; SD – standard deviation; Med – median; CV – coefficient of variation in percent; CACB – cartilage with adjacent compact bone; SB – spongy bone; M-W U – Mann-Whitney U test; p – level of significance; NS – difference non-significant

increased concentrations of Hg in human bones, as suggested by the presented results and the finding of the Scientific Committee on Emerging and Newly Identified Health Risks contained in the report by Mutter [27]. It should be noted, however, that in scientific literature no information was found on the specific concentration of mercury in skeletal pieces in relation to amalgam dental fillings.

Smoking and the concentrations of toxic metals in the bones. As a result of smoking, the human body accumulates significant amounts of Cd and Pb. It has been reported that smoking 20 cigarettes per day causes a gradual increase in Cd content in the blood, and sometimes also in bone [28]. Jurkiewicz et al. [29] found that the concentration of Cd in the spongy layer of the femur in those smoking more than 20 cigarettes per day was more than two times higher than in non-smokers. This metal has a negative impact on calcium metabolism and therefore why smoking is considered to be a major risk factor for osteoporosis and related bone fractures [29, 30, 31, 32]. Brodziak-Dopierala et al. [30] found the greatest concentrations of Pb and Cd in addicted smokers; they amounted to approximately 8 mg/kg and 0.4 mg/kg dw, respectively. In the presented study, the inhabitants of north-western Poland who had smoked over 20 cigarettes a day had a lower concentration of Pb and Cd in the cartilage with adjacent compact bone (0.586 and 0.028 mg/kg dw, respectively), compared to values given by Brodziak-Dopierala et al. [30] for heavy smokers from the Silesian agglomeration. It is possible that the increased accumulation of Pb and Cd in patients studied by Brodziak-Dopierala et al. [30] was due to environmental pollution and also compulsive and long-term cigarette smoking.

In the presented study, Pb concentration was higher in the cartilage of smokers than in non-smokers, similar to Jurkiewicz et al. [33]. In patients from north-western Poland this may reflect the associated increased exposure to this metal with tobacco smoking. In some older patients from other regions of Poland who were life-long smokers, even higher concentrations of Pb and Cd were detected in the spongy bone of the femoral head. Brodziak-Dopierala et al. [34], observed ~4 mg Pb/kg and ~0.2 mg Cd/kg dw in the same material, which is several times more than in the bone material analyzed in this study. In the cartilage of patients studied by Brodziak-Dopierala et al. [34], Pb and Cd concentrations were >10 and >0.65 mg/kg dw, respectively, which was even greater than in the spongy bone of the femur.

For patients from north-western Poland, similar to the inhabitants of Upper Silesia, there was no statistically significant correlation between the concentrations of highly toxic elements in the cartilage or spongy bone and the number of cigarettes smoked [29].

Food preferences and concentrations of toxic metals in the bones. A varied diet, for example, rich in fish and seafood, may increase the accumulation of Hg, Cd and Pb in humans. Research has shown that in recent years a large amount of consumed aquatic invertebrates (e.g. molluscs and crustaceans) can be a source of Cd intoxication [35]. According to the European Community Regulations [5] the acceptable weekly intake of Hg and Cd in food (Provisional Tolerable Weekly Intake, PTWI), is 5 and 7 µg per kg of body weight, respectively, and in the flesh of fish Hg levels should not exceed 1 mg/kg ww. Fish available on the Polish market, with the exception of butterfish, do not demonstrate high exposure to Hg (i.e. not higher than 0.73 mg Hg/kg).

Elevated levels of this metal in the bodies of Poles that are associated with the consumption of fish are seasonal, related to increased consumption during Christmas. In this study, in patients from Szczecin and surrounding areas there existed some differences in the concentrations of Hg in bones between individuals regularly consuming fish and seafood, and people not preferring such a diet. The concentrations of Hg in the cartilage with adjacent compact bone were the lowest and similar in the groups of patients who do not or only rarely eat fish and seafood (<0.0025 mg/kg), and almost twice as high among those consuming fish and seafood several times a month (0.0040 mg/kg). However, the differences were not statistically significant. To some extent, these results may suggest that a diet rich in fish and seafood contributed to the observed differences in the concentrations of Hg in the cartilage with adjacent compact bone derived from femoral heads of inhabitants of Szczecin and the surrounding area.

Bone diseases and the concentrations of toxic metals. Concentrations of Pb and Cd have been determined in different types of human bones. Most studies relate to bone material obtained during hip replacement. Jurkiewicz et al. [29] studied the concentration of Cd in the head of femur bones with symptoms of degenerative changes. They found a higher Cd concentration in the articular cartilage (0.07 mg/kg dw) compared to the spongy bone and compact bone. In both these materials, the concentration of Cd was 0.05 mg/kg dw. This indicates the facilitated penetration of Cd into the affected area of the articular cartilage [29]. A similar study conducted in Szczecin showed a higher Cd concentration in the spongy bone (0.026 mg/kg dw) compared to the cartilage with adjacent compact bone (0.021 mg/kg dw), but the difference between these mean values was not statistically significant.

Pb and Cd in bone tissue were found to be important factors in the occurrence of osteoporosis [36]. In the case of certain medical conditions (*osteoporosis, osteoarthritis*), various researchers report typical, reduced or elevated concentrations of heavy metals, including Pb and Cd. Wiechula et al. [14] found that Pb concentration in the femoral head of patients with *coxarthrosis* was about 3 mg Pb/kg and Cd 0.07 mg Cd/kg. The reported values are consistent with those reported by Jurkiewicz et al. [33] for the spongy bone of the femoral head in patients with osteoporosis. In comparison to the quoted values, in the presented study different toxic concentrations of these metals were detected in spongy bone in the examined north-western Polish population. Concentrations of Pb and Cd in patients were 0.470 and 0.020 mg/kg dw, respectively, i.e. 540% and 250% lower compared to Wiechula et al. [14]. The data is therefore very divergent and interpretation difficult and ambiguous because the increased concentrations of toxic metals in the bones may have been induced not only by diseases, but also by different environmental factors, including diet, smoking and place of residence.

CONCLUSIONS

Analysis of the impact of environmental factors on the concentration of metals in the bones of patients showed the influence of:

- amalgam dental fillings on the concentration of Cu in the cartilage with adjacent compact bone and Hg concentration in spongy bone. The highest concentration of Cu and Hg were found in people with multiple amalgam fillings;
- cigarette smoking on the concentration of Pb in the cartilage with adjacent compact bone and Cd in the spongy bone; a higher concentration of Pb was detected in smokers, and Cd in non-smokers, although these differences were not statistically significant;
- a diet rich in fish and seafood on the concentration of Cd in the cartilage with adjacent compact bone; its concentration was the greatest in people who ate fish or seafood once a month.

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