



## Research paper

### Occurrence of mercury in the knee joint tissues

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#### ABSTRACT

**Introduction:** Mercury is one of the elements that are commonly found in nature. This element is highly toxic, mainly affecting the nervous system, kidneys and lungs. Mercury ions can accumulate in bone and cartilage and build up behind calcium ions in carbonates and hydroxyapatites. High mercury concentrations in the spongy bone compared to the compacted bone were found.

**Aim:** The aim of the manuscript was to assess the mercury content in tibial and femoral tissue taken from patients undergoing knee arthroplasty.

**Material and methods:** Samples were taken from 17 patients (tibial and femoral bone samples), collected from patients who underwent knee arthroplasty. The tested samples were homogenized and determined by atomic absorption spectrometry and the AMA 254 amalgamation technique. Studies have shown mercury presence in all samples tested.

**Results and discussion:** The range of mercury content in the tested samples was 3.3–19.18  $\mu\text{g}/\text{kg}$ . The average for the examined bone tissue samples was 8.71  $\mu\text{g}/\text{kg}$ , while for the tibia it was slightly higher (9.08  $\mu\text{g}/\text{kg}$ ), compared to the femur (8.34  $\mu\text{g}/\text{kg}$ ). There was a high mercury content in men's bone tissue (10.05  $\mu\text{g}/\text{kg}$ ), compared to women (8.15  $\mu\text{g}/\text{kg}$ ). In both sexes, higher levels of mercury in the tibia were found in men (11.08  $\mu\text{g}/\text{kg}$  in men, and 8.24  $\mu\text{g}/\text{kg}$  in women).

**Conclusions:** The dependence between mercury concentration in bone tissue and the patient's age, weight and BMI, the number of cigarettes smoked and the consumption of fish and seafood were checked. There were no statistically significant correlations between these indicators.

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## 1. INTRODUCTION

Mercury and its compounds are considered one of the most dangerous substances. Mercury circulation in the environment is a complicated process as it undergoes changes in many elements of the natural environment: air, soil, water and in living organisms. Due to its high toxicity, bioaccumulation and biomagnification in living organisms, mercury is of interest among scientists.<sup>1-3</sup>

The growing awareness of the harmfulness of mercury prompted the development of legal acts regarding extraction, use and storage of mercury and its compounds. The main assumption is limitation of extraction, replacement in industry with less toxic counterparts, and control of utilization of this element.<sup>4,5</sup>

Food products such as fish and seafood are the main source of mercury exposure for people who are not exposed at work. Thousand to ten thousand times more methylmercury may accumulate in their tissues than in other food products.<sup>1,6,7</sup> Methylmercury is considered the most toxic mercury compound due to its strong neurotoxic activity and ability to bioaccumulate and biomagnify.<sup>1,8</sup> It is soluble in lipids, which is why it accumulates in the nervous system, reproductive system and liver the most, where it has toxic effects.<sup>9-11</sup> What is more, the factor determining the mercury content in fish meat is the place of catch, age, muscle mass and the type of food consumed by fish.<sup>3,5</sup> The European Food Safety Authority (EFSA) has established permissible mercury concentrations in fish and seafood that are between 0.5 µg/kg and 10 µg/kg fresh weight.<sup>12</sup>

Bone is one of the most important target organs for heavy metals. This results in the toxicity of these elements in bone tissue and changes such as degenerative processes, osteoporosis and bone mineral changes that can lead to fractures.<sup>13,14</sup> There is a large number of studies on mercury content in bones from excavations from the Middle Ages, when mercury was widely used.<sup>15-18</sup>

Bone tissue, due to its slow metabolism, allows determining the degree of exposure to metals and metal compounds over several years or more. The long half-lives of metals in bones have made them a good indicator of exposure to heavy metals. By disturbing the elemental balance and affecting other metals, may also accumulate in bone tissue. Mercury ions can accumulate in bone and cartilage and build up behind calcium ions in carbonates and hydroxyapatites. High mercury concentrations in the spongy bone compared to the compacted bone were found. This is due to the larger surface in contact with the blood vessels and the greater metabolic turnover of the spongy bone.<sup>5,19-21</sup>

The mercury content in bones is lower than in other tissues of the human body, e.g. in the kidneys and liver, as confirmed by the results of Garcia et al. (2001).<sup>22</sup> High mercury content was found in bones from excavations.<sup>16</sup>

## 2. AIM

The aim of this manuscript is to assess the mercury content in bone tissue obtained from patients undergoing knee arthroplasty. An analysis of the mercury content in the tissues

examined, depending on sex, age of the patient, weight, addiction to smoking and consumption of saltwater fish was made as well.

## 3. MATERIAL AND METHODS

The material for mercury testing were tibial and femoral bone samples, collected from 17 patients who underwent knee arthroplasty at the Janusz Daab Traumatology Hospital in Piekary Śląskie, Poland. The indication for this surgery was knee osteoarthritis and significant pain that had been felt for 10 years on average. All patients came from the Silesian Province. The studied population was 12 women and 5 men. In 8 patients, samples were taken from the right limb, and in 9 patients – from the left. The average age in the examined group was 70 years, the range was from 55 to 78 years, in women the average age was 70.4 years, and in men 69.8 years. During the procedure, femoral epicondylitis and tibial plateau were cut off, then, after describing the biological material, they were stored in polyethylene containers at –22°C in the freezer. The general characteristics of the samples tested are presented in Table 1. Bone tissue samples were removed from the freezer and left for 8 h at room temperature, then placed in a muffle furnace for 18 h at 90°C to evaporate water from the samples.<sup>24</sup>

**Table 1. General description of the test samples.**

Parameters	Description
Gender, <i>n</i>	
women	12
men	5
Age, years	
whole population	70.24 ± 6.61
women	70.42 ± 6.36
men	69.80 ± 7.47
Place of live, <i>n</i>	
village	5
city up to 10000 population	1
city 10000–100000 population	3
city over 100000 population	8
Knee, <i>n</i>	
right	8
left	9
Height, m*	
whole population	1.64 ± 0.12
men	1.81 ± 0.09
women	1.58 ± 0.05
Body weight, kg*	
whole population	83 ± 13.7
men	94 ± 16.7
women	78 ± 9.6
BMI, <i>n</i>	
correct value	1
overweight	3
I degree of obesity	8
II degree of obesity	2
no information	3
Smokers, <i>n</i>	
smokers	8
nonsmokers	2
no information	7

Comments: \* Numbers are given as arithmetic mean ± SD.

Mercury was determined using atomic absorption spectrometry and the AMA 254 (Altec, Czech Republic) amalgamation technique. This method allows for a direct determination of the total concentration of mercury in solid samples, regardless of their form, using the ease of release of the element from organic and inorganic forms. The method uses the phenomenon of radiation absorption of mercury atoms in a gaseous form. The radiation emitted by the low-pressure mercury lamp absorbs free mercury atoms, and consequently, the radiation intensity is reduced in proportion to the number of mercury atoms released from the sample. The radiation intensity registers the spectrometer and converts absorbance into concentration.<sup>24,25</sup>

The limit of quantification is 0.01 ng total Hg (THg), and the reproducibility of this method is estimated at approximately 1.5%. The analysis time for one sample was approximately 7 minutes, in 3 repetitions each. The time of individual stages was 120 s, 140 s and 60 s.<sup>24–26</sup> The camera is controlled using a PC with Windows 7, a specialized program from Leco to control the current process, calibration curve and statistical analysis of results.

The statistical analysis of the results obtained was made in Statistica 13. The first stage of statistical analysis was to determine the distribution of mercury content in bone tissue using the Lilifors normality test. The distribution of THg content was normal ( $P < 0.01$ ). To develop and describe the obtained results, the values of the arithmetic mean, standard deviation, median and range of changes were used: minimum and maximum, 10<sup>th</sup> and 90<sup>th</sup> percentiles. The one-factor ANOVA test for individual BMI groups and the Student's *t* test for smokers and non-smokers were performed. A scatter diagram was made for mercury content by age and by BMI.

#### 4. RESULTS

The statistical analysis made for the results obtained is given in Table 2. The lowest content of THg was found in the tibia bone sample, its average was 3.31  $\mu\text{g}/\text{kg}$ . A small amount of this element was also observed in sample 6 for tibia, and sample 9 for femur (3.33  $\mu\text{g}/\text{kg}$  and 3.761  $\mu\text{g}/\text{kg}$ , re-

**Table 2. Statistical analysis of THg in bone tissue samples,  $\mu\text{g}/\text{kg}$  d.w.**

Type bone	AM	SD	CV, %	Range
Tibial	9.08	4.58	50	3.30–19.18
Femur	8.34	2.96	36	3.76–14.99
All	8.75	3.77	44	3.30–19.18

Comments: AM – arithmetic mean; SD – standard deviation; CV – coefficient variability.

**Table 3. THg content in smokers and non-smokers ( $\mu\text{g}/\text{kg}$  d.w.)**

	<i>n</i>	AM	SD	Med	Range		Percentyl		CV
					Min	Max	10	90	
Nonsmoking	18	7.87	3.61	6.49	3.31	14.99	3.33	12.65	46
Smoking	4	8.96	1.49	8.92	7.21	10.79	7.21	10.79	17

Comments: AM – arithmetic mean; SD – standard deviation; Med – median; CV – coefficient variability.

spectively). The highest amount of this metal was found in sample 11 for tibia (19.18  $\mu\text{g}/\text{kg}$ ), high concentrations were also found in sample 14 for tibia and sample 5 for femur (17.43  $\mu\text{g}/\text{kg}$  and 14.99  $\mu\text{g}/\text{kg}$ , respectively). The lowest scatter of results characterized the tibia bone sample (0.01  $\mu\text{g}/\text{kg}$ ), and the greatest the tibial bone sample 38 (1.26  $\mu\text{g}/\text{kg}$ ).

The range of changes for all bone tissues was 3.3–19.9  $\mu\text{g}/\text{kg}$ . The arithmetic mean of all samples tested was 8.71  $\mu\text{g}/\text{kg}$  and the SD was 3.77  $\mu\text{g}/\text{kg}$ . The coefficient of variation for all samples was 43%. In total 6 tibia samples were above average and 6 femoral samples.

The distribution of THg content in bone tissue was normal ( $P > 0.05$ ). In the studied group, the most results (29%) were in the range of 6–8  $\mu\text{g}/\text{kg}$ .

Analysing the mercury content in bone tissue by sex, we observe a lower content in women (8.15  $\mu\text{g}/\text{kg}$ ), than in men (10.05  $\mu\text{g}/\text{kg}$ ). However, gender differences are not statistically significant.

There was no dependence between the THg content in bone tissue and the patient's age. The correlation between mercury concentration and age is poor ( $r = -0.0186$ ). The regression equation is as follows:  $y = 9.4624 - 0.0108x$ . With the passage of each year, the THg concentration in bone tissue decreases by 0.0108  $\mu\text{g}/\text{kg}$ . The above chart explains 0.03% of cases.

There was no dependence between THg concentration in bone tissue and the patient's weight. There is a poor correlation between the THg content and the patient's weight ( $r = 0.15$ ). The regression equation is as follows:  $y = 5.0086 - 0.0359x$ . As the weight increases by 1 kg, the THg concentration increases by 0.0359  $\mu\text{g}/\text{kg}$ . The scatter chart explains 2.36% of cases. There is no dependence between the THg content and the patient's BMI ( $P = 0.7$ ). There is a poor correlation between the mercury concentration and the patient's BMI ( $r = 0.075$ ). The regression equation is as follows:  $y = 6.0745 - 0.0625x$ . Comparing BMI values for patients with the one-way ANOVA test, no differences were found between these groups.

There was no dependence between the THg content in bone tissue and the number of cigarettes smoked (Table 3). There is an average correlation between the THg content in bone tissue and the number of cigarettes smoked ( $r = 0.39$ ). The regression equation is  $y = 8.1375 - 0.0137x$ . Along with the increased number of cigarettes smoked, the THg content in bone tissue increases by 0.137  $\mu\text{g}/\text{kg}$ . Smoking and non-smoking groups were also compared using the student's *t* test for independent groups, but no difference was found between the groups studied.

Saltwater fish consumption is the greatest source of exposure. However, this dependence has not been demonstrated in the conducted tests. The highest THg content occurred

in the group of people who occasionally consumed fish (9.22  $\mu\text{g}/\text{kg}$ ), followed by people who consumed fish twice a week (8.30  $\mu\text{g}/\text{kg}$ ). The lowest content concerned people consuming sea fish twice a month (8.03  $\mu\text{g}/\text{kg}$ ). There were no statistically significant differences between the mercury content in the examined bone tissue and the frequency of consumption of saltwater fish. The regression equation is as follows:  $y = 9.6808 - 0.5911x$ .

## 5. DISCUSSION

Bone tissue is an example of a biological material in which remodelling processes occur throughout human life. Through the process of mercury accumulation in bones, they are an important material for assessing mercury exposure over a longer period.

Among all samples, the THg content range was between 3.3–19.18  $\mu\text{g}/\text{kg}$ , while the arithmetic mean was  $8.71 \pm 0.28$   $\mu\text{g}/\text{kg}$ . The scope of literature on works on mercury content in bone tissue is very marginal. In femoral samples, the median was 7.9  $\mu\text{g}/\text{kg}$ , and the change ranged from 3.76  $\mu\text{g}/\text{kg}$  to 14.99  $\mu\text{g}/\text{kg}$ . These results were very similar to those obtained in the knee tissues. In the work of Ziola-Frankowska et al.<sup>27</sup> from Poznań, the median was 17.3  $\mu\text{g}/\text{kg}$ , and the range was 3.6–128.5  $\mu\text{g}/\text{kg}$ , while in the work of Kwapuliński et al.,<sup>28</sup> the average was 0.14  $\mu\text{g}/\text{kg}$ .<sup>27,28</sup> The average THg content in tibia tissue in the tests was 9.08  $\mu\text{g}/\text{kg}$ , median 8.38  $\mu\text{g}/\text{kg}$ , and the range 3.31–19.18  $\mu\text{g}/\text{kg}$ . Lanocha-Arendarczyk et al.<sup>29</sup> calculated the average of 5  $\mu\text{g}/\text{kg}$ , median of 3  $\mu\text{g}/\text{kg}$ , and the range of changes of 1–30  $\mu\text{g}/\text{kg}$ .<sup>29</sup> These values are similar to those obtained in these studies. Rasmussen et al.<sup>30</sup> determined the mercury content of bones from cemeteries in Denmark.<sup>30</sup> Those were skeletons that had pathological symptoms caused by syphilis. Researchers calculated that skeletons contained 40 times more mercury compared to the results of research on modern human bones. Interestingly, in another work published by Rasmussen et al.<sup>31</sup> they compared two male skeletons, one with syphilis and the other with no symptoms<sup>31</sup>. Mercury concentration in the skeletal tissue of the healthy skeleton was comparable to the results of research on bone tissue in modern man according to Rasmussen.<sup>32</sup>

The next stage of the work was to check the correlation between the mercury content in bone tissue and the patient's age. The study showed no correlation. Comparing to literature data, Ziola-Frankowska et al.<sup>27</sup> also did not show statistical significance between the patient's age and mercury concentration in femoral bone from those undergoing hip surgery.<sup>27</sup> In turn, Kim et al.<sup>13</sup> noticed higher mercury levels in young men compared to older ones.<sup>13</sup> The same work also analysed the effect of blood mercury on bone density and osteoporosis in Korean men. The authors suggested that people with higher levels of mercury in the blood have a reduced bone density and more frequent osteoporosis. Lavado-Garcia et al.<sup>14</sup> did not confirm this dependence in studies on a group of women before menopause.<sup>14</sup> Cho et al.<sup>33</sup> conducted a study on 481 postmenopausal women investigating the dependence

between blood concentration and bone density and the occurrence of osteoporosis.<sup>33</sup> They hypothesized the effect of mercury on bone metabolism, thereby reducing their density. However, the research did not confirm the hypothesis, it even showed a protective effect. The incidence of osteoporosis was 0.36 times lower at higher mercury concentrations. Fish and seafood have been identified as the main source of mercury, as they are the main ingredient in Korean women's diet. The authors (2012) stated that bone protection is affected by omega 3 and 6 fatty acids, and arachidonic acid in fish, thus masking the effects of mercury on bone tissue.<sup>33</sup> In the conducted tests there was no dependence between the THg content and the consumption of salt-water fish and seafood. Lanocha-Arendarczyk et al.<sup>29</sup> confirmed this dependence with a correlation coefficient of 0.42.<sup>29</sup>

The correlation between mercury concentration in bone tissue and the patient's weight and BMI values were subsequently analysed. There were no statistically significant correlations between these indicators. Cho et al.<sup>31</sup> in their work showed a dependence between the concentration of mercury in the blood and these indicators on Korean women in the postmenopausal period, Ziola-Frankowska et al.<sup>27</sup> also showed a dependence between the mercury content in the femur and the patient's mass and BMI.<sup>27,33</sup>

There was no correlation between the number of cigarettes smoked and the THg content, and no statistically significant difference was found between the group of smokers and non-smokers. Referring to the literature references of Ziola-Frankowska et al.,<sup>27</sup> Lawado-Garcia et al.<sup>14</sup> and Kim et al.<sup>13</sup>, the possible dependence between the number of cigarettes smoked and the concentration of THg in the body is indicated.<sup>13,14,27</sup>

Summing up the above discussion, mercury concentration tests in bone tissue seem to be appropriate research material. Many of the listed literature positions indicate a correlation between the increased amount of mercury in the body's tissues and selected factors.

## 6. CONCLUSIONS

- (1) The determined THg content in the tibia was slightly higher than in the femur.
- (2) The bone tissue of men had a higher THg content than that of women.
- (3) There was no correlation between the THg content in bone tissue and the patient's age, body weight, BMI and smoking.
- (4) The consumption of sea fish does not affect the THg content in the examined bone tissue.
- (5) Studies need to be continued.

## Conflicts of interest

The authors declare no conflict of interest.

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## Ethics

Biological material for research was obtained with the consent of the Bioethics Committee Resolution No. 2/2013 of 18.06.2013.

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