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## Factors affecting the aluminium content of human femoral head and neck

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## ABSTRACT

Tissues for the study were obtained intraoperatively during hip replacement procedures from 96 patients. In all the cases, the indication for this treatment was primary or secondary degenerative changes in the hip joint. The subject of the study was the head and neck of the femur, resected in situ. Aluminium concentrations measured in femoral head and neck samples from patients aged between 25 and 91 were varied. Statistical methods were applied to determine the variations in relation to the parameters from the background survey. Significant differences in the aluminium content of femoral head samples were observed between patients under and over 60 years of age. Based on the results, it was confirmed that the aluminium accumulates in bones over a lifetime. The study showed that the content of aluminium in the head and neck of the femur depends on the factors such as: type of medicines taken, contact with chemicals at work, differences in body anatomy and sex. The study on the levels of aluminium in bones and the factors affecting its concentration is a valuable source of information for further research on the role of aluminium in bone diseases. Based on the investigations, it was found that the GF-AAS technique is the best analytical tool for routine analysis of aluminium in complex matrix samples. The use of femoral heads in the investigations was approved by the Bioethics Committee of the University of Medical Sciences in Poznań (Poland).

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## 1. Introduction

The studies on aluminium showed that it is biologically available and related to many human diseases including neurological disorders [1], e.g. Alzheimer's disease [2]. Aluminium may also cause osteomalacia when the gastro-intestinal barrier to absorption is bypassed or renal excretion impaired [3–5]. Its toxic effect is accompanied by strongly elevated levels of this metal in serum, brain and bone tissues [6]. The aluminium can disturb normal bone growth and may also lead to renal osteodystrophy. Besides, the accumulation of aluminium in bones was shown to result in bone loss. This element can also inhibit bone turnover and reduce the activity and differentiation of osteoblasts [7–9]. Moreover, some studies revealed that aluminium might be implicated in bone fragility in sufferers from Alzheimer's disease [10].

There are various sources of human exposure to aluminium, including: diet, dermal application of personal care products, use of antacids and aluminium based adjuvants in vaccinations [1,11]. The average human intake is about 10 mg Al/day (range: 10–1000 mg Al/day), mainly in medicines, water, food and by inhalation [11,12]. About 4% of aluminium is retained from the diet by intestinal absorption [13].

Additionally, the aluminium can be partially accumulated in bones, which are the main storage site of this element, for years under continuous intake conditions [13]. Besides, it was found that in elderly patients as well as in the particularly young patients with Alzheimer-type pathology, the intestinal absorption of aluminium is increased, thus enhancing the alleged accumulation in bone tissue [14].

Human bone analysis can provide useful information on age-related chronic diseases and the health status of renal and bone cancer patients [15]. The determination of aluminium in bones may be particularly helpful in getting direct information on its accumulation in the organs of patients with renal osteodystrophy and the evidence of the range of chronic exposure in studies of Alzheimer's disease [10].

The main objective of this study was to determine the concentration of aluminium in the proximal femoral head (cancellous bone) and femoral neck (cortical bone) of patients attending the Clinic of Orthopaedics and Traumatology (Poznań, Poland) and undergoing total hip replacement for osteoarthritis, using GF-AAS technique. The present study was conducted to assess differences in the concentration of aluminium between the femoral head and neck, according to sex, and also to examine, by statistical analysis, a possible correlation between various factors, including age, place of residence, tobacco and alcohol consumption, contact with chemicals in the workplace, physical activity, diet, medicines and the content of aluminium in the femoral bone. Moreover,

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our additional aim was to investigate whether the determined concentration levels of aluminium in the femoral head and neck may pose a risk to human health.

## 2. Materials and methods

### 2.1. Ethics statement

The study was approved by the Bioethics Committee of the Karol Marcinkowski University of Medical Sciences in Poznań, Poland (approval no. 172/14). Patients who participated in the study were informed about the research and gave their consent to use their tissues for research purposes. All the necessary information was collected from each patient in order to fill in the questionnaire used for subsequent statistical analyses.

### 2.2. Patients

The sample consisted of 96 patients operated on for total hip replacement (THR) in the Orthopaedic Clinic in Poznań, Poland. Table 1 shows the characteristics of patients enrolled in the study, with particular emphasis on the factors that possibly affect the concentration of aluminium in the femoral bone. The patients involved in the study were not diagnosed with dementia-related diseases, nor with Alzheimer's disease and renal disease.

### 2.3. Materials

Tissues for the study were obtained intraoperatively during hip replacement procedure. In all cases, the indications for this treatment were primary or secondary degenerative changes in the hip joint. The subject of the study was the head and neck of the femur, resected in situ with an orthopedic oscillating saw.

In the total hip replacement procedure, the head of the femoral bone is replaced with a metal ball and a cup is placed into the acetabulum. The examined proximal ends of the femurs had undergone degenerative processes and changed their shape. The femoral heads were flattened and bony excrescences could be observed at the head-neck border. Bone condensation could be observed in the superolateral part of the femoral head, as well as cysts in the femoral head and neck. Articular surfaces revealed defects that were more noticeable in the loading area. The articular cartilage was not present pathologically, or was changed, softened, disintegrated, with areas of local destruction due to vascular congestion of subchondral bone and blood vessel infiltration.

The subject of the study was the head and neck of the femur, resected in situ with an orthopedic oscillating saw. Directly after acquisition of the biological material (head and neck of the femur), bone sub-samples were excised under sterile conditions from the head and neck of the femur in order to obtain approximately 1 g of the material. The sub-samples were taken down the femoral head in such a way as to collect a full section of cancellous bone without articular capsule and without articular cartilage. In the case of the femoral neck, the thickness of a collected section was 1–2 mm, and for the cortical bone it was 5 mm and the slices had a shape of a triangle. The aluminium-free surgical equipment was used during the sampling procedure.

### 2.4. Methods

The frozen bone samples were freeze-dried using a Lyovac lyophilizer GT2e (Steris, Germany), for 24 h. After drying, approximately 0.5 g of the sample was weighed, placed in a Teflon bomb and put in the Mars 5 Xpress microwave oven (CEM, USA). A 10 ml volume of supra-pure nitric acid (V) (Merck, Germany) was added to the digestion bombs. The prepared samples were allowed to stand for 8 h for slow mineralization. Then, they were mineralized in the microwave oven, using a modified EPA method 3051 [16]. After cooling, the samples were placed

**Table 1**  
Information on patients included in the study.

Factors	Sample of patients (n = 96) operated on for total hip replacement (THR)	
Age [years]	AM ± SD/Med. (range)	
	Women (n = 57)	64.5 ± 14.2/65 (25–87)
Body weight [kg]	Men (n = 39)	63.2 ± 10.2/61 (42–91)
	Women (n = 57)	69.8 ± 14.2/68 (45–115)
Height [cm]	Men (n = 39)	84 ± 16.1/81 (45–114)
	Women (n = 57)	159.9 ± 6.8/160 (139–174)
Width of the femur [cm]	Men (n = 39)	172.2 ± 6.4/173 (155–185)
	Women (n = 57)	2.98 ± 0.29/3 (2.4–3.7)
Place of residence	Men (n = 39)	3.31 ± 0.3/3.4 (2.5–3.8)
		Number of patients (percent)
Type of hip osteoarthritis	Village	24 (25%)
	City > 10,000	16 (16.7%)
	Town < 10,000	56 (58.3%)
Cigarette smoking	Primary – idiopathic	54 (56.3%)
	Secondary – developmental dysplasia of the hip	42 (43.8%)
Alcohol drinking	Non-smoker	73 (76%)
	Irregular smoker	6 (6.3%)
	Regular smoker	17 (17.7%)
Physical activity	Non-drinker	46 (47.9%)
	Occasionally	23 (24%)
	Often	27 (28.1%)
Environment pollution	During puberty	60 (62.5%)
	Before the disease	30 (31.3%)
	Constant	23 (24%)
Contact with chemicals in the workplace	Air	5 (5.2%)
	Water	3 (3.1%)
	Soil	2 (2.1%)
Diet	Type of chemicals: fertilizers, plant protection arable, heavy metals, paints, adhesives, plaster, tannery articles, photographic chemicals, reagents used in metallurgy, other chemicals, detergents	30 (31.3%)
	Meat	5 (5.2%)
	Fish	8 (8.3%)
	Vegetarian	1 (1.0%)
	High consumption of tea	17 (17.7%)
	High consumption of coffee	9 (9.4%)

in polyethylene volumetric flasks and filled up to 50 ml with demineralized water. The extracts of bone samples after digestion were clear and colorless or slightly yellow, with no visible sediment and fat residues. The same observations during the preparation of the samples of human breast and human brain tissues for the determination of aluminium were found by [17,18].

The concentration of Al was determined using the Shimadzu AA7000 Graphite Furnace AAS analytical technique (Shimadzu, Japan). The optimized parameters of the atomic absorption spectrometer with graphite furnace atomization have been presented in Tables 2 and 3. Standard reference material (SRM) of bone ash from the National Institute of Standards and Technology (NIST) was used to check the accuracy of analyte mass fraction determination.

The statistical analysis of the collected data was performed with Statistica PL v.7.0 (StatSoft) software. In order to determine compliance with the expected normal distribution of results we used a Shapiro-Wilk test ( $p < 0.05$ ), and to examine significant differences, a Mann-Whitney U-test was performed ( $p < 0.05$ ).

## 3. Results

In total, 96 patients – 57 women and 39 men – with a mean age of 64 years (range: 25–91) were included in this study. Figs. 1 and 2 show the content of aluminium in the femoral head and neck of men and women, depending on age.

For women, aluminium content was determined in all six age groups (A.20–40; B.41–50; C.51–60; D.61–70; E.71–80; F > 80), both in the

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