

Original Article



Chromium Content in the Human Hip Joint Tissues*

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Abstract

Objective Chromium has many important functions in the human body. For the osseous tissue, its role has not been clearly defined. This study was aimed at determining chromium content in hip joint tissues.

Methods A total of 91 hip joint samples were taken in this study, including 66 from females and 25 from males. The sample tissues were separated according to their anatomical parts. The chromium content was determined by the AAS method. The statistical analysis was performed with U Mann-Whitney's non-parametric test, $P \leq 0.05$.

Results The overall chromium content in tissues of the hip joint in the study subjects was as follows: 5.73 $\mu\text{g/g}$ in the articular cartilage, 5.33 $\mu\text{g/g}$ in the cortical bone, 17.86 $\mu\text{g/g}$ in the cancellous bone, 5.95 $\mu\text{g/g}$ in the fragment of the cancellous bone from the intertrochanteric region, and 1.28 $\mu\text{g/g}$ in the joint capsule. The chromium contents were observed in 2 group patients, it was 7.04 $\mu\text{g/g}$ in people with osteoarthritis and 12.59 $\mu\text{g/g}$ in people with fractures.

Conclusion The observed chromium content was highest in the cancellous bone and the lowest in the joint capsule. Chromium content was significantly different between the people with hip joint osteoarthritis and the people with femoral neck fractures.

Key words: Chromium; Femur head; Hip joint; Cortical bone; Cancellous bone

Biomed Environ Sci, 2015; 28(2): 89-96

doi: 10.3967/bes2015.011

ISSN: 0895-3988

www.besjournal.com (full text)

CN: 11-2816/Q

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INTRODUCTION

Chromium is counted among trace elements that are obligatory for the energy metabolism of humans and animals^[1]. It affects the metabolism of glucose and lipids. It is a component of the glucose tolerance factor (GTF)^[1]. Moreover, it has influence on certain enzymes that regulate the synthesis of cholesterol^[2-3]. When absorbed by blood, chromium

binds to globulin. Bound to transferrin it is transported to tissues^[4]. Chromium contained in blood is absorbed into the bone quite quickly. It also accumulates in the spleen, liver and kidneys^[4-6].

Chromium is subject to the process of exchanges between the plasma and the surface of bones^[6]. Like lead, though with much lower efficiency, it can be introduced into the bone structure during bone remodelling processes^[7]. Bones are an important reservoir of chromium after

*This work was financed by the Medical University of Silesia in Katowice (contract No. KNW-1-124/K/4/0).

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its oral or intraperitoneal administration^[8-9].

Weber^[10] conducted long-term degradation studies of soluble chromate-51 in rats and found that radioactive chromium administered orally was mainly accumulated in the area of epiphysis of long bones. O'Flaherty^[7,11] published a research on chromium as an essential and toxic metal and model of chromium kinetics in rats. The distribution of radioactive chromium compounds was considered similar to Ca45 or Sr89 which have a high affinity for bones^[11].

Berry et al.^[12] showed that prosthesis-released chromium can be a reason for the extensive osteolysis around a cementless knee prosthesis. In such a situation, chromium may induce a lower level of activity of osteoblasts which may result in prosthesis loosening^[13].

Chromium alloys are applied in various types of orthopedic prostheses and implants^[14-15]. However, over time these alloys corrode and soluble chromium ions (VI) are released. Toxicity of these metal ions contributes to hypersensitivity reactions, neurological disorders and bone diseases^[14]. Accumulation of chromium in the skeleton may interfere with bone formation and resorption by modulating key enzymes which are involved in these processes^[11]. Based on experiments on rats, Sankaramaqnivel et al.^[16] presented a hypothesis that chromium disturbs the bone remodelling process. Chromium exerts its influence on bones by modulating biochemical parameters of bone: alkaline phosphatase (ALP), tartrate-resistant acid phosphatase (TRAP), calcium and phosphorus. A significant accumulation of chromium and reduction of the alkaline phosphatase activity in the skeleton were observed in this study. This resulted in changes in the bone formation rate^[16].

Some reports show that chromium added to mixtures of ceramic prostheses stimulates bone remodelling as shown by the increased cellular activity (osteoblastic cells) and bone resorption. As a result, the implant may grow into the surrounding tissues^[14-15,17].

It has been demonstrated that chromium ions can be added to the hydroxyapatite crystal during mineralization and affect the parameters of the crystal lattice and the size of the crystals. The bone tissue around the metallic implants containing chromium may be changed^[14,18]. Studies using the electron spin resonance (ESR) suggested that chromium is associated with the organic constituent of the calcified tissues^[19].

It can be concluded that the adult rats' exposure to K₂Cr₂O₇ in their prenatal and postnatal periods impairs their growth and decreases the mineral density of bones^[20]. The study showed that K₂Cr₂O₇ changes biochemical parameters related to the bone tissue and histopathological parameters of the femur^[20].

Chromium has been determined as a component of cigarette tobacco with the concentration ranging from 0.4 to 10.0 µg/g^[21]. Antilla et al.^[22] observed that average lung tissue levels of chromium was 6.4 µg/g in smokers and 2.2 µg/g in nonsmoking referents^[22]. The pulmonary metal concentrations were compared with smoking history, pulmonary emphysema, age, and occupation^[22]. The mean chromium concentrations for the non-smokers, smokers, and ex-smokers were 1.3, 4.3, and 4.8 µg/g dry wet, respectively. The pulmonary chromium content increased with age and smoking time, but showed no connection with occupation^[23].

The content of elements in bones is dependent on several factors, including age, sex, place of residence, health status, smoking, or diet pattern^[1,2,6,9,24-27]. After reaching the peak bone mass, calcium loss is observed with age. Moreover, accumulation of heavy metals derived from environmental and occupational exposures occurs^[1,28]. The difference in bone mass between women and men is quite significant, therefore sex is an important factor to influence the content of trace elements in bones^[29]. At the same age, bone mass in women is lesser than bone mass in men^[28]. Women show a greater tendency to develop osteoporosis, especially in the postmenopausal period, which is confirmed by many long-term studies^[30]. Additional factors that may have influences on the content of elements in bones of women are pregnancy and lactation^[30]. Food components, depending on their origin, may provide the human body with essential substances but also elements that can accumulate in bones^[29].

The role of chromium in bone metabolism has not been fully understood yet. Therefore, it is advisable to trace the changes in chromium content in chosen elements of the hip joint which was removed in arthroplasty. Chromium content was determined in tissues of patients (female, male) with different disease status, due to which joint replacement had been conducted, and in smokers and non-smokers. The novelty of this study was to determine the chromium content in both the bone

and the connective tissue of the joint capsule.

MATERIAL AND METHODS

Subjects of the Study

The samples of the study were elements of hip joint from people living in cities of the Upper Silesian Industrial District ($n=85$) and unpolluted areas ($n=6$) in Poland. The samples were collected in the Municipal Hospital in Siemianowice Slaske, which were obtained intraoperatively during hip replacement procedures based on consent of the Bioethics Committee L.dz.NN-6501-160/I/06.

In total, 91 hip joint samples were taken, 66 from females and 25 from males. The mean age of the patients was 65.7 ± 10.5 years; in females it was 67.3 ± 8.6 years and in males it was 61.4 ± 13.6 years. The patients were divided into two groups according to the reason for endoprosthesis procedure. The first group included people with fractures of the femoral neck ($n=7$), the other one included the people with degenerative changes of the hip joint ($n=84$). The study subjects consisted of smokers ($n=17$) and non-smokers ($n=74$). Non-smokers were mainly females ($n=58$). However, in the group of smokers, there were slightly more males ($n=9$) than females ($n=8$).

The patients included in the study group, do not have personal questionnaire forms of chronic diseases, such as diabetes.

Method of Division Tissue for Study

This type of surgery is recommended, in most cases, for degenerative changes of hip.

Further analysis was conducted on the following samples:

1. Femoral head excised *in situ*.
2. Anterolateral aspect of the joint capsule, which was routinely excised in order to open the hip joint during surgery.
3. A box-shaped fragment of cancellous bone from the intertrochanteric area (this fragment was routinely chiselled out from the femoral bone in order to create a starting point for preparation of the proximal femur before implantation of the prosthetic stem).

The femoral heads were debrided from residual soft tissues. Fragments of joint capsule, ligament of the head of the femur, and femoral neck (especially the medial calcar) were removed with various instruments, such as Liston bone cutting forceps,

Luer bone rongeur and bone curette. In the next stage, bone curette and Luer rongeur were used to remove articular cartilage and then the subchondral bone until a rounded 'center' was obtained made of the cancellous bone only. In some patients, the cartilage was absent or scarce due to progression of osteoarthritis. The subchondral layer of bone had different widths: from virtually non-existent to a fraction of a millimetre to a few millimetres of eburniated bone in advanced coxarthrosis. The remaining part of the femoral head consisted mostly of the cancellous bone, sometimes with heterogeneous microarchitecture, with areas of osteosclerosis and geodes filled with a fibrous connective tissue. The collected samples were placed in polyethylene bags, labelled and stored in a freezer at a temperature of -20 ± 1 °C.

Mineralization and AAS Analysis

In order to conduct the marking of the content of elements in femoral bone head samples using the AAS method, the samples with known weight were subject to gradual ashing to constant weight in a muffle furnace at a temperature of 100 °C (initially), and 420 °C (later). The weight of the wet samples was about 1 g, the weight of the ash was some 0.5-0.8 g. A quantity of acid used, on average, amounted to about 2 mL sample, but if ash has been duly solubilised was used more acid. The analytical sample of ash (approximately 1 g) was digested in 2 cm³ of spectrally pure HNO₃ (V) (Supra pure) by Merck. The formed solution was transferred into a flask with a volume of 25 cm³, then filled up with distilled water to the scale mark. In samples prepared this way, chromium content was determined using the atomic absorption spectrometry method (AAS) and Pye Unicam SP9 apparatus. The correctness of the applied methodology was tested using the method of standard addition.

Statistical calculations were made using the Statistica software for Windows 10. For the needs of the statistical analysis, the samples were taken from 5 individual elements of the hip joint in both females and males. According to the reason for the endoprosthesis procedure, the study subjects were divided into 2 groups: a group of femoral neck fractures and a group of hip osteoarthritis. The study subjects consisted of people living in cities throughout the Upper Silesian Industrial Region and people living in unpolluted areas in Poland. The statistical analysis of chromium occurrence was

conducted with U Mann-Whitney's non-parametric test. The level of significance when analysing the differences was at $P\leq0.05$.

RESULTS

Chromium content in particular parts of the hip joint was as follows: 5.73 $\mu\text{g/g}$ in the articular cartilage, 5.33 $\mu\text{g/g}$ in the cortical bone, 17.86 $\mu\text{g/g}$ in the trabecular bone, 5.95 $\mu\text{g/g}$ in the fragment of the cancellous bone taken from the intertrochanteric region of the femoral bone and 1.28 $\mu\text{g/g}$ in the joint capsule (Figure 1).

There were no statistical significant differences in chromium content in particular parts of the hip joint between males and females in the study subjects. Chromium content in the articular cartilage was 6.34 $\mu\text{g/g}$ in females and 4.42 $\mu\text{g/g}$ in males, and

in the cortical bone it was 5.36 $\mu\text{g/g}$ and 5.26 $\mu\text{g/g}$, respectively. The chromium content in the cancellous bone was highest in the whole hip joint, in females it was 17.08 $\mu\text{g/g}$ and in males it was 19.93 $\mu\text{g/g}$. In the fragment of the cancellous bone from the intertrochanteric region and in the fragment of the joint capsule, the chromium contents in females and males were 6.05 $\mu\text{g/g}$ and 5.75 $\mu\text{g/g}$, and 1.13 $\mu\text{g/g}$ and 1.67 $\mu\text{g/g}$, respectively (Table 1), the differences had no statistical significance.

Statistical significant difference in chromium content were observed between the people with osteoarthritis and the people with fractures. In the former group, the chromium content was 7.04 $\mu\text{g/g}$ and in the latter group, the chromium content was 12.59 $\mu\text{g/g}$ (U Mann-Whitney's Test $P<0.05$) (Table 2).

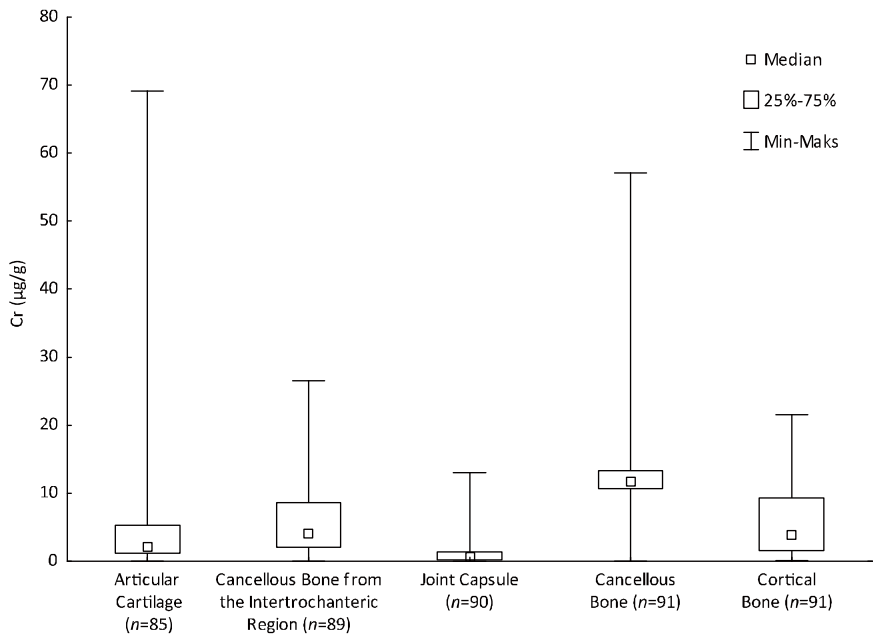


Figure 1. Some statistical parameters the chromium content in selected elements of the hip joint.

Table 1. Concentration of Chromium in Particular Parts of the Hip Joint in Female and Male

Parts	Female (n=66) (µg/g)		Male (n=25) (µg/g)		Female vs. Male (UM-W)
	AM±SD	Med	AM±SD	Med	
Articular cartilage	6.34±13.08	2.25	4.42±5.57	1.8	NS
Cortical bone	5.36±4.60	3.89	5.26±4.55	3.72	NS
Cancellous bone	17.08±15.48	11.8	19.93±20.18	11.78	NS
Cancellous bone from the intertrochanteric region	6.05±5.50	4.01	5.75±5.81	4.14	NS
Joint capsule	1.13±1.47	0.62	1.67±2.91	0.48	NS

Note. AM, arithmetic mean; SD, standard deviation; Med, median; UM-W, U Mann-Whitney test; NS, non-significant ($P>0.05$).

For tobacco smoking, the chromium content was 7.87 µg/g in the non-smokers and 6.07 µg/g in the smokers, the difference was not statistically significant. In the females, there was no statistical significant difference in chromium content between smokers and non-smokers, while in the males the difference was statistically significant (U Mann- Whitney’s Test, $P<0.002$). In the males, the chromium content was higher in smokers than in non-smokers (Table 3).

The chromium content was 7.55 µg/g in people living in polluted areas and 7.36 µg/g in people living in rural areas, the difference was not statistically significant.

No significant correlations were also observed in the occurrence of chromium among particular parts of the hip joint and patients' age.

DISCUSSION

Humans are exposed to various toxic substances which can accumulate in the body. As a result of significant increase of the concentration they can lead to a variety of pathological changes in the tissues. Bones are susceptible to accumulation of metals due to the long recovery time of bone

injury^[24-29]. Therefore, they often reflect the total level of toxic metals in the body^[24-25].

In our study, the chromium content in patients with fractures (12.59 µg/g) was approximately 80% higher than that of patients with osteoarthritis (7.04 µg/g), suggesting that chromium has influence on the change in the structure of the bone tissue and reduction of mechanical strength. In their research Kuo et al.^[24] showed that for various disorders of the hip joint, the lowest content of this element was observed in patients with fractures (4.96 µg/g) and the highest in different diseases (21.33 µg/g).

In the literature, there is a lot of information on the metal content in the bones taken from excavations^[25,31]. Kosugi et al.^[25] determined the content of chromium in bones (ribs) taken from excavation areas of Japan from the 5000 period- 130 years BC. The average chromium content was 23 µg/g, there was no statistical significant differences in the occurrence of chromium among different periods^[25]. These values are significantly higher compared with the results obtained in our study. This could be explained by the different burial conditions and the type of soil in which the bones were contained.

Table 2. Concentration of Chromium in Particular Parts of the Hip Joint in Patients with Femoral Neck Fractures and Hip Osteoarthritis

Parts	Femoral Neck Fractures (n=7) (µg/g)		Hip Osteoarthritis (n=84) (µg/g)		Femoral Neck Fractures vs. Hip Osteoarthritis (UM-W)
	AM±SD	Med	AM±SD	Med	
Articular Cartilage	4.65±3.67	4.55	5.85±11.82	2.09	P=0.03
Cortical Bone	7.77±4.54	9.64	5.07±4.51	3.78	
Cancellous Bone	35.67±24.09	47.68	16.38±15.37	11.78	
Cancellous Bone from the Intertrochanteric Region	9.79±8.11	8.69	5.54±5.12	3.82	
Joint Capsule	5.14±4.81	5.11	0.99±1.23	0.53	

Note. AM, arithmetic mean; SD, standard deviation; Med, median; UM-W, U Mann-Whitney test; *P*, level of significance.

Table 3. Concentration of Chromium in Particular Parts of the Hip Joint in Smoker and Nonsmoker Patients

Variabilities	AM±SD (µg/g)	Med (µg/g)	UM-W
Smoker (n=17)	6.07±9.83	3.00	NS
Non-smoker (n=74)	7.87±11.92	3.55	
Smoker male (n=9)	4.15±9.11	1.17	P=0.002
Non-smoker male (n=17)	9.34±13.09	4.45	
Smoker female (n=8)	8.20±10.29	4.21	NS
Non-smoker female (n=58)	7.40±11.52	3.40	

Note. AM, arithmetic mean; SD, standard deviation; Med, median; UM-W, U Mann-Whitney test; NS, non-significant ($P>0.05$). *P*, level of significance.

Chromium content in bones taken from inhabitants of highly industrialized Taiwan, China was 11.9 $\mu\text{g/g}$ ^[24]. It is higher than the average value of all elements of the hip joint in our study (7.53 $\mu\text{g/g}$).

Considering chromium content in different age groups, it can be observed that the lowest chromium content was in age group <40 years (3.14 $\mu\text{g/g}$), and the highest chromium content was in the oldest age group-over 80 years (11.64 $\mu\text{g/g}$). However, no significant correlations between age and chromium content were observed in particular parts of the hip joint. According to Kuo et al.^[24], the lowest chromium content in Taiwanese was found in age group <40 years (5.36 $\mu\text{g/g}$) and the highest in age group 41-60 years (21.68 $\mu\text{g/g}$). The differences between own results and the results of Kuo et al.^[24], might be duo to the fact that in their own research, the sample size with fractures was small ($n=7$). In addition, Kuo et al.^[24] does not explain what other diseases can be found in the group.

Garcia et al.^[32] determined that the average chromium content in bones of people living in the region of Tarragona in Spain was 0.33 $\mu\text{g/g}$ (range: 0.2-5.8 $\mu\text{g/g}$). This content is much lower in comparison to our study. In his study, Garcia confirmed that there were no statistical significant differences in chromium content in the bone tissue among different age groups. In our study, there was no statistical significant difference in chromium content between males and females. And in males, the chromium content was higher in non-smokers than in smokers. The samples size of smokers in our

study was small, but most of the general population were non-smokers. Perhaps, this is why such results were obtained.

D'Haese et al.^[33] have determined that the chromium content in the bones was higher in people after dialysis (0.5 $\mu\text{g/g}$) compared with that in people with normal renal function (0.2 $\mu\text{g/g}$).

Hirayama et al.^[34] measured the chromium content in the femur of rats, the average content was 1.4 $\mu\text{g/g}$.

The analysis of chromium content in terms of other factors included the group similarity analysis. Tree diagrams (dendrogram) are illustrations of this method. The measure of similarity is the euclidean distance. When analysing the similarity of occurrence of chromium in the groups of female and male, other similarity groups can be observed. In the males, the greatest occurrence similarity concern the cortical bone and cancellous bone and the highest dissimilarity was observed in the articular cartilage. However, in the females, the greatest similarity of the occurrence was related to the articular cartilage and the fragment of the joint capsule and the highest dissimilarity concern the cortical bone (Figure 2). Moreover, the sex specific euclidean distance varied, but in females the distance was greater. In patients with fractures, the greatest similarity of occurrence was observed for the joint capsule and the cancellous bone. In patients with osteoarthritis, the similarity of the occurrence of chromium was related to the joint capsule and the cancellous bone from the intertrochanteric region (Figure 3).

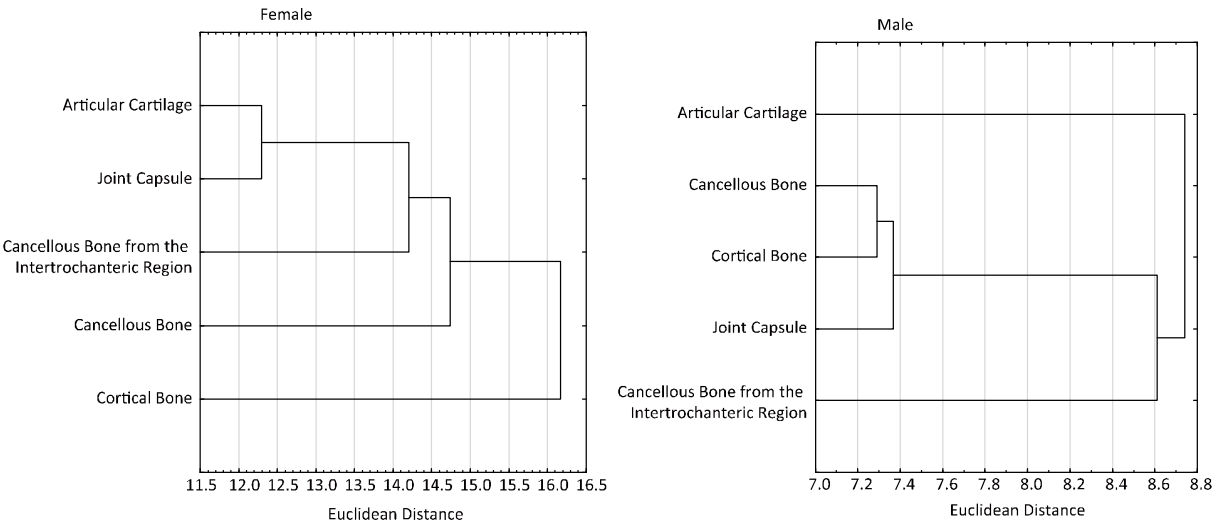


Figure 2. Analysis of group similarity in the population of female and male.

When analysing dendrograms in the group of smoking and non-smoking females and males, the similar changes in the dendrograms in smoking females and males was observed. The greatest similarity of occurrence of chromium concern the articular cartilage and cortical bone, then the joint capsule. The greatest dissimilarity was related to the cancellous bone of the male smokers and the fragment of the cancellous bone taken from the intertrochanteric region of the female smokers. In the non-smoking females, the greatest similarity concern the articular cartilage and the joint capsule, whereas in non-smoking males, the greatest similarity was in the cortical bone and cancellous bone.

Due to the difficulty in obtaining test samples, as it is necessary to take the samples intraoperatively, the tissue is rarely analysed. Most of the previous studies used the samples taken from corpses of people or samples from excavations. The samples used in our study were bones of living people, which were obtained intraoperatively during hip replacement procedures^[9,26-27,29].

A high chromium content was observed in the cancellous bone, on average, it was approximately three times higher compared with other parts of the hip joint and the lowest content was observed in the joint capsule. The cancellous bone is the most metabolically active type of bone tissue, therefore its chromium content is high. In contrast, the joint capsule is made of a different type of tissue-the connective tissue in which no accumulation of

elements occur, this is why chromium content in this tissue was lowest.

Yoshinaga et al.^[35] marked the chromium content in the ribs of Japanese was under 6 µg/g^[35], similar to the value obtained in our study.

CONCLUSION

The highest chromium content was observed in the cancellous bone and the lowest in the joint capsule. No statistical significant differences in chromium content between males and females was observed.

Statistical significant difference in chromium content was observed between patients with fractures and patients with degenerative changes, whereas the chromium content in people with fractures was higher.

The correlation in chromium content between chosen elements of the hip joint and age has not been confirmed.

There were no statistical significant difference in chromium content between smokers and non-smokers. In males, the chromium content was higher in the non-smokers than in the smokers.

A study on the content of chromium in the tissues of the hip joint is one of the primary biomonitoring research. Because of the sampling way they do not have direct application in medicine, however, can provide valuable information for reference on the content of chromium in osseous tissue.

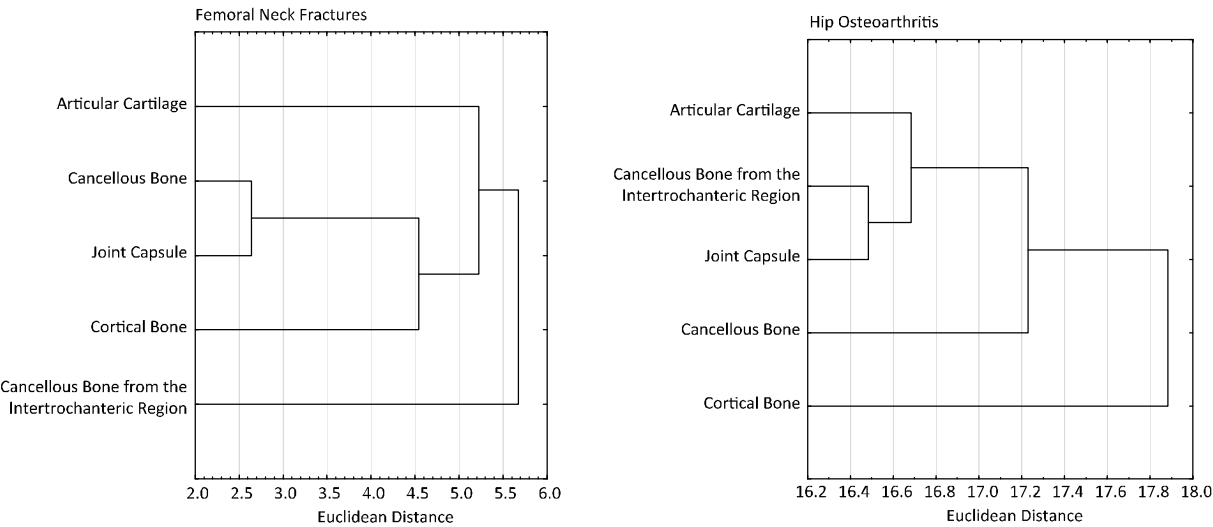


Figure 3. Analysis of group similarity in patients with femoral neck fractures and hip osteoarthritis.

Reviewed: June 25, 2014;

Accepted: December 9, 2014

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