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Epidemiology

The content of manganese and iron in hip joint tissue

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ABSTRACT

Manganese and iron are elements that constitute components of bone tissue. The aim of this study was to determine presence of manganese and iron in hip joint tissue and interdependencies between these elements. The objects of the research were hip joint elements from people residing in cities on the territory of the Upper Silesian Industrial District. The number of people in the study group was 91 samples, including 66 samples from women and 25 from a man. The examined tissues were obtained intraoperatively during hip replacement procedures. The content of manganese and iron was determined using the atomic absorption spectrophotometry (AAS) method. The lowest content of manganese and iron was found in the cortical bone, and the largest, in the case of manganese, in the articular cartilage, whereas in the case of iron in a fragment of the cancellous bone from the intertrochanteric area. The content of iron in selected elements of the hip joint decreased with age. Higher content of manganese in hip joint tissue of women compared to men was confirmed. What is more, higher content of iron in hip joint tissue of men was confirmed as well.

Introduction

Bone tissue has many elements for which bones are a storehouse. Our body releases them in different conditions of the organism. Variable content of elements in bone tissue may be caused by many factors, such as race, sex, age, diet, diseases, environmental and work exposure. Exchange of elements in bone takes place slowly. This is why bones are a pool of free exchange of some metals in our body. Determined contents of selected metals in bones may indicate the size of exposure. The information about the content of elements in human bones is useful for evaluating nutrition rules, prevention and control of various diseases caused by a lack of balance of trace elements [1–3].

Manganese is an essential component of bones and it takes part in the synthesis of proteins involved in connective tissue regeneration. Its deficiencies cause bone deformities, growth retardation and impaired motor coordination [4]. Prolonged manganese deficiency has been reported to produce osteoporosis, and congenital disorders of the skeletal system, such as chondrodystrophy [5–7]. What is more, manganese supports the activity of magnesium in bones, as it displaces it in junctions in enzymatic systems, but, contrary to calcium and phosphorus, it does not block these enzymes, but stimulates them to an even more active work than magnesium ions. The physiological function of manganese is also closely associated with some enzyme activities (superoxidase dismutase – SOD, arginase) and with metallothionein [4,8]. Manganese is an activator of different enzymes that control the metabolisms of carbohydrates, proteins and lipids (including cholesterol), and nitrogen metabolism [9].

The manganese contents in human tissues, especially in bones, decrease with age, which can cause with fractures of the bone (osteoporosis), dermatitis and hypochotesterolemia. In addition to skeletal deformities and testicular dysfunction can result from a manganese deficient [10]. Manganese metabolism in the body is closely related to iron metabolism, such as women with low ferritin is greater absorption of manganese. This indicates the influence of the level of iron in the body with the metabolism of manganese [9].

Literature data shows that the intake of iron in women's diets is too low. However, the level of manganese in the tested diets is appropriate [11].

Iron is an essential component for all organisms. The metabolic function of iron is related to with its influence on other elements. Research on animals has shown that sideropenia increases the absorption of lead from the gastrointestinal tract. In children, the exposure to excessive absorption of lead is associated with iron deficiency leads to cognitive and behavioral in the early stages of infantile development. Iron reacts antagonistically with cadmium, manganese, lead and zinc [9–13].

Iron inhibits bone reconstruction by reducing the formation of osteoblasts, and thus – the synthesis of new bone tissue. The natural endogenous globular protein from the transferrin group – lactoferrin, may be useful in osteoporosis prevention [12].

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For example, in the group of 38 untreated patients with hemochromatosis, HFE-related, 79% had osteopenic and 34% osteoporotic. In these patients, vitamin D levels in serum were normal, there was no dysfunction of the parathyroid glands, and only 13% occurred hypogonadal. The decrease in BMD was more pronounced at the femoral neck than at the lumbar spine. Bone remodeling was decreased in patients with cirrhosis. The hepatic iron concentration seemed to have a negative impact on BMD. Thus, HFE-related genetic hemochromatosis appears to be associated with significant bone loss [14].

In the group of 17 adult patients with sickle cell anemia, who had done blood transfusions, 47% had osteopenia. The serum ferritin level was significantly higher in osteopenic than in non-osteopenic patients [15].

Osteoporosis accompanying iron overload was also observed in patients with African siderosis. In the group of femoral neck fractures in 50 black patients, occurred in 88% of the population increased iron content. The authors noted that iron overload accounted for a reduction in the rate of bone mineralization while ascorbic acid deficiency caused a decrease in bone volume [16].

During HIV infection, particularly in its more advanced stages, often observed in the accumulation of iron in places such as bone marrow, brain, muscle, liver and spleen [17]. Bones can also be storage of this metal. In the group of 161 HIV-positive patients, 80 had osteopenia or osteoporosis [18].

Lifestyle behavior that prevents iron loading should be associated with good bone mineral density (BMD). Postmenopausal women who have the habit of drinking green tea, have higher BMD than that of non-tea drinkers. Mineralization has been observed to stimulate mesenchymal cells of murine bone marrow by a component of green tea, a potent antioxidant and iron chelating compound – (−)-epigallocatechin-3-gallate [19].

Natural iron-binding proteins – lactoferrins, are likely to hold the balance of iron in the process of bone reconstruction [20]. It is suggested that iron-deficient rats have lower bone mass than animals with a sufficient amount of iron. Such a connection between bone tissue condition and iron has not been noticed in humans. More and more studies suggest that there is a relationship between oxidation of lipids and bone metabolism [21].

Iron availability to cells also depends on haptoglobin (Hp) phenotypes. The postmenopausal osteoporotic women with fractures may be regarded as patients with a genetically determined, calcium-phosphorus and iron "malnourished" phenotype and an increased oxidative damage. The Hp phenotype is a genetic marker of iron metabolism and of oxidative stress, whereas a specific marker for calcium-phosphorus metabolism remains to be found. A candidate gene has been identified in vitamin D receptor polymorphisms. In adolescent girls' relationship was observed between bone mineral density in the distal radius, and serum ferritin [22].

More and more research suggests a the relationship between oxidative stress and bone tissue, increased oxidative stress favors bone loss [21,23,24].

Due to the importance of manganese and iron for bone tissue, this study aims at determining the presence of manganese and iron in hip joint tissue and assessing interdependencies that exist between these elements.

Materials and methods

The objects of the studies were elements of hip joint from people residing in cities of the Upper Silesian Industrial District. The material was collected in the Municipal Hospital in Siemianowice Slaske. The examined tissues were obtained intraoperatively during hip replacement procedures based on consent of the Bioethics Committee. Recommendations for this type of surgery were, in most cases, 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. Surgical instruments that were used during collection and distribution of the samples were made of corrosion-resistant steel in accordance with international standards. Iron and manganese contamination was rather impossible. In the next stage, bone curette and Luer rongeur were used to remove articular cartdeterminedilage and then the subchondral bone until a rounded "core" 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 various widths: from virtually non-existent to a fraction of a millimeter to a few millimeters 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 $-20 \pm 1\,^{\circ}C$.

Ninety-nine hip samples were taken from the patients, including 66 from women and 25 from men. The average age was 65.7 ± 10.5 in the general population, including 67.3 ± 8.6 in women and 61.4 ± 13.6 in men. Two groups were distinguished based on the cause for which endoprosthetics was conducted. The first group is people with fractures of femoral bone neck ($n = 7$). The other group are patients with degenerative changes of hip ($n = 84$). The studied population was divided into four age groups: up to 40, aged 41–60, aged 61–80, and after 80. The numbers in each group were, respectively: up to 40 $n = 5$, aged 41–60 $n = 22$, aged 61–80 $n = 58$ and after 80 $n = 6$. The studied population did not include people who would be exposed to manganese or iron in their working conditions.

In order to conduct the marking of the content of elements in femoral bone head samples using the AAS method. Bone samples of known weight were burned into ashes in a muffle furnace in porcelain crucibles. The ashing process was conducted in two stages – at first, at a temperature of 100°C for about 12 h and then at a temperature of 420°C for 12 h.

The analytical sample of ash (approximately 1 g) was digested in 2 cm^3 of spectrally pure HNO_3 (V) (Supra pure) by Merck. The formed solution was transferred into a flask with a volume of 25 cm^3, then filled up with distilled water to the scale mark. In samples prepared this way, manganese and iron 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.

The procedure was additionally confirmed by measuring the concentration of iron and calcium in the reference material NIST-1400 (bone ash). Differences between the measured value and the reference value ranged from 2.0 up to 7.6% for iron and from 5.1 up to 9.8% for calcium.

Statistical calculations were made in StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10

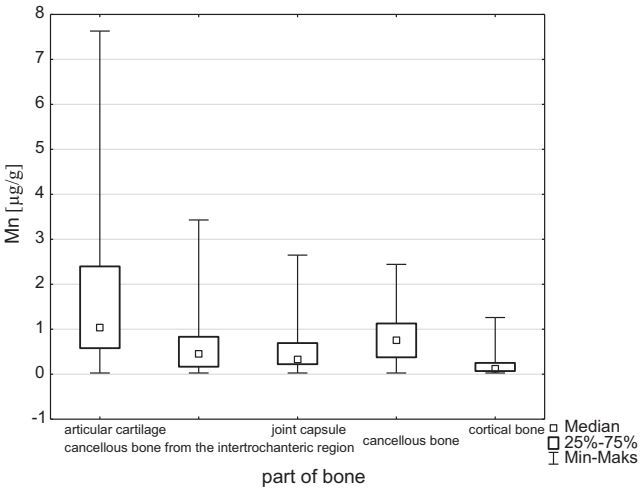

Fig. 1. The average content of manganese in the hip joint tissue.

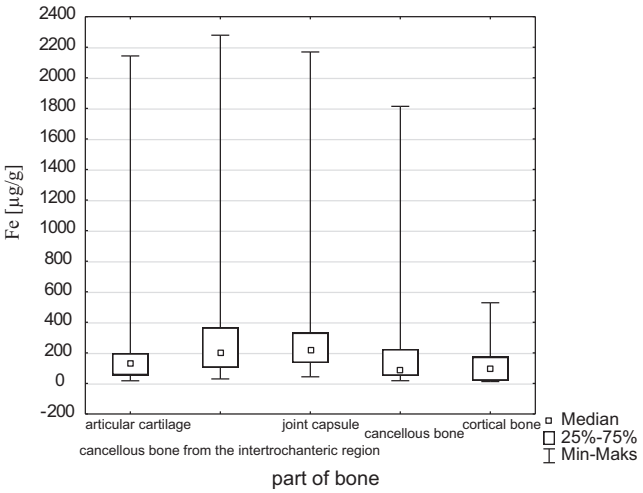

Fig. 2. The average content of iron in the hip joint tissue.

(www.statsoft.com). *U* Mann–Whitney's test was applied and the levels of significance when analyzing differences were $p > 0.05$.

Results

Distribution of iron and manganese content in different elements of hip did not show characteristics of a normal distribution (test Shapiro–Wilk, $p > 0.05$).

Table 2
Correlations of iron from manganese in selected elements of hip men and women.

	Women	Men
Articular cartilage	0.26	−0.02
Cortical bone	−0.31*	−0.68*
Cancellous bone	−0.03	0.14
Cancellous bone from the intertrochanteric region	−0.14	−0.52*
Joint capsule	0.20	−0.16

* Statistically significant, $p < 0.05$.

The content of manganese in different parts of hip joint was as follows (μg/g): cortical bone 0.19, the articular capsule 0.50, the cancellous bone from the intertrochanteric area 0.63, the cancellous bone 0.80, articular cartilage 1.79 – Fig. 1. Respectively, 95% confidence intervals in the cortical bone amounted to 0.15–0.24 μg/g, in the articular capsule 0.40–0.60 μg/g, in the cancellous bone from the intertrochanteric area 0.48–0.78 μg/g, in the cancellous bone 0.68–0.93 μg/g, in the articular cartilage 1.37–2.21 μg/g.

Whereas, the number of iron occurrence was as follows (μg/g): cortical bone 111, the cancellous bone 163, articular cartilage 184, the articular capsule 356, the cancellous bone from the intertrochanteric area 453 – Fig. 2. The 95% confidence intervals for iron were: in the cortical bone 86–136 μg/g, in the articular capsule 269–443 μg/g, in the cancellous bone from the intertrochanteric area 308–598 μg/g, in the cancellous bone 117–209 μg/g, in the articular cartilage 120–247 μg/g.

The content of manganese did not differ significantly in the group of patients with degenerative changes and fractures (test *U* Mann–Whitney, $p < 0.05$). In the case of iron, its content in the group of patients with degenerative changes differed significantly compared to the group with fractures (test *U* Mann–Whitney, $p > 0.05$). In patients with fractures, the content of iron was much higher, averaging 347.71 μg/g, as compared to people with degenerative changes 242.82 μg/g.

The content of manganese in the articular cartilage and the cancellous bone did not differ significantly among women and men (test *U* Mann–Whitney, $p < 0.05$). However, in cortical bone, in the cancellous bone from the intertrochanteric area and the articular capsule there are statistically significant differences between women and men – Table 1. In the group of studied women and men, the manganese content is, respectively: in cortical bone 0.22 and 0.13 μg/g, in the cancellous bone from the intertrochanteric area – 0.75 and 0.38, and in articular capsule 0.55 and 0.39 μg/g.

In the case of iron, the content in men and women in different parts of hip joint did not differ significantly – Table 1.

In order to observe changes in the content of manganese and iron, the surveyed population was divided into 4 age groups in the age function. The content of manganese increased from a value of 0.42 μg/g, then 0.65 μg/g, to 0.86 μg/g in the age group of 61–80

Table 1
Concentration of manganese and iron in women and men hip joint tissue (μg/g).

	Mn			Fe		
	AM ± SD	Med	Range	AM ± SD	Med	Range
Articular cartilage	1.88 ± 1.40	1.08	0.13–7.63	190 ± 151	115	17–2143
Cortical bone	0.22 ± 0.22	3.89	0.13–21.55	96 ± 76	58	12–396
Cancellous bone	0.84 ± 0.45	0.82	0.03–2.44	163 ± 128	77	18–1813
Cancellous bone from the intertrochanteric region	0.75 ± 0.53	0.55	0.03–3.43	402 ± 372	185	29–2234
Joint capsule	0.55 ± 0.34	0.35	0.03–2.65	334 ± 250	214	43–2169
Articular cartilage	1.61 ± 1.30	0.88	0.03–6.85	171 ± 82	155	46–547
Cortical bone	0.13 ± 0.09	0.07	0.03–0.40	148 ± 91	138	12–528
Cancellous bone	0.72 ± 0.47	0.65	0.03–2.30	165 ± 103	142	25–547
Cancellous bone from the intertrochanteric region	0.38 ± 0.26	0.26	0.03–1.41	567 ± 584	215	35–2279
Joint capsule	0.39 ± 0.30	0.24	0.03–1.44	412 ± 330	255	62–1246

AM: arithmetic mean, SD: standard deviation, Med: median.

Table 3
The average mean of iron and manganese in the bones.

References	Mn [μg/g]	Fe [μg/g]	Country
Kuo et al. [25]	0.70	20.30	Taiwan
Garcia et al. [26]	0.17	–	Spain
Sumino et al. [27]	0.074	–	Japan
D'Haese et al. [13]	–	150–250	Belgium, Greece, Czech, Argentina, Egypt
Scancar et al. [28]	–	100–300	Slovenia

years, and in the age group above 80 years of age, it decreased to the value of 0.60 μg/g. However, in case of the content of iron, the largest occurred in the group up to 40 years of age (291.83 μg/g), in the next group it was 267.24 μg/g, in the group of 61–80 – 249.05 μg/g, and in the oldest group it was 162.80 μg/g.

The correlation analysis showed a dependency between iron and manganese in cortical bone of women ($r = -0.31$) and men ($r = -0.68$) and the cancellous bone from the intertrochanteric area in men ($r = -0.52$). The group of men showed generally higher values of the correlation factor between manganese and iron – Table 2.

Discussion

Studies on metal content in bone tissue are often found in literature. Due to cumulative capacity of bone tissue, it is a good material to conduct studies on the content of elements. Very few studies include other tissues to determine the content of metals.

In the conducted studies, the content of iron and manganese in bone tissue (cortical and cancellous bone from femoral head bone and cancellous bone from the intertrochanteric area of femular bone) and articular cartilage as well as articular capsule was determined. These studies have shown which tissue has a greater ability to accumulate manganese and iron, and what interdependencies occur between these elements. It was shown that the highest manganese content was in articular cartilage and the smallest in cortical bone. This means that the cartilaginous tissue has a higher storage capacity of this element than bone tissue. In the case of iron, its content was the lowest in cortical bone, as in the case of manganese, whereas the largest was in articular capsule and in the cancellous bone from the intertrochanteric area. In previously conducted studies on the content of nickel, the highest content of these elements was in the cancellous bone of femoral head bone and the smallest was in a fragment of the articular capsule [2,3]. This may indicate that nickel and lead have accumulative abilities in bone of femoral head bone, whereas manganese and iron do not [2,3].

The determined iron content was significantly higher compared with the results which were given by Kuo et al. [25]. The content of manganese was at a similar level. The determined content of manganese and iron in bones of people living in Taiwan was respectively 0.7 and 20.3 μg/g [25]. The average content of manganese was higher in comparison with the results of Garcia et al. [26] – 0.17 μg/g and Sumino et al. [27] (Japonia) – 0.074 μg/g. The content of iron determined by Scancar et al. [28] in the iliac crest, where the range of iron content variation was 100–300 μg/g, was also at a similar level. D'Haese et al. [13] marked the content of iron in patients with a normal renal function at the level of 70–170 μg/g, while in dialysis patients the content of iron was higher and amounted to 150–250 μg/g (Table 3).

Kosugi et al. [29] determined the content of elements in bones (ribs) taken from excavation areas of Japan from the 5000 period – 130 years BC. Iron content was the lowest in the Kofun era (104 μg/g) and the highest in the Edo era (10970 μg/g). In the case of manganese, the lowest content appeared in the Jomon era (103 μg/g) and the highest in the Edo era (1167 μg/g). Hisanaga et al. [30] as well as Kosugi et al. [29] determined manganese and iron content in ribs taken from excavations and in bones of people living today. The content of iron was the highest in the Edo era, as in studies by Kosugi et al. [29] (1900.4 μg/g), and the lowest in people living today – 532.5 μg/g. Manganese content was also the highest in the Edo era (2794 μg/g), and the lowest in people living now 6.8 μg/g.

Vuorinen et al. [31] determined the content of iron and manganese in ribs of skeletons from the 16th and 17th century. It amounted to, respectively: manganese 1120 μg/g and iron 5691 μg/g. Diagenetic processes taking place in soil undoubtedly influenced such high contents of iron and manganese. Information about the contents of iron and manganese in the bone from the literature data has been collected and presented in the Table 3.

In this study, iron content was higher in men than in women, whereas in case of manganese it was the other way round. Vuorinen et al. [31] reported a lower content of iron in comparison with men and an inverse relationship for the content of manganese as well.

In literature, there is information that the content of manganese in bones decreases with age. However, this dependence seems to be different in the studies. Manganese content increases in the age group of up to 40, 41–60 and 61–80, while in the age group of above 80 it decreases. In studies by Kuo et al. [25], manganese content increased to a value of 0.96 μg/g in the age group of 41–60 and then decreased in the group of 61–80 to 0.59 μg/g. The smallest value was in the group of over 80 years of age – 0.40 μg/g.

In this study, we observed a decrease in iron content with increasing age. As for the content of iron in different age groups, in studies by Kuo et al. [25], iron content varied the same way as the content of manganese, so it increased to the range of 41–60 years (from 23.36 to 23.79 μg/g), and in the next two age groups it decreased from 20.13 to 9.33 μg/g.

The dependency that occurs between manganese and iron in animal organisms is often of an antagonistic nature [9]. In studies, such dependency occurred in cortical bone of men and women and in the cancellous bone from the intertrochanteric area of men. In the results by Kuo et al. [25], the correlation between manganese and iron had a synergistic character and the correlation coefficient was 0.38. A similar dependence was observed by Hisanaga et al. [30].

Conclusions

The lowest content of manganese and iron was in cortical bone, the largest manganese content was found in articular cartilage, and iron – in the cancellous bone from the intertrochanteric area.

With age, the content of iron decreased in chosen elements of the hip joint.

Manganese content in hip joint tissue of women was as compared with men, while iron content was higher in tissues of men.

Research on the content of manganese and iron will be continued in the population living in rural areas.

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References

[1] Lanocha N, Kalisińska E, Kosik-Bogacka DI, Budis H, Sokołowski S, Bohatyrewicz A. Concentrations of trace elements in bones of the hip joint from patients after hip replacement surgery. J Trace Elem Med Biol 2012;26:20–5.

[2] Brodziak-Dopierała B, Kowol J, Kwapuliński J, Kusz D, Cieliński Ł. Lead and calcium content in the human hip joint. Biol Trace Elem Res 2011;144:6–16.

[3] Brodziak-Dopierała B, Kwapuliński J, Sobczyk K, Kowol J. The occurrence of nickel and other elements in tissues of the hip joint. Ecotoxicol Environ Saf 2011;74(4):630–5.

[4] Brandi K, Müller AS, Pallauf MWJ. Influence of manganese deficiency of manganese enzyme activity and gene expression in growing rats. In: 22 workshop, macro and trace elements, vol. 2. 2004. p. 1296–302.

[5] Pabbruwe MB, Standard OC, Sorrella ChC, Howlett CR. Bone formation within alumina tubes: effect of calcium, manganese, and chromium dopants. Biomaterials 2004;25(20):4901–10.

[6] Strause LG, Hegenauer J, Saltman P, Cone R, Resnick D. Effects of longterm dietary manganese and copper deficiency on rat skeleton. J Nutr 1986;116:135–41.

[7] Odabasi E, Turan M, Aydin A, Akay C, Kutlu M. Magnesium, zinc, copper, manganese, and selenium levels in postmenopausal women with osteoporosis. Ann Acad Med Singapore 2008;37:564–7.

[8] Floriańczyk B. Trace elements as constituents of antioxidative proteins. J Pre-Clin Clin Res 2008;2:25–7.

[9] Kabata-Pendias A, Mukherjee AB. Trace elements from soil to human. Berlin, Heidelberg: Springer-Verlag; 2007.

[10] Schäfer U, Anke M, Seifert M, Müller R. (Jena, Germany): Manganese. Nutritional intakes, nutritional and toxicological standards – environmental exposures and standards – are there health risks?, vol. 906. Jena, Germany: Friedrich-Schiller-University of Jena; 2002. p. 911.

[11] Marzec Z, Marzec A, Zaręba S. Daily food rations as a source of iron and manganese for adults. Rocz Panstw Zakl Hig 2004;55:29 (in Polish).

[12] Weinberg ED. Iron loading: a risk factor for osteoporosis. BioMetals 2006;19:633–5.

[13] D'Haese PC, Couttenye MM, Lamberts LV, Elseviers MM, Goodman WG, Schrooten I, et al. Aluminium, iron, lead, cadmium, copper, zinc, chromium, magnesium, strontium and calcium content in bone of end-stage renal failure patients. Clin Chem 1999;45(9):1548–56.

[14] Guggenbuhl P, Deugnier Y, Boisdet JF, Rolland Y, Perdriger A, Pawlotsky Y, et al. Bone mineral density in men with genetic hemochromatosis and HFE gene mutation. Osteoporosis Int 2005;16:1809–14.

[15] Shah FT, Chatterjee R, Owusu-Asante M, Porter JB. Adults with severe sickle cell anemia and iron overload have a high incidence of osteopenia and osteoporosis. In: 46th annual meeting of the American Society of Hematology. 2004. p. 468A.

[16] Schnitzler CM, Schnaid E, MacPhail AP, Mesquita JM, Robson HJ. Ascorbic acid deficiency, iron overload and alcohol abuse underlie the severe osteoporosis in black African patients with hip fractures a bone histomorphometric study. Calcif Tissue Int 2005;76(2):79–89.

[17] Weinberg GA, Boelaert JR, Weinberg ED. Iron and HIV infection. In: Friis H, editor. Micronutrients and HIV infection. Boca Raton, FL: CRC Press; 2002. p. 135–80.

[18] Fausto A, Bongiovanni M, Cicconi P, Menicagli L, Ligabò EV, Melzi S, et al. Potential predictive factors of osteoporosis in HIV-positive subjects. Bone 2006;38(6):893–7.

[19] Chen CH, Ho ML, Chang JK, Hung SH, Wang GJ. Green tea catechin enhances osteogenesis in a bone marrow mesenchymal stem cell line. Osteoporosis Int 2005;16(12):2039–45.

[20] Lorget F, Clough J, Oliveira M, Daury MC, Sabokbar A, Offord E. Lactoferrin reduces in vitro osteoclast differentiation and resorbing activity. Biochem Biophys Res Commun 2002;296:261–6.

[21] D'Amelio P, Cristofaro MA, Tamone C, Morra E, Di Bella S, Isaia G, et al. Role of iron metabolism and oxidative damage in postmenopausal bone loss. Bone 2008;43(6):1010–5.

[22] Ilich-Ernst JZ, McKenna AA, Badenhop NE, Clairmont AC, Andon MB, Nahhas RW, et al. Iron status, menarche, and calcium supplementation in adolescent girls. Am J Clin Nutr 1998;68:880–7.

[23] Altindag O, Erel O, Soran N, Celik H, Selek S. Total oxidative/anti-oxidative status and relation to bone mineral density in osteoporosis. Rheumatol Int 2008;28:317–21.

[24] Yousefzadeh G, Larijani B, Mohammadirad A, Heshmat R, Dehghan G, Rahimi R, et al. Determination of oxidative stress status and concentration of TGF-beta 1 in the blood and saliva of osteoporotic subjects. Ann NY Acad Sci 2006;1091:142–50.

[25] Kuo HW, Kuo SM, Chou CH, Lee TC. Determination of 14 elements in Taiwanese bones. Sci Total Environ 2000;255:45–55.

[26] Garcia F, Ortega A, Domingo JL, Corbella J. Accumulation of metals in autopsy tissues of subjects living in Tarragona County, Spain. J Environ Sci Health 2001;36(9):1767–86.

[27] Sumino K, Hayakawa K, Shibata T, Kitamura S. Heavy metals in normal Japanese tissues – amounts of 15 heavy metals in 30 subjects. Arch Environ Health 1975;30:487–94.

[28] Scancar J, Milacic R, Bendikt M, Bukovec P. Determination of trace elements and calcium in bone of the human iliac crest by atomic absorption spectrometry. Clin Chim Acta 2000;293:187–97.

[29] Kosugi H, Hanihara K, Suzuki T, Himeno SI, Kawabe T, Hongo T, et al. Elemental composition of ancient Japanese bones. Sci Total Environ 1986;52:93–107.

[30] Hisanaga A, Hirata M, Tanaka A, Ishinishi N, Eguchi Y. Variation of trace metals in ancient and contemporary Japanese bones. Biol Trace Elem Res 1988;22:221–31.

[31] Vuorinen HS, Pihlman S, Rauhamaa-Mussalo H, Tapper U, Varrela T. Trace and heavy metal analyses of a skeletal population representing the town people in Turku (Abo), Finland in the 16th–17th centuries: with special reference to gender age and social background. Sci Total Environ 1996;177:145–60.