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Metal content in medieval skeletal remains from Southern Croatia

Angela Stipisic [a,*], Maja Versic-Bratincevic [b], Zlatka Knezovic [a], Davorka Sutlovic [b,c]

[a] Public Health Institute of Split – Dalmatia County, Vukovarska 46, 21 000 Split, Croatia
[b] Department of Forensic Medicine, University of Split, School of Medicine, 21 000 Split, Croatia
[c] Department of Pathology and Forensic Medicine, University Hospital Centre Split, 21 000 Split, Croatia

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ABSTRACT

This work is a contribution to the existing knowledge of lifestyle and diet of the South Croatian population who lived in Early Medieval Period. The one hundred samples dating from 9th century were discovered and collected at the burial sites Ostrovica and Naklice. Concentrations of metals and their mutual relationships were examined in regards to gender and age of skeletal remains. Differences were observed in diet between men and women as well as among age groups.

For a correct interpretation of the results it is necessary to determine the metal content in the soil. Namely, metals in archaeological bones are influenced by changes in soil – *diagenesis*, which is confirmed by our results. We concluded that there were no influences of *diagenesis* on lead, calcium, strontium and zinc content while cadmium, iron, manganese and copper are most exposed.

1. Introduction

Environmental conditions, dietary habits and uptake of some elements from the surrounding soil may reflect on heavy metal content in human bones. This statement is supported in several papers with results of metals in archaeological human bones from different locations and different periods (Martinez-Garcia et al., 2005; Dobrovolskaya, 2005; Shafer et al., 2008; Donno et al., 2010; Nakashima et al., 2010; Yamada et al., 1997).

Martinez-Garcia et al. (2005) determined the content of lead, copper, zinc, cadmium and iron in archaeological bones from the area of Cartagena in Spain from different historical periods. According to their study, the lowest concentrations of lead were in the Neolithic period (median Pb 45 μg/g), the values from the Bronze Age were higher (median Pb 80 μg/g), and maximum were recorded during the Roman (median Pb 742 μg/g) and the Byzantine era (median Pb 898 μg/g). The concentrations of copper were highest in Byzantine era bones (median Cu 111 μg/g), and the concentrations of iron during the Ottoman Empire (median Fe 15 383 μg/g) (Martinez-Garcia et al., 2005).

Schutkowski and Herrmann (1999) analyzed metals in archaeological human bones from 6th to 8th century, from Northwestern Germany (Schutkowski and Herrmann, 1999). They found low zinc content, increased content of copper and strontium as well as enlarged ratio of Sr/Ca. Strontium is not an essential element. It is found in plant origin food. Ratio of Sr/Ca in bones is very important. Higher ratio indicates that foods of plant origin (cereals and legumes) were predominated in the diet, while a smaller ratio suggests higher incidence of animal origin food (milk and dairy products) (Schutkowski and Herrmann, 1999).

According to Mays (2003), strontium is mostly located in the skeleton, with a strong relationship between Sr/Ca ratio in diet and in the bones of the consumer (Mays, 2003). The analyses of Sr/Ca ratio in human skeletal remains from medieval times from England were carried out, particularly in archaeological bones of breastfeeding mothers. Mays found association not only between Sr/Ca ratio and diet from that period, but also with metabolic changes during lactation. During lactation, transfer of calcium is faster than strontium, as well as through the placenta during pregnancy. Potentially, according to Mays, bone Sr/Ca ratios is a sensitive technique for detecting the administration of low-protein or animal-milk supplements into infant diets. This is important as historical sources indicate that such foods were frequently used as infant feeding supplements in the past (Mays, 2003).

In archaeological studies analysis of metals in bones reflect diet and lifestyle habits of archeological populations. Contamination and *diagenesis* of archaeological bones can compromise the credibility of this information (Martinez-Garcia et al., 2005; Nielsen-

* Corresponding author. Tel.: +385 021401175; fax: +385 021539825.
E-mail address: astipis@gmail.com (A. Stipisic).

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Marsh and Hedges, 2000). *Diagenesis* is a series of changes in the soil, including: dissolution, precipitation, crystallization and exchange of minerals between the soil and bones. According to Nielsen-Marsh et al. (2006), there are three important alterations to bone apatite that can be used as indicators of diagenetic change: porosity increase, crystallinity increase and inclusion of exogenous ione (Nielsen-Marsh et al., 2006). These processes can significantly affect the archaeological bones.

Preservation of archaeological bone depends on the composition and acidity of the soil (Hedges and Millard, 1995; Hedges, 2002). Archaeological sites that are located on gentle hills and not being exposed to groundwater are less exposed to the influence of *diagenesis* (Hedges and Millard, 1995; Nielsen-Marsh and Hedges, 2000). The composition of calcareous soil with slightly alkaline pH values is appropriate for preservation of osteological material over thousands of years. The alkaline soil can preserve bone far better than the sour one, which easily destroys hydroxyapatite, an integral part of the bone (Hedges, 2002). Previous studies in Croatia indicated that the osteological material is best preserved at the archaeological sites along the Adriatic coast, under the condition that their location is not close to the sea (Slaus, 2006c; Novak and Slaus, 2010). These sites have soil with neutral to slightly acidic pH values and high content of sand, whereas in Central and Continental Croatia, level of preservation varies depending on the acidity of the soil and the amount of groundwater (Slaus, 2006c).

Selection of archaeological bone is also very important for reducing the impact of *diagenesis* (Vuorinen et al., 1990a; Price et al., 1992). Long parts of femur are less exposed to changes in the soil than the short bones. Trabecular bone is more exposed to the *diagenesis* than compact cortical bone (Carvalaho et al., 2004; Ambrose and Krigbaum, 2003).

Contamination of archaeological bones can occur with the long laying in the soil, but the sampling and preparation techniques may also cause it (Price et al., 1992). The part of the skeletal remains must be selected very carefully for chemical analysis before metal determination. During the preparation of samples, metal accessories such as saws, forceps, etc. should not be used.

Contemporary archaeological research in Croatia became more intensive at the beginning of nineties. Analysis included anthropological and archaeological research (determination of gender, age, trauma, dental analysis, as well as osteological changes indicating different diseases) both for the continental and coastal Croatian (Slaus, 1997a, 2000b, 2006c, 2008d; Novak and Slaus, 2010). During the 90-ies of the 20th century the Museum of Croatian Archaeological Monuments in the excavations carried out a number of sites from the early Middle Ages, or the 9–10th century (Delonga and Buric, 1998). However, in the aforementioned studies in Croatia so far no one has dealt with the analysis of metals in archaeological bones.

In our study the content of metals was analyzed in 100 samples of archaeological bones in compact part of the bone – cortical femoral. Archaeological bones were collected from two archaeological sites of Southern Croatia: Ostrovica and Naklice (Fig. 1). The influence of *diagenesis* on the distribution of metals in archaeological samples was investigated by determining metals and pH in soil samples which were collected from the burial site during the archaeological excavations.

The aim of our study was to determine the difference of metal distribution in regards to gender and age, as well as potential differences in social status between men and women, and children and adults. We also tried to determine whether there are significant differences referring to metal distribution in the bones of the early Croatian population for two different archaeological sites from the same historical period.

2. Methods

2.1. Instrumentation and operating conditions

Lead and cadmium measurements were carried out by using a Model AAS vario 6 GFAAS atomic absorption spectrometer (Analytik Jena AG, 2001) equipped with a transversely heated graphite atomizer with autosampler (Model MPE 50), a deuterium background correction system and a hollow cathode lamp for lead operated at 3 mA (wavelength 283.3 nm) and for cadmium operated at 3 mA (wavelength 228.8 nm). Pyrolytic coated graphite tubes with PIN-platform (Analytik Jena, Part No. 407-A81.025) were used during the anlytical determination (Analytik Jena AG, 2001). The injection volume was 20 μL and integrated absorbance (peak area) was used for signal evaluation.

Calcium, strontium, zinc, copper, iron and manganese determinations were carried out by using a Model AAS vario 6 FAAS atomic absorption spectrometer equipped with deuterium background correction system and a hollow cathode lamp for calcium, strontium, zinc, copper, iron and manganese (wavelength 422.7 nm for Ca; 460.7 nm for Sr; 213.9 nm for Zn; 324.8 for Cu nm; 248.3 nm for Fe and 279.5 nm for Mn). Concentrations of Zn, Cu, Fe and Mn were determinated by Flame AAS with C_2H_2/air burner, while Ca and Sr were determinated with C_2H_2/N_2O_2 burner (Analytik Jena AG, 2001).

Samples were weighted on the Mettler Toledo balance – Model AX 205DR (Mettler, Germany) with resolution of 0.01 mg. A closed microwave system, CEM Model Mars 5 was used for wet digestion (CEM Corporation, 2006).

pH values of the soil samples were determined with pH-meter Model inoLab – pH720; measuring range and resolution – 2.000. + 19.999 with accuracy of ±0.005 (Wissenchaftlich Technishe Werkstatten WTW, 2004).

2.2. Reagents

Working standards of Pb, Cd, Ca, Sr, Zn, Cu, Fe and Mn for measurements were prepared from Merck (Darmstadt, Germany) stock solutions (1000 ± 2) mg/L, suprapur. Standard solutions were prepared in range of expected concentration values.

We applied the same technique for the metal analysis in two different matrices: soil and human bones. To confirm the success of the method performed, we used the available Certified Standard Reference soil material: SRM – 2710a Montana I Soil, from the National Institute of Standards and Technology (NIST, 2009).

Pyrolysis and atomization curves were established in the presence of chemical modifier – 0.1% $Pd(NO_3)_2$ + 0.05% $Mg(NO_3)_2 \times 6H_2O$. Modifier was prepared from Merck stock solution: Art.1.07289 palladium matrix modifier for graphite furnace AAS and Art.1.05855 magnesium nitrate hexahydrate. Volumen of added modifier was 5 μL.

2.3. Samples preparation

After archaeological excavations, bone samples were classified according to the anthropological parameters: gender, age (0–15, 16–25, 26–39 and above 40 years) as well as geographical location. For chemical analysis, bone samples were prepared with special attention to avoid the post-mortem contamination.

A part of a dense cortical bone was sawed for analysis. Bone pieces were crushed into small fragments using razor blades and stored in sterile polypropylene tubes at −20 °C until analyzed. After drying to a constant weight samples were washed in 6 ml 65% nitric acid (HNO_3) over night, subsequently washed in distilled water and finally dried at room temperature.