Legal Medicine 20 (2016) 18–22

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Bone mineral density of skeletal remains: Discordant results between chemical analysis and DXA method

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ARTICLE INFO

Article history:
Received 9 November 2015
Received in revised form 3 March 2016
Accepted 15 March 2016
Available online 15 March 2016

Keywords:
Bone density
DXA scan
Calcium and phosphorus concentration
Skeletal remains

ABSTRACT

Dual-energy X-ray absorptiometry (DXA) scanning is a gold standard for bone mineral density measurement and diagnosis of primary and secondary osteoporosis in living persons. DXA is becoming widespread when analysing archaeological material, and is considered to provide an accurate diagnosis of osteoporosis in skeletal samples.

The aim of this study was to explain the differences in results between bone mineral density (obtained with DXA) and chemical determination of calcium and phosphorus concentrations in skeletal remains. We examined bone mineral density (BMD) and mineral content of femoral bone samples exhumed from mass graves of the Second World War. BMD was determined by Hologic QDR 4500 C (S/N 48034) Bone Densitometer. Concentrations of calcium and phosphorus were determined with AAS (Atomic absorption spectroscopy) and UV/VIS (Ultraviolet–visible) spectroscopy.

The results obtained in this study do not support the hypothesis according to which BMD measured by DXA scan has positive correlation with chemically determined concentrations of calcium and phosphorus in bones, especially in acidic soils where there was significant impact of diagenesis observed.

1. Introduction

The DXA (dual-energy X-ray absorptiometry) technique uses X-rays of two different energies to determine bone mineral density (BMD, g/cm^2) and bone mineral content (BMC, g). The Dual-energy X-ray absorptiometry is considered as a gold standard in measuring bone mineral density and is most widely used diagnostic tool for primary osteoporosis (usually age related and associated with post menopause) as well as for secondary osteoporosis (osteoporosis caused by other conditions, such as hormonal imbalances, diseases or medications) [1]. In Croatia, the method is also used for that purpose, exclusively. However, previous research have shown that DXA is also becoming used from the aspect of forensic medicine in other parts of the world. Namely, several authors have used DXA scan for gender and age prediction [2,3], stress indication [4] and osteoporosis [5] of skeletal remains.

Densitometer measures the quantity of hydroxyapatite in bone, as bone mineral content (BMC) in grams, and calculates the areal BMD, expressed as grams of mineral per unit area scanned [6].

Mature bone is composed mainly of proteins and minerals. Approximately 60% of bone weight is mineral. Bone mineral content is in 80–90% composed of calcium and phosphorus, which form the inorganic component of bone matrix [7]. Calcium (Ca) and inorganic (i) phosphorus (P) are the two main constituents of hydroxyapatite, bone mineral that strengthens the mechanical resistance of the organic matrix. Over 99% of calcium and 85% of phosphorus in the human body exist as a complex within bone where the ratio between Ca/P mass is 2.2 [8].

Diagenesis is a natural process that alters the proportions of organic (collagen) and inorganic components (mainly hydroxyapatite) of bone exposed to environmental conditions. It is accomplished by the exchange of natural bone constituents, deposition

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http://dx.doi.org/10.1016/j.legalmed.2016.03.008
1344-6223/

in voids or defects, adsorption onto the bone surface and leaching from the bone [9]. Hydroxyapatite is insoluble in water, but it breaks down into soluble salts of calcium and phosphorus in the presence of an acid environment. If the soil is neutral or basic, a skeleton may persist for centuries in good condition. Mineral dissolution will also occur in a corrosive soil environment (irrespective of taphonomy) [10]. In order to understand post-mortem chemical changes in bone, geochemical conditions at a burial site must be taken into account. The most important variable for demineralisation and decomposition of hydroxyapatite in soil is pH, while several other factors such as organic matter content, mineralogy, and texture, soil solution fluoride and carbonate concentration, temperature regime, abundance and distribution of precipitation, local groundwater movement, microbial activity, and duration of interment can also affect the skeleton remains [11].

To our knowledge, no studies have yet been performed to assess BMD and correlation with calcium and phosphorus concentrations (and ratio) in human skeletal remains. Respectively, this is the first study to measure BMD with Dual-energy X-ray absorptiometry, and Ca and P content with Atomic absorption spectroscopy and Ultraviolet–visible spectroscopy (respectively) of bone samples exhumed from mass graves dating from World War II. Also, this is a novel research in Croatia, considering the use of DXA method from forensic aspect.

Our expectation was positive correlation of bone mineral density and mineral content (calcium and phosphorus) with the concentrations of calcium and phosphorus, but only the results obtained for phosphorus acted in predicted manner, while values of calcium were higher than expected.

2. Experimental

2.1. Sample collection and screening

Femoral samples found in mass graves are often not preserved as a whole, but partially fragmented. Fragments of the right and the left femur bones of middle aged men were excavated from mass graves at two locations during our previous studies [12,13]. The left and the right femur bones were paired according to their location in the mass grave, anthropometric measurements, and DNA analysis. Out of 104 excavated femur samples (52 possible pairs) 72 were successfully matched (36 pairs). The samples were taken of the cortical bone from the upper shaft femur (under the minor trochanter), from equal femur positions of the right and the left femur bones. The average fragment length was 4.59 cm. BMD was determined on these isolated fragments.

2.2. Bone mineral density (BMD) determination

Bone density scanning (DXA scan) was performed on 72 femoral bone samples (36 pairs) at the Department of Endocrinology, University Hospital Center Split. The study was approved by the Ethical Committee of the University of Split School of Medicine, Split. BMD was measured using Hologic QDR 4500 C (S/N 48034; Bedford, MA 01730, USA) Bone Densitometer, and results were expressed as BMD (g/cm^2). Femur fragments of specific appearance and size were aligned in series of four (Fig. 1) in order to reproduce lumbar spine. Femur samples were scanned with Caucasian men normative programme (weight 75 kg, height 175 cm), followed by the analysis with Lumbar spine programme.

2.3. Grouping of the samples from DXA results

After *the bone* mineral density measurement with *DXA*, chemical analysis was performed to quantify calcium and phosphorus content in bone samples, and their mutual ratios. DXA results showed differences in BMD values between left and right femur fragments of the same person. Following the criteria of congruence obtained by densitometry, 20 femoral pairs of bones were chemically analyzed. Samples for chemical analysis were divided in two groups: fragments with the smallest BMD difference (10 matching pairs, Group 1) and fragments with the biggest BMD difference (10 matching pairs, Group 2) (Table 1).

2.4. Chemicals

Reagents used for the extraction and measurement such as standard metal solutions were of suprapur quality (Merck, Darmstadt, Germany). Standard solutions were prepared in range of expected concentration values.

2.5. Sample preparation

After the DXA measurements, compact bone pieces from femur fragments 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, the samples were washed in 6 ml of 65% nitric acid (HNO_3) overnight, washed in distilled water, and dried at the room temperature [14,15]. 0.5 g of the sample was placed in a teflon-TFM vessel, 65% nitric acid and hydrogen peroxide wet-ashed was added to the sample, and digested in automated (temperature regulated) microwave digestion unit (CEM, USA Model Mars 5 with 1600 W power). Digested samples were diluted with deionized water, and metal content was quantitatively determined. The same analysis was repeated twice using samples from different parts of the same femur fragment.

2.6. Calcium and phosphorus determination

Concentrations of elemental calcium (Ca) were determined with aModel AAS vario 6 FAAS atomic absorption spectrometer (AnalytikJena AG) in flame mode. Phosphorus was determined using UV/VIS spectrometer (model Lambda 25, Perkin Elmer, Waltham, MA USA) double beam on 650 nm wave lengths. Tungsten lamp was used as a source with 1 nm slit.

2.7. Soil analysis

The influence of *diagenesis* on calcium and phosphorus content in bone samples was determined through metal content and pH value in soil samples collected from the burial site during the excavations. Soil sample analysis (pH value in the 5% water solution of soil and quantitative Ca and P analysis) was carried out to eliminate the impact of the soil on bone contamination. To compare soil composition samples should be taken in vicinity of the bones, and from the wider area of the exhumation. Two samples were taken per each location: the first sample was from the skeletal remains level and the second sample was 20 cm below skeletal remains. Samples were prepared by microwave digestion in the same manner as the bone samples (CEM Corporation), and subjected to quantitative analysis on Atomic Absorption Spectrometer (AAS).

2.8. Method validation

Certified Standard Reference Material SRM-2710a Montana I Soil, from the National Institute of Standards and Technology (NIST, USA) was used to verify the method (National Institute of Standard and Technology, 2009). The reproducibility of sample preparation and analysis (for five times) was expressed as relative standard deviation. All the values of relative standard deviation (coefficient of variation) were less than 10%, indicating good preci-