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The effect of age, anthropometric parameters, vertebral bone densitometry and ash density on iliac crest bone volume and microstructure

OLGA CVIJANOVIĆ[1]
SANJA ZORIČIĆ CVEK[1]
GORDAN GULAN[2]
VELJKO ŠANTIĆ[2]
LUKA ĐUDARIĆ[1]
TAMARA ŠOIĆ-VRANIĆ[1]
MARINA NIKOLIĆ[1]

[1]Department of Anatomy, Medical Faculty of Rijeka, University of Rijeka, Croatia

[2]Clinic for Orthopedics Lovran, Medical Faculty of Rijeka, University of Rijeka, Croatia

Correspondence:
Olga Cvijanović, MD, PhD
Department of Anatomy
Medical Faculty of Rijeka, University of Rijeka
Braće Branchetta 20, 51000 Rijeka
Email: olga.cvijanovic@medri.uniri.hr

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Abstract

***Background and Purpose**: The purpose was to compare iliac crest bone static bone histomorphometry and vertebral bone densitometry as two methods frequently used in clinical practice.*

***Patients and Methods**: Cylindrical bone samples of the iliac crest bone (N=100) for bone histomorphometry and the whole bodies of the third lumbar vertebra (L3) (N=100) for bone densitometry and atomic absorption spectrometry were collected. Multiple regression analyses were carried out and results were considered significant when p<0.05 and 0.01.*

***Results**: Age was inversely proportional to all histomorphometric parameters except for Tb.Sp. Age predicted BV/TV with the largest share of contribution of 68%. Gender showed the highest share of contribution for the Tb.Th (32%), while BMD showed it for the Ct.Th (10%).*

***Conclusions**: After age and gender, BMD is the third strongest variable to predict iliac crest bone histomorphometric parameters, and thus we can conclude that iliac crest bone histomorphometry and vertebral bone densitometry are correlated, which is important for monitoring osteoporosis in good clinical practice.*

INTRODUCTION

Lumbar vertebra and iliac crest bone are functionally different skeletal organs *(1)*. Lumbar vertebra is load-bearing and therefore exposed to more pronounced structural changes dependent on age or hormone deprivation. It is inaccessible for bone biopsy and, along with the hip and forearm, it is the most susceptible skeletal organ for fracture. In contrast, iliac crest bone is a plate-like bone with an easily palpable ridge close to skin surface, which is why bone biopsy is easily performed at this site *(2)*. Bone densitometry (dual x apsorptiometry, DEXA) is the most commonly used technique in bone status estimation and it is therefore considered a gold standard in assessing bone mass and therapeutic action in osteoporosis. Also, numerous prospective studies have been performed with results of bone mineral density showing a good correlation with the occurrence of fractures in women. Drawing on these results, the values of bone mineral density began to be increasingly used in clinical practice in assessing fracture risk *(3, 4)*. However, interpretation of the bone mineral density (BMD) value is sometimes illogical and cannot be primarily used to assess a risk of fractures. This is supported

by the fact that 50% of women who have experienced a fracture have good bone density, with no possibility to diagnose osteoporosis (T>-2,5 SD) *(5)*. Bone biopsy is followed by bone histomorphometry which is a very precise method in quantitative evaluation of the bone structure and bone mass, but it is also an invasive method and, if not necessary, it is avoided in good clinical practice. Another minus of the bone histomorphometry is inability to measure three-dimensional properties of bone, which is done in vitro and in vivo by use of the micro-CT and MRI, respectively *(6, 7)*. The most commonly performed is transiliacal bone biopsy, when bone cylinder contains both cortexes in the diameter of 6 to 9 mm *(2, 8)*. Previous studies have established positive impact of body weight on bone mass and structure, whereas the findings on the body height are contradictory. One of the risk factors for osteoporotic fracture is low body mass index and, if bone densitometry could not be performed, BMI may be the only indicator of the bone mass condition *(9)*.

Ash Density (AD) of dry bone substance is a direct reflection of bone mineralization, which is an important indicator of bone quality. Deviations in terms of high or low degree of bone mineralization, as identified in women with vertebral fractures, indicate that such bone is physically weaker and therefore cannot respond to biomechanical requirements *(10)*.

By comparing bone densitometry with static histomorphometry, our aim was to compare these methods that are used in clinical practice for diagnosis and monitoring osteoporosis, and to try to explain whether bone mass and bone structure of the crista iliaca is correlated to the bone mineral density of lumbar vertebra.

PATIENTS AND METHODS

The materials for this study comprised the whole body of the third lumbar vertebra (L3) and a cylindrical bone specimen obtained by transiliacal biopsy. Half of the specimens were obtained from 50 women and another half from 50 men. The individuals had no history of musculoskeletal, malignant, liver or kidney diseases. The bodies of L3 were removed by cutting the vertebral arch, after which the specimen was frozen at –20 °C. Cylindrical bone sample obtained from the iliac crest bone sample was fixed in 4% paraformaldehyde, and embedded in methylmethacrylate without being decalcified. Two consecutive (5 mm thick) sections were cut every 150 mm using a Leica RM550 circular microtome (Leica, Vienna, Austria) equipped with a tungsten carbide knife. The sections were stained with toluidine blue, Masson trichrome and Von Kossa staining which gives a good contrast between bone and bone marrow. Data on body height and body weight of subjects were obtained from their health cards. This study was approved by the local ethics committee.

Bone histomorphometry

The system for microscopy of the bone sections consisted of Olympus BHA microscope (Olympus, Tokyo, Japan) and Pulnix digital camera (Pulmix, Yokohama, Japan) connected to the personal computer. Digital images were captured under 40X magnification and stored until measurements were performed. For histomorphometric analysis, a semiautomatic image analysis system, equipped with Issa software (VAMS, Zagreb, Croatia), was used. According to the American Society of Bone and Mineral Research, bone histomorphometric parameters were derived from 2D measurements as explained by O. Cvijanović *et al (11)*.

Bone densitometry

Prior to atomic absorption spectometry, the body of L3 was immersed in a pot with a saline liquid and scanned using a device for bone densitometry (Hologic, Bedford, MA, USA). By this analysis, anterior – posterior (AP) and and lateral (L) scans were obtained and quantified as bone mineral content (BMC, g) and bone mineral density (BMD, g/cm^2) *(11)*.

Atomic absorption spectrometry

After being placed in a weighed porcelain pot, the body of L3 was weighed using electronic scales (Gilbertini Elettronica, Milano, Italy). In this way, wet weight of the bone specimen was obtained. Bone specimens were dried by autoclave at 105 °C to constant weight, after which the electronic scale measured their dry weight. In the next step, bone specimens were burned in muffon oven to 800 °C for 48 hours with the temperature gradually rising. The weight of dry matter obtained is expressed in g/ml *(11)*.

Statistics

Statistica 8.1 (StatSoft) computer software (StatSoft-Inc., Tulsa, OK) was used for statistical analyses. After data was tested for normal distribution, multiple regression analysis was employed. Results were considered statistically significant at the level P<0,05 and P<0,01.

RESULTS

Age, anthropometry and bone parameters of the iliac crest and lumbar vertebra of the study participants are presented in Table 1. Bivariant correlations of all measurements were calculated in order to avoid collinearity between variables (Table 2). Since BMI is calculated from the known values of body weight and body height, and due to high correlation between them, these variables were excluded from further analyses.

Multiple regression analysis revealed that all the iliac crest bone histomorphometric parameters were significantly predicted by model 1 (age, gender, BMI, AD and BMD) (Table 3).

TABLE 1
Descriptive statistics of the study group (N=100).

Anthropometric Parameters and Age	Minimum	Maximum	Mean ± SD
Age (years)	31	78	53.31 ± 13.99
Body Weight (kg)	50	120	74.80 ± 11.25
Body Height (cm)	155	190	172.15 ± 8.11
Body Mass Index, BMI (kg/m^2)	18.37	33.30	25.17 ± 2.71
Lumbar vertebra			
Bone Mineral Density; BMD (g/cm^2)	0.18	0.68	0.41± 0.10
Ash Density; AD (g/ml)	0.11	0.31	0.17± 0.04
Iliac Crest bone			
Bone Volume; BV/TV (%)	11.38	23.13	16.99 ± 2.83
Bone Surface; BS/TV (/mm)	2.38	4.16	3.29 ± 0.32
Trabecular Thickness; Tb.Th (µm)	71.49	161.94	105.34 ± 15.13
Trabecular Number; Tb.N (/mm)	1.19	2.06	1.62 ± 0.17
Trabecular Separation; Tb.Sp (µm)	391.42	742.99	519.03 ± 67.46
Cortical Bone Volume; CV (%)	71.15	97.79	86.59 ± 5.75
Cortical Thickness; Ct.Th (µm)	266.91	752.19	467.11 ± 79.97

(Decimal point should be used instead of a comma!)

TABLE 2
Bivariant Correlations.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Age (years)	1.00													
2. Gender (M/F)	-0.02	1.00												
3. Body height (cm)	-0.23*	0.79#	1.00											
4. Body weight (kg)	-0.21	0.58#	0.70#	1.00										
5. Body mass index (kg/m^2)	-0.08	0.09	0.05	0.75#	1.00									
6. BV/TV (%)	-0.82#	0.41#	0.51#	0.44#	0.15	1.00								
7. BS/TV (/mm)	-0.31#	-0.06	-0.01	-0.06	-0.08	0.36#	1.00							
8. Tb.Th (µm)	-0.51#	0.61#	0.56#	0.51#	0.21*	0.80#	-0.15	1.00						
9. Tb.N (/mm)	-0.67#	-0.22*	0.00	0.06	0.08	0.46#	0.53#	-0.00	1.00					
10. Tb.Sp (µm)	0.74#	0.06	-0.14	-0.09	-0.01	-0.70#	-0.78#	-0.15	-0.76#	1.00				
11. CV (%)	-0.45#	0.11	0.28#	0.25	0.08	0.39#	0.05	0.28#	0.24*	-0.28*	1.00			
12. Cth (µm)	0.01	0.11	0.14	0.29#	0.24*	0.06	-0.19	0.17	-0.12	0.16	0.01	1.00		
13. BMD (g/cm^2)	-0.40#	0.51#	0.44#	0.50#	0.32#	0.57#	-0.01	0.61#	0.15	-0.21*	0.21*	0.23*	1.00	
14. AD (g/ml)	-0.27*	0.35#	0.39#	0.29#	0.06	0.34#	0.07	0.31#	0.17	-0.20	0.19	-0.05	0.61#	1.00

*P<0.05; #P<0.01; M-male; F-female

DISCUSSION

This investigation demonstrated that all the iliac crest bone histomorphometric parameters were significantly predicted by the common influence of age, gender, BMI, AD and BMD (Table 3). When independent variables were analyzed, then the largest share of contribution was found for age, gender and BMD (Table 4).

Age was inversely proportional to all histomorphometric parameters except Tb.Sp (Table 4). Age predicted BV/TV with the largest share of contribution of 68%. It is

TABLE 3

A common influence of prediction variables (age, gender, BMI, AD, BMD) on bone histomorphometric parameters of the iliac crest bone.

Crista Iliaca	R	R^2	F	P
BV/TV (%)	0.91	0.82	77.24	0.00
BS/TV (/mm)	0.36	0.13	2.37	0.04
Tb.Th (µm)	0.81	0.65	31.04	0.00
Tb.N (/mm)	0.72	0.51	17.37	0.00
Tb.Sp (µm)	0.75	0.56	21.06	0.00
CV (%)	0.46	0.21	4.51	0.00
Ct.Th (µm)	0.37	0.13	2.53	0.04

R – coefficient of multiple correlation
R^2 – coefficient of determination
P – statistical significance

TABLE 4

Statistically significant effects of single prediction variables on bone histomorphometric parameters of the iliac crest bone.

Criterion variables	Prediction variables	β	r	P	Share of contribution
BV/TV (%)	Age	-0.79	-0.86	0.00	68%
	Gender	0.36	0,59	0.00	21%
BS/TV (/mm)	Age	-0.37	-0,33	0.00	12%
Tb.Th (µm)	Age	-0.45	-0.55	0.00	25%
	Gender	0.53	0.60	0.00	32%
	BMD	0.24	0.25	0.02	6%
Tb.N (/mm)	Age	-0.68	-0.65	0.00	44%
	Gender	-0.23	-0.27	0.01	6%
Tb.Sp (µm)	Age	0.77	0.72	0.00	55%
CV (%)	Age	-0.46	-0.42	0.00	19%
Ct.Th (µm)	BMD	0.38	0.25	0.02	10%

β – beta ponder
r – coefficient of the partial correlation
P – statistical significance

common knowledge that age-related changes lead to deterioration of the cortical and trabecular bone which is more pronounced at the load bearing sites of the skeleton *(11-14)*. Also, the shares of contribution age showed for the structural parameters, Tb.N and Tb.Sp were 44% and 55%, respectively (Table 4).

Transilical bone biopsy is usually used for the purpose of diagnosing a number of metabolic bone diseases, as well as in order to monitor osteoporotic changes, especially in resistant osteoporosis *(2, 8)*. Indications for transiliacal bone biopsy do not involve age-related changes in bones, so the question arises whether the age-related changes in bone are comparable to findings in bones caused by osteoporosis. The answer should be sought in the results of research carried out on samples of healthy bones, obtained *post mortem* from different age groups, and also from the results of histomorphometric studies carried *iv vivo*, from the bone samples obtained from individuals with osteoporosis. By comparing both it can be concluded that, in principle, similar changes but different mechanisms are responsible for a much greater range of changes in osteoporotic bones. These changes are most often due to the lack of estrogen, as compared to aging changes that are slower in flow, because they are part of involutional processes in the human organ systems *(1, 12, 13, 15)*. Nevertheless, when one speaks of osteoporotic bone or senile bone, then the following changes are present in the trabecular compartment: thinning of the trabeculae, reduction in the number of trabeculae, increase in the distance between the trabeculae, reduced connec-

tivity and increased degree of anisotropy of trabecular bone. Thinning of the bone trabeculae does not significantly affect the strength of the trabecular bone, whereas loss of bone trabeculae significantly weakens the structure of the trabecular bone *(16, 17)*.

Numerous studies have documented that age-related changes in trabecular bone compartment differently affect men and women. While aging, men are prone to trabecular bone thinning, while women are disposed to loss of single trabeculae. Even if we have not presented separated data for men and women, our result of exactly proportional relationships between gender and BV/TV or Tb.Th, but inversely proportional between gender and Tb.N, suggest predominantly bone structure changes, rather than bone loss, in both sexes *(1, 11)*.

To answer the question whether the iliac crest bone histomorphometric data reflects bone mineral density of the lumbar vertebra, our result on directly proportional relationship between BMD with Tb.Th and Ct.Th could help. In clinical practice, BMD is the most commonly used parameter of bone densitometry and describes the amount of minerals in the scanned area of bone surface. However, by use of this parameter, it is not possible to monitor the changes of geometry, architecture, volume and degree of mineralization of bone. Numerous studies have investigated and found differences between iliac crest and lumbar vertebra *(1, 18-20)*. Women who have suffered from the vertebral fracture had hypo- or hypermineralization of the iliac crest trabecular bone *(10)*. It seems that, regardless of the different function, there is a connection in changes that affect different skeletal organs during aging. Our results revealed that Tb.Th and Ct.Th were significantly predicted by BMD, with the share of contribution amounting to 6% and 10%, respectively. After age and gender, vertebral BMD is the third strongest variable to predict iliac crest bone histomorphometric parameters. According to correlations between iliac crest bone histomorphometry and vertebral bone densitometry, it can be concluded that these two methods are comparable, which is useful in diagnosis and follow-up of osteoporosis.

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