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Correlations between Microcomputed Tomography and Bone Histomorphometry in Korean Young Females

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The bone mass and microarchitecture are important determinants of bone strength, with microarchitectural deterioration being one of the specific changes associated with osteoporosis. The purpose of this study was to evaluate and compare the results of microcomputed tomography (micro-CT) and histomorphometry of biopsied specimens. A bone biopsy was performed on the iliac crest of 10 normal premenopausal Korean women. Measurements of the bone mineral density (BMD), micro-CT, and bone histomorphometry were performed. The bone volume, as determined by both micro-CT and histomorphometry, was significantly correlated (r=0.88, $p<0.01$). The osteoid surface was correlated with both the bone volume (r=0.84, $p<0.01$) and the structure model index (SMI) (r=-0.89, $p<0.01$) measured by micro-CT. The SMI was correlated with both the bone volume (r=-0.85, $p<0.01$) and the total hip BMD (r=-0.65, $p<0.05$). In conclusion, some, but not all of the parameters of the micro-CT, were well correlated with the bone histomorphometric results. Micro-CT and histomorphometry appear to be complementary techniques in the study of bone microarchitecture.

Key Words: Microarchitecture, microcomputed tomography, histomorphometry, bone mineral density, bone biopsy

INTRODUCTION

Osteoporosis is a disease that is characterized by a low bone mass and a microarchitectural deterioration, which may result in a fracture.[1] The microarchitecture and bone mass are important determinants of the mechanical bone strength.[2,3] To investigate the bone microarchitecture, classical histomorphometric methods have been applied. However, bone histomorphometry has several disadvantages. First, it takes considerable time and effort to prepare samples. Second, it usually cannot provide a three dimensional structure. Third, some of the microarchitectural parameters cannot be measured. Microcomputed tomography (micro-CT) allows these limitations to be overcome, as it can directly assess trabecular thicknesses, trabecular separations, and the trabecular number, and also allows the derivation of parameters, such as the structure model index (SMI), and the degree of anisotropy (DA) to be calculated. The SMI and DA are used as previously defined.[4,5]

The roles of micro-CT and histomorphometry for the evaluation of the bone microarchitecture were studied. If, the parameters obtained from a micro-CT show good correlation with those from bone histomorphometry, the former could replace the latter, at least with selected parameters. The purpose of this study was to evaluate and compare the results of micro-CT and histomorphometry in biopsied bone specimens.

MATERIALS AND METHODS

Ten female college students volunteered for a bone biopsy. Their ages ranged from 20 to 26 years. The subjects were all had normal menstrual

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cycles. A detailed medical history and a physical examination revealed no specific illness or medication history related to metabolic bone diseases. These studies were conducted in accordance with the Declaration of Helsinki.

The bone biopsies were performed in an outpatient setting. Tetracycline was given, at 250 mg, four times a day, for three days. Tetracycline was administered for another three days fourteen days later, and the bone biopsy scheduled for after the second tetracycline administration. The biopsy was taken at the iliac crest, two centimeters posterior, and two centimeters inferior, to the anterior superior iliac spine, following premedication, with meperidine, and local anesthesia with bupivacaine. A transiliac bone biopsy was performed with a Rochester trephine (Gauthier Medical Inc., Rochester, MN, USA).

Undecalcified bone sections were prepared as follows. Briefly, the biopsy specimens were subjected to serial alcohol dehydration, embedded in methyl methacrylate, and sectioned at 5 μm. The sections were stained with Goldner's modified Masson's trichrome, and histomorphometrically examined using a microscopic image analyzer system (Bioquant, Bioquant-R&M Biometrics, Inc., Nashville, TN, USA). Indirect indices of the traditional bone histomorphometry were calculated by assuming that the bone trabeculae formed a plate structure.[4,6,7]

Microcomputed tomography (SkyScan 1072, SkyScan, Belgium) was used to analyze the bone volume, trabecular thickness, trabecular number, trabecular separation, SMI, and DA.[4,5] Cubic voxels, 19.9 μm in dimension, were used to represent the measured object.

Measurements of the BMD were performed by dual energy x-ray absorptiometry (Expert-XL, Lunar Co, Madison, WI, USA), at the lumbar spine, femur, and total body. The body fat content was automatically obtained, using dual energy x-ray absorptiometry, during the total body BMD measurements.

The data were statistically analyzed by using a Pearson's correlation test. A values of $p < 0.05$ as considered significant.

RESULTS

The descriptive statistical parameters for the anthropometry, BMD, histomorphometry, and micro-CT are detailed in Table 1. The T scores of the total body, lumbar spine, and total hip BMD were 1.05 ± 0.76, 0.26 ± 0.86, and 0.83 ± 0.83, respectively.

The correlation coefficients between the histomorphometric, micro-CT, and BMD measurements are detailed in Table 2. The bone volumes, as measured by the micro-CT, were highly correlated with those measured by the histomorphometry (r=0.876, p=0.001). The histomorphometric osteoid surface was correlated with the bone volume, as measured by the micro-CT (r=0.841, p=0.009) and the SMI (r=-0.893, p=0.003). The SMI was negatively correlated with the bone volume, as measured by histomorphometry (r=-0.714, p=0.020), and with that measured by the micro-CT (r=-0.846, p=0.002). The total hip BMD was positively correlated with the osteoid surface (r=0.822, p=0.012) and the SMI (r=-0.646, p=0.044). The BMD of other sites (total body and lumbar spine) and the BMC were not correlated with any other micro-CT or histomorphometric parameters. The indirectly calculated indices from the bone histomorphometry did not correlate with the directly measured micro-CT indices.

DISCUSSION

Micro-CT has several advantages : first, it is non-destructive and the sample can be used for further study, second, it avoids the sample preparation problems occasionally associated with the histomorphometric procedure, such as the deforming or shrinkage of plastic embedded bone slices during the preparation of the glass slides, third, no specimen preparation or staining is required, which resultings in savings of time and effort, and fourth, specimen can be rotated in any direction, three dimensional images created, and fracture simulations are possible. A disadvantage of micro-CT is that this system cannot measure biological parameters, such as the osteoid thickness, erosion depth, or osteoclast number.

The dynamic parameters were measured, including the mineral apposition rate (MAR), eroded surface / bone surface (ES/BS), and osteoid surface / bone surface (OS/BS). The OS/BS was

Table 1. Parameters of Anthropometry, Bone Mineral Density, Histomorphometry, and Microcomputed Tomography

	Mean	SD	Min	Max	N
Height (cm)	160.8	4	155.7	167.2	10
Weight (kg)	57.6	5.78	50.8	68.3	10
Body fat (%)	32.93	5.86	22.4	42.1	10
Total body BMD (g/cm^2)	1.184	0.06	1.082	1.296	10
Lumbar BMD (g/cm^2), L2-4	1.153	0.104	1.011	1.279	10
Total hip BMD (g/cm^2), proximal femur	1.034	0.1	0.881	1.200	10
Bone mineral content (mg)	2282	182	1865	2513	10
BV-H (%)	24.45	6.07	14.4	35.4	10
Cortical thickness (μm)	1175	306.6	846	1706	10
Osteoid thickness (μm)	32.22	13.99	11.34	62.77	10
OS/BS (%)	1.51	1.59	0.02	4.00	8
Mineral apposition rate (μm/day)	0.985	0.042	0.77	1.16	10
Calculated trabecular thickness (mm)	0.086	0.018	0.067	0.100	7
Calculated trabecular separation (mm)	0.351	0.143	0.235	0.644	7
Calculated trabecular number (mm^{-1})	2.781	0.776	1.44	3.72	7
BV-CT (%)	24.54	6.88	16.3	37.20	10
Measured trabecular thickness (mm)	0.051	0.027	0.004	0.102	10
Measured trabecular separation (mm)	0.236	0.023	0.207	0.262	10
Measured trabecular number (mm^{-1})	3.483	0.4	2.757	4.106	10
Structure model index	0.88	0.389	0.024	1.389	10
Degree of anisotropy	0.177	0.045	0.124	0.268	10

BMD, bone mineral density; BV-H, bone volume measured by histomorphometry; OS/BS, osteoid surface / bone surface; BV-CT, bone volume measured by microcomputed tomography.

significantly, and positively, correlated with the bone volume and total hip BMD. These correlations could be explained by the increased new bone formation contributing to the gain of bone mass. Conversely, the correlations might be applicable only to normal healthy young females, and have not been confirmed in osteoporosis or metabolic bone diseased patients. The bone histomorphometry has advantages in terms of the measurement of the dynamic parameters, and thus complements micro-CT.

In this study, the BMD was not correlated with the bone volume measured by the micro-CT or with that measured by histomorphometry. In general, about 2/3 of the bone strength is represented by the bone volume, which is correlates with the bone density, with the other 1/3 explained by the bone quality. Our observation that the BMD was not correlated with the bone volume could be due to the two dimensional area of the BMD measurements. A three dimensional volumetric BMD might show correlation with the bone volume. In this study, the BMDs were systemically measured, rather than the biopsy sample itself. Published studies, reporting the BMD measurements of the specimen itself, have shown

Table 2. Correlations among the Histomorphometry, Microcomputed Tomography, and Bone Mineral Density

	BV-H	OS/BS	BV-CT	Tb.N	Tb.Th	SMI	Total	Lumbar	Femur
OS/BS	0.85†	1	0.84†	-0.16	0.3	-0.89†	0.21	0.48	0.82*
BV-CT	0.88†	0.84†	1	0	0.58	-0.85†	0.18	-0.16	0.47
Tb.N	0.41	-0.16	0	1	-0.5	-0.06	-0.35	0.03	0.08
Tb.Th	0.33	0.3	0.58	-0.5	1	-0.32	0.31	-0.15	0.21
SMI	-0.76	-0.89†	-0.85†	-0.06	-0.32	1	-0.4	-0.07	-0.65*
Total	0.02	0.21	0.18	-0.35	0.31	-0.4	1	0.51	0.70*
Lumbar	-0.05	0.48	-0.16	0.03	-0.15	-0.07	0.51	1	0.66*
Femur	0.52	0.82*	0.47	0.08	0.21	-0.65*	0.70*	0.66*	1
Pelvis	0.04	0.27	0.01	-0.12	-0.05	-0.24	0.76*	0.67*	0.78†

BV-H, bone volume measured by histomorphometry; BV-CT, bone volume measured by microcomputed tomography; OS/BS, osteoid surface / bone surface; Tb.N, trabecular number; Tb.Th, trabecular thickness; SMI, structure model index; Total, total body bone mineral density; Lumbar, lumbar bone mineral density; Femur, total hip bone mineral density; Pelvis, pelvis bone mineral density.
*$p<0.05$, †$p<0.01$.

good correlations with the micro-CT parameters.[8-12] In the present study, the pelvic BMD did not correlate with the histomorphometric or micro-CT parameters. Of the micro-CT parameters, only the SMI was significantly correlated with the total hip BMD.

The indirectly calculated histomorphometry parameters did not correlate with the directly measured indices of the micro-CT, even though the bone trabeculae were likely to form plate-like structures (mean value of SMI was less than 1) in the iliac bone. According to the present study, it is not possible to obtain the three dimensional parameters for the trabecular thickness, trabecular separation, or trabecular numbers from the traditional two dimensional histomorphometry. Studies of the microarchitecture of trabecular bone must be carried out by directly measuring the parameters using techniques such as micro-CT. Uchiyama, et al. and Ito, et al. reported relatively good correlation between the histomorphometric and micro-CT variables for the iliac bone.[8,9] Moreover, the microarchitectural parameters measured by micro-CT were useful for determining the differences between the fracture and non-fracture groups,[13] and for demonstrating the effects of drugs on osteoporosis.[14]

Our study has been conducted with small number of subjects, and therefore its statistical strength may be limited. It is relatively hard to find volunteers in Korea, as in many other Asian countries, due to cultural influence.

In this study, young Korean females were examined, and the role of micro-CT was found to be similar to that reported in Caucasians[4,10] and other Asians.[8,9,11] Micro-CT technology can be applied to both Caucasians and Asians, and in the young and old. In conclusion, microcomputed tomographic and histomorphometric analyses may be used in a complementary fashion to study the bone microarchitecture.

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