Estimation of Bone Mineral Density and Bone Loss by Means of Bone Metabolic Markers in Postmenopausal Women

Hiroaki MIURA, Itsuo YAMAMOTO, Itsuaki YUU, Yusuke KIGAMI, Toyotsugu OHTA, Yasuyo YAMAMURA, Yasuo OHNAKA, and Rikushi MORITA

Department of Radiology, Shiga University of Medical Science, Otsu 520-21, Japan

Abstract. We have examined healthy women (51 premenopausal women and 30 postmenopausal women; age 28-59) for lumbar bone mineral density (BMD) by dual energy X-ray absorptiometry (DXA) and assessed metabolic bone markers, such as type I procollagen carboxy-terminal propeptide (P1CP), pyridinoline (PYR), deoxypyridinoline (DPYR), osteocalcin (BGP) and alkaline phosphatase (ALP). BMD was assessed once a year in three consecutive years. Correlations among the BMD, BMD changes and levels of bone markers in samples at the first DXA assessment were studied. In pre-menopausal women, none of the biochemical markers were correlated with the BMD or changes in BMD. In contrast, BMD in post-menopausal women correlated (negatively) well with levels of P1CP, DPYR, PYR and ALP declining in this order, and a significant positive correlation was observed between the rate of bone loss in postmenopausal women and the P1CP concentration. PYR and DPYR also had a tendency to correlate. Combinations of several bone markers improved the correlation. These results show that by measuring several bone specific biochemical markers in postmenopausal women, one can estimate their rates of bone loss as well as their present BMDs. The measurement of biochemical bone markers will therefore be very useful in evaluating bone status and would be applicable in screening postmenopausal osteopenia.

Key words: BMD, Bone metabolic marker, Postmenopause, Bone loss

POSTMENOPAUSAL osteoporosis is a process occurring in a subset of postmenopausal women who are characterized by exaggerated bone loss related to estrogen deficiency [1-3]. The loss of ovarian hormones causes an increase in bone turnover and results in a negative bone balance due to a relatively high bone resorption rate compared to bone formation rate, which is also enhanced [1]. Several lines of evidence suggest that a high rate of bone turnover causes great loss of bone [4-7]. "Fast losers" have a high bone turnover rate, which is reflected not only in concentrations of metabolic bone markers specific to bone resorption but also in those specific to bone formation, and could be identified. Bone loss, reportedly, could be predicted by the assessment of bone markers such as urinary hydroxyproline, serum alkaline phosphatase and more recently, urinary pyridinoline [5-7]. To identify fast losers is important in preventing postmenopausal osteoporosis in an early stage and also essential in selecting appropriate treatment [7]. In addition, bone mineral density is reportedly low in subjects with high levels of metabolic bone markers such as osteocalcin [8]. A close relationships between biochemical bone markers and bone mineral density has therefore been reported.

Many specific assays for metabolic bone mark-
ers have recently been developed [9]. Among them, the measurement of metabolites of type I collagen cross-links is shown to be more sensitive and more specific to bone resorption than conventional measurements such as urinary hydroxyproline and urinary calcium excretion [10]. An assay for propeptide of type I procollagen, on the other hand, is shown to be specific to bone formation [11]. Employing these new metabolic bone markers, we attempted to assess bone turnover in pre- and post-menopausal women and examined whether bone loss or bone mineral density could be predicted by these measurements.

Materials and Methods

Subjects

Fifty one premenopausal women aged 28–59 years (mean ± SD; 42 ± 6) and 30 post-menopausal women aged 42–59 (mean ± SD; 52 ± 4) were examined. In the group of post-menopausal women, 7 women who were perimenopausal at the start of this study, and underwent menopause during the study period, were included. All subjects were sport-loving volunteers who agreed to undergo sequential bone density measurement in the hospital of Shiga University of Medical Science. They had no abnormal urinary or serum biochemical values, except a slight increase in serum alkaline phosphatase in some of the postmenopausal women. Termination of menstrual events for more than one year was judged as menopause, and the mean period after menopause in postmenopausal women was approximately 2.5 years. During 3 years of bone mineral monitoring, none of the subjects was given particular tutelage on eating habits such as calcium intake.

Measurement of bone mineral density

Bone mineral density (BMD) was assessed in the lumbar spine (L2–L4) in the supine position with a dual energy X-ray absorptiometer (QDR-1000, Hologic Inc., USA) for all subjects once a year for 3 consecutive years, from 1991 to 1994 (4 times altogether). Preliminary examination showed 1.2% in vivo precision in measuring lumbar BMD with this machine. The precision of the machine was always monitored by the standard calibration phantom at the start of the examination, showing a variation less than 0.5% throughout the examination period.

Measurement of bone metabolic markers

At the first time of BMD measurement, urine and blood samples were obtained and stored at −20 °C before the assay. Pyridinoline (PYR) and deoxypyridinoline (DPYR) in urine were measured by high performance liquid chromatography (intra- and interassay variations in both markers: 10% and 10–15%, respectively) [12]. Peptide-unbound DPYR (intra- and interassay variations: less than 10% and 15%, respectively) in urine was measured by an enzyme-linked immunoassay with specific antibodies to free type DPYR (Metra, San Francisco) [13]. Bone gla-protein (BGP) was measured by a radioimmunoassay detecting the C-terminal portion of the peptide (CIS lab., Tokyo) [14]. Type I procollagen carboxy-terminal propeptide (PICP; intra- and interassay variations: 8.5% and less than 4.2%, respectively) were assessed in serum by a radioimmunoassay (Orion Diagnostica, Finland) [15]. Alkaline phosphatase (ALP) was measured by a colorimetric assay with a kit (Sigma, St. Louis). Other routine biochemical analyses, including calcium and phosphorus measurement in the urine and serum, were also performed. The excretion of urinary PYR, DPYR and free-DPYR were expressed as a ratio to urinary creatinine excretion.

Statistical analysis

The analyses were performed by means of a Stat View J-4.02 program on a Macintosh computer. BMD data were expressed as the mean ± SD and were statistically analyzed by Welch’s t-test. Correlations among bone metabolic markers, BMD and bone loss were calculated by Pearson’s method. Multiple regression analyses were performed to estimate bone mass and bone loss. A P value less than 0.05 was considered significant.

Results

Sequential measurements of lumbar BMD in premenopausal women showed a 1.3 ± 3.2% (mean ± SD; not significantly decreased from the initial BMD) decline in three years, while those in post-
menopausal women showed 5.6 ± 5.6% (mean ± SD; significantly decreased from the initial BMD, \(P<0.05\)) a decline during the same period (Fig. 1).

A comparison of the decline in lumbar BMD in three years with the levels of biochemical markers is shown in Table 1. Among the biochemical markers examined, only P1CP showed a significant (\(r=0.476; P<0.05\)) positive correlation with the decrease in lumbar BMD, and PYR and DPYR showed a tendency to correlate with the decline in lumbar BMD (\(r=0.447\) and 0.343, respectively; in both, \(P<0.1\)).

In a comparison of the levels of biochemical markers with the initial values for lumbar BMD shown in Table 1, significant negative correlations were observed with DPYR (\(r=-0.663; P<0.001\)), P1CP (\(r=-0.639, P<0.001\)), PYR (\(r=-0.599, P<0.05\)) and ALP (\(r=0.430, P<0.05\)), while no significant correlations were observed with free-DPYR and BGP.

A combination of several markers improved the correlation. As shown in Table 2, the best correlation was obtained in lumbar BMD with a combination of P1CP, ALP and DPYR. The employment of DPYR and P1CP always produced high \(r\) values. Figure 2 shows the scattered plots of correlation between estimated BMD values and measured BMD values. From the regression analysis, the following formula for the estimation of BMD is obtained: Estimated BMD=1.467−0.023 × DPYR−0.018 × ALP−0.0012 × P1CP (\(r=0.841, P<0.0001\)).

To estimate of bone loss, only one combination of P1CP, DPYR, BGP and the initial BMD showed a significant correlation, as shown in the following formula; Estimated bone loss in three years=0.00077 × DPYR + 0.0013 × BGP + 0.00044 × P1CP−0.0077 (\(r=0.762, P<0.01\)). Figure 3 shows the scattered plotting of the estimated bone loss and the actually measured bone loss.

**Discussion**

Postmenopausal bone loss is caused by an imbalance between bone formation and bone resorption. In contrast to bone loss by aging, both bone formation and bone resorption are increased in postmenopause [1]. If the rate of imbalance, namely the ratio of bone formation to bone resorption is constant, the amount of imbalance should be greater when the absolute bone turnover rate is high. Some previous studies suggested that this hypothesis might be true [4, 16, 17]. In this study, we attempted to prove the hypothesis by using DXA rather than DPA or SPA, as in the previous studies [16, 17], and by using more specific meta-

![Fig. 1. Changes in BMD in pre- and postmenopausal women during 3 years. Each BMD value represents the mean ± SEM. BMD in postmenopausal women decreased excessively relative to premenopausal women. PRE, premenopausal women; POST, postmenopausal women.](image)

**Table 1.** Correlation between bone metabolic markers and BMD or bone loss in postmenopausal women

<table>
<thead>
<tr>
<th></th>
<th>PYR</th>
<th>DPYR</th>
<th>free-DPYR</th>
<th>P1CP</th>
<th>ALP</th>
<th>BGP</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD (&lt;1991) (first measurement)</td>
<td>(r=-0.599)</td>
<td>(r=-0.663)</td>
<td>(r=-0.307)</td>
<td>(r=-0.639)</td>
<td>(r=-0.430)</td>
<td>(r=-0.214)</td>
</tr>
<tr>
<td></td>
<td>(P&lt;0.01)</td>
<td>(P&lt;0.001)</td>
<td>NS</td>
<td>(P&lt;0.001)</td>
<td>(P&lt;0.05)</td>
<td>NS</td>
</tr>
<tr>
<td>Bone loss (BMD; 1991–1994)</td>
<td>(r=0.447)</td>
<td>(r=0.343)</td>
<td>(r=0.169)</td>
<td>(r=0.476)</td>
<td>(r=0.276)</td>
<td>(r=0.208)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>(P&lt;0.05)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data represents the coefficient (\(r\)) and probability (\(P\)) in simple linear regression. PYR, pyridinoline; DPYR, deoxypyridinoline; P1CP, type 1 procollagen carboxy-terminal propeptide; ALP, alkaline phosphatase; BGP, bone gal-protein; NS, not significant.
bolic bone markers, PYR, DPYR and P1CP, rather than hydroxyproline or ALP. Our results show that biochemical markers of bone turnover are good predictors not only of bone loss but also of BMD itself. Apparently there were no differences between bone resorption markers and bone formation markers in predicting bone loss or BMD. P1CP is a specific maker for type I collagen synthesis and its levels are shown to correlate with bone formation by histomorphometry [11], whereas DPYR and PYR are metabolites of collagen cross-links and representatives of a bone resorption marker. Both bone formation markers and bone resorption markers correlated with BMD, and even better in bone formation markers with bone loss. Since both bone formation and bone resorption are markedly enhanced after menopause, differences of the levels of biochemical markers between premenopause and postmenopause would be a more important determinant than the qualities of those markers in terms of specificity to bone resorption or to bone formation. In this sense, specificity of biochemical markers to bone turnover is the most important.

Our unexpected results show a close correlation between bone markers and bone density, although Delmas et al. have reported a weak correlation between them [18]. The correlation of lumbar BMD with PYR, DPYR, and P1CP was good, again showing no difference between bone formation markers and bone resorption markers. We found no such

Table 2. Multiple regression analyses of various combinations of bone metabolic markers for BMD in postmenopausal women

<table>
<thead>
<tr>
<th>Independent 1</th>
<th>Independent 2</th>
<th>Independent 3</th>
<th>Coefficient (r)</th>
<th>Probability (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1CP</td>
<td>ALP</td>
<td>DPYR</td>
<td>0.841</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P1CP</td>
<td>ALP</td>
<td>PYR</td>
<td>0.820</td>
<td>0.0018</td>
</tr>
<tr>
<td>P1CP</td>
<td>BGP</td>
<td>DPYR</td>
<td>0.807</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P1CP</td>
<td>free-DPYR</td>
<td>PYR</td>
<td>0.794</td>
<td>0.0039</td>
</tr>
<tr>
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<td>free-DPYR</td>
<td>0.744</td>
<td>0.0006</td>
</tr>
<tr>
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<td>ALP</td>
<td>BGP</td>
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<td>0.0009</td>
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<tr>
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<td>ALP</td>
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<tr>
<td>DPYR</td>
<td>BGP</td>
<td>PYR</td>
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<td>0.0411</td>
</tr>
</tbody>
</table>

See Table 1 for abbreviations.

![Fig. 2. Relationship between BMD in postmenopausal women and their estimates, calculated by the multiple regression formula. Dependent: BMD (the initial value in 1991). Coefficient: intercept=1.467, DPYR=−0.023, ALP=−0.018, P1CP=0.0012, r=0.841, P<0.001.](image)

![Fig. 3. Relationship between bone loss in postmenopausal women and their estimates, calculated by the multiple regression formula. Dependent: bone loss (the decrease in BMD from 1991 to 1994). Coefficient: intercept=−0.00777, DPYR=0.00077, P1CP=−0.00044, BGP=0.0013, BMD (the initial value in 1991)=0.135, r=0.762, P<0.01.](image)
correlation in premenopausal women. We observed in two samples, taken at a one year interval, that levels of bone markers are relatively constant (data not shown), and therefore a high bone turnover state might persist for a certain period of time, which would be enough to reduce bone mineral density. The better correlation with the BMD than the changes in BMD (bone loss) might be attributable to smaller errors in measuring BMD once than in measuring it twice (differences between two measurement) and also to smaller changes in BMD. If monitoring period were extended, the correlation with bone loss would be improved.

The present study demonstrated a promising role of the assessment of bone specific biochemical markers in the evaluation of BMD. By measuring some specific bone markers such as PICP, DPYR and PYR in the postmenopausal period (the sixth decade of life), one would be able to estimate their present bone mineral densities as well as the amount of density which their bones will lose in the following years. Thus, it would be reasoned to use bone markers as a method for mass screening of osteoporosis. The employment of recently developed biochemical bone markers [19, 20], possibly more specific than DPYR and PYR, will further improve accuracy in estimating BMD or the rate of bone loss, and this remains to be investigated.

References


18. Delmas PD, Wahner HW, Mann KG, Riggs BL
