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Early Changes in Biochemical Markers of Bone Turnover Predict Bone Mineral Density Response to Antiresorptive Therapy in Korean Postmenopausal Women with Osteoporosis

SANG WAN KIM*,**, DO JOON PARK*,**, KYONG SOO PARK*,***, SEONG YEON KIM*,***, BO YOUN CHO*,***, HONG KYU LEE*,*** AND CHAN SOO SHIN*,***

**Department of Internal Medicine, Seoul National University College of Medicine, Seoul 110-744, Korea*
***Seoul National University Hospital Clinical Research Institute, Seoul 110-744, Korea*
****Institute for Endocrinology, Nutrition and Metabolism, Seoul National University Medical Research Center, Seoul 110-799, Korea*

Abstract. Biochemical markers of bone turnover have been suggested to be useful in monitoring the efficacy of antiresorptive therapy. In this study, we investigated the predictive value of bone turnover markers to determine short-term response in bone mineral density (BMD) and to identify nonresponders in 138 postmenopausal women (mean age 58 years) with osteoporosis given with either hormone thearpy (HT) or alendronate. Urinary type I collagen N-telopeptide (NTx) and serum osteocalcin (OC) at baseline, 3, and 6 months after treatment as well as spine and femoral neck BMD at baseline and 12 months were measured. Significant decreases in both NTx and OC were evident in women on treatment with antiresorptive agents as early as 3 months ($p<0.01$). Percent change of NTx at 3 months correlated with the percent change of spinal BMD at 12 months of treatment. When bone turnover markers were stratified by tertiles, the average rate of lumbar spine BMD gain increased significantly with increasing tertiles of baseline value ($p<0.05$) and percent change ($p<0.05$) of urinary NTx at 3 month of treatment. In terms of BMD response, urinary NTx at 3 months decreased significantly more in BMD responders group than in nonresponders group. Logistic regression analysis demonstrated that percent change of NTx at 3 months is an independent predictor to identify BMD nonresponders, defined as those whose BMD gain remained within the precision error range of dual energy X-ray absorptiometer (DXA). We conclude that biochemical markers of bone turnover, especially percent change in urinary NTx levels, can be used to determine BMD response to antiresorptive therapy in Korean postmenopausal women with osteoporosis.

Key words: Bone turnover marker, Bone mineral density, Responders, Logistic regression

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BONE turnover is characterized by two tightly coupled activities, the degradation of old bone by osteoclast followed by the formation of new bone by osteoblasts. The rate of resorption and formation of bone matrix can be assessed by measuring bone matrix components released into the circulation during remodeling, *i.e.*, the biochemical markers of bone turnover. In osteoporosis, bone turnover markers have been known to be useful in predicting the rates of bone loss in postmenopausal women [1–3]. Moreover, some markers of bone resorption predict the subsequent risk of hip fracture independently of bone mineral density (BMD) [4, 5], which is still the single most important predictor for osteoporotic fracture [6–8]. Recently, it has been also suggested that the biochemical markers of bone turnover may be used to monitor the efficacy of antiresorptive therapy in patients with osteoporosis [9–13]. Patients with high turnover at baseline have a significantly higher increase in spinal BMD in response to injectable or nasal calcitonin than those with low turnover [14]. A similar trend has been observed in patients treated with hormone thearpy (HT) or alen-

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Correspondence to: Dr. Chan Soo SHIN, Department of Internal Medicine, Seoul National University College of Medicine, 28 Yungun-Dong, Chongno-Gu, Seoul, 110-744 Korea

dronate [15]. Moreover, in addition to the baseline values, the decrease in bone turnover markers after antiresorptive therapy is strongly correlated to the increase in BMD. Antiresorptive therapy induces a 30%–60% decrease in markers of resorption and formation that fall within the premenopausal range within only 3–6 months [9–13]. In contrast, given the 1–2% precision error of bone mass measurement by dual-energy X-ray absorptiometry (DXA) and the expected change in bone mass induced by antiresorptive treatment, it is usually necessary to wait up to 1–2 years after initiating therapy to determine whether treatment is effective. Thus, several studies have suggested that changes in bone markers after 3–6 months of treatment could be used to predict the amplitude of BMD response after 1–2 years in postmenopausal women treated either with estrogen or alendronate [9, 10, 12, 16]. Reported correlation coefficients between percent change in bone turnover markers and percent change in BMD are typically of 0.4–0.6, implying that less than 40% of the inter-individual variance in long-term BMD changes can be predicted from early changes in bone turnover markers. For the clinician, however, identification of the nonresponder, *i.e.*, those who fail to gain a significant BMD after 1–2 years of treatment, would be the primary concern of practice. To identify nonresponders, defining a cut-off value for each bone turnover markers is necessary and should be performed in each population. This study was undertaken firstly, to investigate whether the baseline values or percent change of biochemical marker of bone turnover after initiating treatment can predict the response in BMD after 1 year of antiresorptive therapy, and secondly, to identify the cut-off value for the prediction of nonresponders of BMD in Korean postmenopausal women with osteoporosis.

Experimental Subjects

One hundred thirty-eight postmenopausal women, aged 42–80 years (mean age 58.0 ± 6.6 years), who were all at least 3 years past menopause (mean 9.5 ± 6.0 years) and had lumbar spine BMD<–2.5 SD below the normal mean for premenopausal Korean women, were studied. Eighty-eight of these osteoporotic women received conjugated equine estrogen 0.625 mg and contiuous or cyclic medroxyprogesterone acetate 2.5–10 mg per day and for the other 50 patients, alendronate (4-amino-1-hydroxybutylidene-1,1-bisphosphonate) 10 mg were given orally once daily in the morning. All subjects also received 500 mg/day of elemental calcium (as carbonate or citrate) and 400 IU of vitamin D3. Blood and urine samples for biochemical marker measurements were obtained at baseline, 3 and 6 months after initiation of therapy. BMD was determined by DXA at baseline and at 12 months of treatment. The study protocol was approved by the local institutional review boards and all patients gave written informed consent before undergoing study procedure.

Material and Methods

Measurement of biochemical markers of bone turnover

Venous blood was taken from the subjects after an overnight fast and the serum samples were obtained by centrifugation and stored at –20°C. Urine samples were collected from a second voiding and stored at –20°C.

Cross-linked N-telopeptides of type I collagen (NTx) in urine were measured by a competitive inhibition ELISA (Osteomark, Ostex International, Seattle, WA). NTx is an indicator of bone resorption. Assay values were corrected for creatinine and expressed as nM BCE/mM Cr. The minimum detection limits were 1 nM BCE/mM creatinine. Intra- and inter-assay variation for NTx were 7.6% and 4.0% respectively. Serum osteocalcin (OC) was measured by competitive RIA kits (CIS Bio International, Cedex, France). OC is a marker of bone formation. The minimum detection limit was 0.1 nmol/L. Intra- and inter-assay variation for osteocalcin were 4.0% and 5.1%, respectively.

Measurement of BMD

BMD was measured in grams per square centimeter at the lumbar spine (L_1–L_4) and femoral neck using a Lunar Expert System (GE Medical Systems, Madison, WI). A single technician measured the BMD of all subjects using the identical equipment throughout the study. The *in vivo* coefficient of variation was 1.8% for the lumbar spine and 1.9% for the femoral neck. The change of BMD with time was expressed in percent change from baseline.

Statistical analysis

The changes in biochemical markers of bone turnover with time under antiresorptive therapy were evaluated by repeated measured ANOVA. Correlations between the percent change in BMD at 12 months, and the actual levels and the percent change of bone turnover markers were assessed by Pearson correlation analysis. Tests for linear trend in BMD change across tertiles of each marker were also performed.

Logistic regression was used to determine the ability of the changes in bone turnover markers to predict the spine BMD response and to compute the probability of each patient to be non-responder. Given the precision error (CV) of the DXA equipment we used in this study (around 1.8%), a change in BMD would be significant if it exceeds 1.96 $\sqrt{2}$ * CV (ie. 4.02%) at the lumbar spine. This value approximately corresponds to the cut-off value (4.05%) between upper two tertiles and the lowest tertile in our study subjects. Thus, non-responders to therapy was defined as women who belong to the lowest tertiles of rates of the percent changes in BMD at 12 months.

Results

Patients characteristics

General characteristics of the studied population and results of measurements are shown in Table 1. The mean and median of year since menopause (YSM) were 9.5 years and 10.0 years, respectively. The women on alendronate were older (56.4 years vs 60.3 years, $p<0.05$), have greater YSM (12.7 years vs. 8.3 years, $p<0.05$) and have lower BMD at both lumbar spine and femoral neck. Other characteristics did not show any significant differences between two groups.

Table 1. Characteristics of subjects at baseline

	HT	Alendronate	*P*
Number	88	50	
Age	56.4 ± 5.8	60.3 ± 8.0	<0.05
Years since menopause	8.3 ± 5.7	12.7 ± 5.8	<0.05
Body mass index (kg/m^2)	23.9 ± 3.0	23.0 ± 3.1	ns
Bone marker level			
serum osteocalcin (ng/ml)	54.7 ± 5.48	39.1 ± 4.1	ns
urine N-telopeptide (nM BCE/mM Cr)	80.0 ± 9.2	111.2 ± 19.1	ns
Bone mineral density (g/cm^3)			
lumbar	0.869 ± 0.141	0.761 ± 0.106	<0.01
femur neck	0.753 ± 0.117	0.674 ± 0.100	<0.01
T-score			
lumbar	–2.10 ± 1.17	–2.99 ± 0.90	<0.01
femur neck	–1.23 ± 0.98	–1.85 ± 0.87	<0.01

HT: hormone thearpy

Longitudinal changes in bone turnover markers and BMD

After 3 month, urinary NTx was 32.3 ± 49.3% below baseline in the treatment gruoups combined, 46.1 ± 53.8% below baseline in the alendronate group, and 25.0 ± 45.8% below baseline in the hormone group (Fig. 1). Urinary NTx decreased significantly more in women on alendronate therapy compared with women on hormone ($p<0.05$). Serum OC decreased 17.1 ± 52.8% below baseline in the treatment gruoups combined, 32.8 ± 32.3% below baseline in the alendronate group, and 10.1 ± 58.5% below baseline in the hormone group after 6 month (Fig. 1). The mean percent decrease in OC in women on alendronate was significantly more than that in women on hormone ($p<0.05$).

Following treatment with antiresorptive therapy, BMD increased by 7.8–8.5% at the lumbar spine , 2.7–3.4% at the femur neck and 4.7–8.8% at the trochanter in the two treatment groups (all $p<0.001$, Fig. 2).

Correlation between bone turnover markers and percent change in bone mineral density after antiresorptive therapy

The correlations of the actual levels and the percent changes in bone turnover markers at 3 and 6 months, with the rate of BMD changes at both lumbar spine and femoral neck were investigated (Table 2). The values of urinary NTx at baseline ($r = 0.259$, $p<0.05$) and percent change at 3 months after treatment ($r = –0.316$, $p<0.05$) were significantly correlated with the percent change of BMD at lumbar spine, whereas the urinary NTx at baseline ($r = 0.347$, $p<0.001$), percent change at 6 months in NTx ($r = –0.344$, $p<0.05$) and serum OC ($r = –0.262$, $p<0.05$) at baseline correlated with the BMD change at the femur neck.

Stratification of the levels or percent change of the biochemical markers showed that the average rate of lumbar spine BMD gain increased significantly with increasing tertiles of baseline level ($p<0.05$) and per-

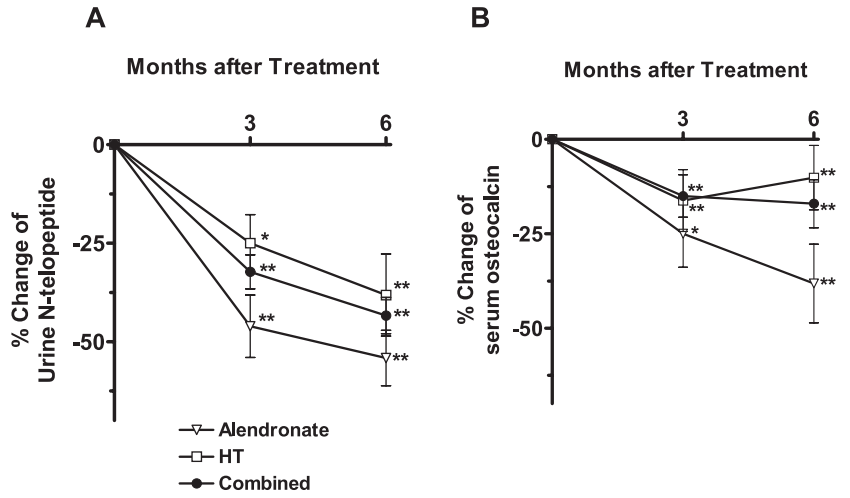

Fig. 1. Mean percent change (±SEM) from baseline in (A) urinary Cross-linked N-telopeptides of type I collagen and (B) serum osteocalcin in women receiving HT or alendronate for 12 months. *: p<0.05, **: p<0.01 from baseline.

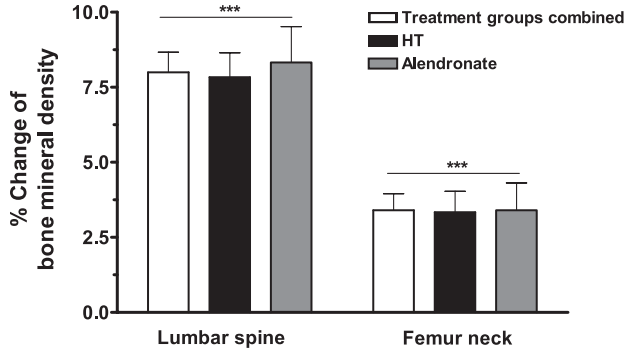

Fig. 2. Mean percent change (±SEM) from baseline in lumbar spine and femoral neck BMD after 12 months therapy with HT or alendronate. ***: p<0.001 from baseline.

cent change (p<0.05) of urinary NTx at 3 months of treatment (Table 3). The average BMD gain at femoral neck increases with increasing tertiles of percent change of urinary NTx at 6 months (p<0.05).

There were no significant differences in the background characteristics in each tertile except for YSM. Stratification of the baseline value or percent change at 3 or 6 months of serum OC levels did not show any significant differences in the change of BMD among subjects with each tertile.

Prediction of BMD gain by biochemical markers of bone turnover

According to our definition for the BMD nonresponders (described in Subjects and Methods) 33% of women from the combined HT- and alendronate-treated group were classified as BMD nonresponders. When we compared percent decreases of biochemical markers between BMD responders and nonresponders, significant decrease was observed only in NTx at 3 months (–41.2 ± 39.5% vs –11.1 ± 63.8%, p<0.05). The significant difference in NTx level at 3 months was also evident within subgroups of patients according to treatment, *i.e.*, among alendronate group (–61.2 ± 17.6% vs –21.7 ± 87.6%, p<0.05) or among hormone group (–32.52 ± 43.2% vs –2.6 ± 48.4%, p<0.05). Given the results of linear trend, we included the baseline urinary NTx level and percent change of NTx at 3 months and the combination of these two variables to discriminate BMD nonresponders from the responders in logistic regression model. Only the percent change of urinary NTx at 3 months in this model was independent predictors of BMD change at 1 year and contributed to spine BMD change variation (p<0.05, Table 4). On the other hand, for the prediction of BMD gain at the femur neck, the percent change of urinary NTx at 6 months of treatment has the discrimination power (p<0.05). We investigated the sensitivity, positive and negative predictive value of the biochemical markers to discriminate BMD nonresponder at the lumbar spine. When the cut-off value was set to provide 90% specificity, sensitivity of serum OC was very low ranging from 11.8% to 29.3% (data not shown). In contrast, urinary NTx had higher sensitivity with cut-off value of 102.8 (nM BCE/mM Cr) for baseline value and a decrease of >45.3% at 3 months of therapy (Table 5).

Table 2. Correlations of the percent changes in biochemical markers with the percent change in BMD at 12 months for women on hormone and alendronate therapy

BMD site	Basal		Percent change			
			3 months		6 months	
	NTx	OC	NTx	OC	NTx	OC
Lumbar spine						
Treatment group combined	0.259[a]	0.109	–0.316[a]	–0.111	–0.024	–0.075
ALN group	0.362[a]	0.054	–0.244	–0.253	0.011	0.319
HT group	0.170	0.137	–0.289[a]	0.010	–0.039	–0.262
Femur neck						
Treatment group combined	0.347[c]	0.262[b]	–0.150	–0.008	–0.344[a]	–0.260[a]
ALN group	0.209	0.048	–0.019	–0.146	–0.376[a]	–0.135
HT group	0.451[c]	0.328[b]	–0.240	0.036	–0.298	–0.304[a]

ALN, alendronate, HT, hormone therapy, NTx, Cross-linked N-telopeptides of type I collagen; OC, osteocalcin.
[a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$

Table 3. Lumbar spine and femur neck BMD change according to tertiles of actual values and percent changes in bone turnover markers in postmenopausal women treated with antiresorptive therapy

		Lumbar spine BMD change (%)				Femur Neck BMD change (%)			
		Lowest T	Middle T	Highest T	*p*	Lowest T	Middle T	Highest T	*p*
Actual values									
NTx	basal	5.434 ± 1.413	8.442 ± 1.195	11.029 ± 1.538	0.020	1.352 ± 1.195	2.862 ± 0.959	5.659 ± 1.622	0.060
	3 months	6.464 ± 1.755	7.841 ± 1.674	11.886 ± 2.185	0.121	2.707 ± 1.022	2.833 ± 1.028	4.899 ± 2.367	0.545
	6 months	6.024 ± 1.474	9.673 ± 1.525	10.698 ± 2.532	0.204	2.996 ± 1.412	0.212 ± 2.142	4.725 ± 2.855	0.344
OC	basal	7.195 ± 1.141	8.494 ± 1.203.	8.543 ± 1.175	0.649	1.788 ± 0.852	2.082 ± 1.169	3.298 ± 1.213	0.582
	3 months	7.795 ± 1.915	7.019 ± 1.645	9.640 ± 1.506	0.542	1.464 ± 1.171	2.660 ± 1.337	5.911 ± 1.878	0.097
	6 months	6.866 ± 1.334	7.902 ± 1.159	9.104 ± 2.003	0.590	1.862 ± 1.383	2.058 ± 1.232	1.288 ± 1.955	0.936
Percent changes									
NTx	3 months	4.298 ± 2.196	9.523 ± 1.648	12.122 ± 1.555	0.012	1.297 ± 1.189	4.469 ± 1.650	4.436 ± 1.741	0.258
	6 months	8.324 ± 2.711	10.572 ± 1.554	8.546 ± 1.787	0.692	–1.135 ± 1.412	2.210 ± 1.652	7.355 ± 2.874	0.022
OC	3 months	7.246 ± 1.843	8.362 ± 1.549	8.804 ± 1.734	0.803	2.815 ± 1.679	4.327 ± 1.336	2.902 ± 1.549	0.743
	6 months	7.753 ± 1.386	7.418 ± 1.707	8.933 ± 1.511	0.765	–0.966 ± 1.061	3.500 ± 1.317	2.974 ± 2.004	0.076

NTx, Cross-linked N-telopeptides of type I collagen; OC, osteocalcin

Table 4. Percent changes of bone markers as independent predictors of BMD change at 1 year in a logistic regression model

Predictors (independent variable)	Regression coefficient (±SE)	Correlation coefficient	significance (*p* value)
Spine			
Percent change of NTx at 3 months	–0.016 ± 0.006	–0.367	0.010
Femur neck			
Percent change of NTx at 6 months	–0.020 ± 0.009	–0.343	0.038

NTx, Cross-linked N-telopeptides of type I collagen

Table 5. Sensitivity and positive and negative predictive values of N-telopeptide % change at 3 months to predict 1 year lumbar spine bone mineral density (BMD) response for a selected 90% specificity

	BMD response			
	Cutoff for 90% specificity	sensitivity (%)	PPV(%)[a]	NPV(%)[b]
Basal NTx level	≥102.8 nM BCE/mM Cr	32.8	73.1	43.5
NTx % change at 3 months	≤–45.3%	61.5	87.5	54.5

NTx, Cross-linked N-telopeptides of type I collagen
[a]: positive predictive value [b]: negative predictive value

Discussion

In this study, we have demonstrated that both the baseline value and early change in bone turnover, as reflected by urinary NTx level, after antiresorptive therapy could predict a significant increase in BMD at the spine and femur neck at 12 months of therapy. Changes in serum OC at 6 months correlated with, albeit less strong than that of urinary NTx, the percent change in femur neck BMD.

Our study shows that the urinary NTx, a marker of resorption, rapidly decreased at 3 months with average reduction of 32.3%, whereas serum OC, a marker for bone formation, gradually decreased during 6 months with average 17.1%. The delayed response of serum OC reflects the physiologic coupling of bone formation to resorption and shows good agreement with previous observations [14, 15, 17]. We found the trends in NTx and OC to be similar. That is, the women who were on alendronate had a significantly greater decrease in NTx and OC than the women on hormone therapy.

We also found that baseline levels of urinary NTx significantly correlated with the rate of BMD gain at both lumbar spine and femur neck at 12 months of therapy, whereas baseline serum OC levels correlated with BMD gain at the femur neck after treatment with antiresorptive agents. These results indicate that the higher the bone turnover at baseline, the more bone mass will be gained after treatment with antiresorptive agents, confirming the results of earlier studies [14, 15]. In addition to the baseline values, percent change of NTx at 3 months and 6 months correlated with percent change of BMD at the lumbar spine and femur neck, respectively, suggesting that early change in bone turnover rate can also predict the BMD change at 1 year post-treatment. These results are also in line with previous observations, which identified the value of decreased bone turnover markers as a predictor for response of anti-resorptive agents [9–13].

To examine further the predictive value of biochemical markers, we stratified the levels or the percent changes of biochemical markers into tertiles and analyzed differences in the rate of BMD gain at 12 months of treatment. We found a strong relationship between increasing tertiles of baseline urinary NTx and the rate of spinal BMD gain, with a difference between highest and lowest tertiles of 5.6%. Moreover, when participants on treatment were separated into three tertiles based on decreases in NTx at 3 months and 6 months, those (in the tertiles) with the largest decreases in NTx had the greatest gain in BMD at lumbar and femur neck, respectively. Although we were able to note similar trends for all women on treatment, the response to alendronate was generally associated with greater reductions in NTx than the response to HT. In terms of BMD response, the reduction of the urinary NTx level at 3 months was significantly greater in BMD responders than that in nonresponders. Indeed we found that women with greater change in urinary NTx level after 3 months of treatment gained 2.8 times more bone compared with low turnover individuals. Using a logistic regression model that included both urinary NTx level at baseline and percent NTx change at 3 months, we found that the percent change of NTx at 3 month is the most significant and independent predictors of BMD response. Therefore, biochemical markers of bone turnover reflects bone turnover rate as well as treatment efficacy to normalize it, as an independent predictor of BMD response. The baseline urinary NTx levels in the highest tertile (91.1 ± 8.9 nM BCE/mM Cr) in this study were more than twice the average premenopausal concentrations (35.0 nM BCE/mM Cr) or two SDs greater than the premenopausal values (46.7 nM BCE/mM Cr) in Korean women [18]. Thus, postmenopausal women who had values of the biochemical markers outside the normal range for premenopausal women may be those experiencing a greater rate of bone gain over 12 months. However, in clinical practice, identifying nonresponder to antiresorptive therapy using a single cut-off value would be more meaningful. In the present investigation, we have suggested cut-off values for urinary NTx values at 3 months after treatment to provide 90% specificity. Although further studies will be needed to achieve consensus on optimal cut-off levels in Koreans, with the corresponding sensitivity of 61.5% and the estimated positive and negative predictive values of 87.5% and 54.5%, respectively, these figures should provide adequate reference data for routine clinical settings.

In our study, the baseline values and percent change of serum OC showed correlation only with femur neck BMD change, and the tertile analysis of serum OC failed to predict the future BMD response. The reason for this weak power is not clear. It may be explained by the fact OC can reflect bone turnover only when resorption and formation are coupled. If for some reason formation and resorption are uncoupled, the change of OC cannot be interpreted as a change in bone turnover.

It may also result from the difference in the assay method for the measurement of OC. ELISA procedure of OC measurement used in this study detects intact OC alone, whereas the radioimmunoassay (RIA) technique in the other studies measures both intact and N-terminal fragments accounting for the discrepancies reported in the literature [19, 20].

There were several limitations to this study. First, although this study was conducted in a prospective design, placebo group was not included who may have reflected noncompliant patients in clinical practice. Second, the study subjects are rather heterogenous including women receiving both HT and alendronate and consisting of women with 4 months to 17 years since natural menopause. Third, the precision error of bone mass measurement by our DXA was relatively high, resulting in a relatively high cut-off value to discriminate the nonresponders. Indeed the non-responders defined in this study did gain some BMD within the precision error range, and thus, are better to be called as poor responders. Finally, although we determined the value of early change in biochemical markers as predictors of BMD response to treatment, accumulating evidence suggest that increase in BMD with treatment could only partially account for the reduction of in the osteoporotic fracture risk [21–23]. Therefore, the changes in BMD are just a surrogate of anti-fracture efficacy of osteoporotic therapy. Whether the changes in bone turnover markers could be the determinant for future fracture risk independent of BMD changes will be the challenges for future research.

In conclusion, we have demonstrated that biochemical markers of bone turnover can be used to determine BMD response to antiresorptive therapy in Korean postmenopausal women with osteoporosis. In addition, we developed a model using the logistic percent change of urinary NTx after short-term treatment period to identify patients who will subsequently demonstrate a poor BMD response. Verification of our data by large-scaled longitudinal studies will lead to better utilization of selected biochemical markers to guide practice for postmenopausal women with osteoporosis.

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