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Correlation of new bone metabolic markers with conventional biomarkers in hemodialysis patients

Abstract

Background: New bone metabolic markers have become available clinically for evaluating chronic kidney disease mineral and bone disorders (CKD-MBD). The aim of this study was to correlate these new bone metabolic markers with conventional markers in regular hemodialysis (HD) patients.

Methods: One hundred forty three HD patients underwent cross-sectional assessment. Two bone formation markers, bone-specific alkaline phosphatase (BAP) and osteocalcin (OC), and one bone resorption marker, amino-terminal telopeptides of type 1 collagen (NTx), were selected for study.

Results: Both circulating OC and NTx levels showed positive correlations with serum intact parathyroid hormone (iPTH) levels. The levels of NTx and OC showed a strongly positive correlation, although they are known to be markers of different aspects of bone metabolism: bone formation and resorption. Patients with high iPTH (≥ 300 pg/mL) had significantly higher levels of all the three bone markers compared with patients with low or normal iPTH.

Conclusion: Serum OC and NTx levels may be useful markers of serum iPTH levels for evaluating bone turnover in HD patients and may eventually prove useful in the management of patients with CKD-MBD.

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Dysregulation of mineral and bone metabolism occurs frequently in chronic kidney disease (CKD), causing increased risk of cardiovascular disease (CVD) [1,2]. High CVD morbidity and mortality are observed to link with hyperphosphatemia, the calcium-phosphorus product ($\text{Ca} \times \text{P}$) and secondary hyperparathyroidism (SHPT) [3,4]. The Kidney Disease Outcomes Quality Initiative (K/DOQI) of the US National Kidney Foundation introduced practice guidelines for bone metabolism and disease in CKD, recommending tight control of serum calcium (Ca), phosphorus (P), $\text{Ca} \times \text{P}$ and intact parathyroid hormone (iPTH) levels [5]. In addition to the role these markers play in bone metabolism, they also contribute to the progression of vascular calcification, leading to high mortality by CVD in hemodialysis (HD) patients [3,4,6]. The mechanisms of CKD mineral and bone disorders (CKD-MBD) are not fully understood; both traditional and nontraditional CKD and CVD risk factors are thought to contribute to CKD-MBD.

Bone remodeling is an ongoing cyclical process and is characterized by two different activities: bone formation and bone resorption. Clinically, several different biochemical markers are used to diagnose CKD-MBD, make follow-up of HD patients and initiate appropriate treatment. Several studies have shown that bone formation and bone resorption markers are well correlated with progression of CKD-MBD in HD patients [7]. Among those markers, the most frequently used is bone-specific alkaline phosphatase (BAP). The increased levels of iPTH are not always synonymous with high turnover bone disease, probably because iPTH secretion is dependent on serum Ca and P levels and because iPTH is not a direct marker of bone remodeling. Serum BAP concentration is reportedly more sensitive than iPTH and osteocalcin (OC) in distinguishing the type of bone disease and estimating bone formation rate [8]. It is unclear whether nontraditional markers for bone remodeling are more useful than traditional markers for better maintenance of CKD-MBD in HD population.

To clarify the issue, new bone metabolic markers were correlated with conventional markers for KD-MBD. Two bone formation markers, BAP and OC, and one bone resorption marker, amino-terminal telopeptides of type 1 collagen (NTx), were measured. Serum levels of the new markers were also compared in two groups that were divided by serum iPTH levels, according the K/DOQI guidelines.

Patients and Methods

Subjects

The cross-sectional study enrolled 143 patients who were all undergoing HD therapy in our center. The causes of CKD included diabetes mellitus (37.8%), renal sclerosis (2.8%) and chronic glomerulonephritis (52.4%). An additional thirteen patients were excluded from the study, either because they were treated with more than one blood purification therapy during the three month regime or because they were medically unstable.

The patients were treated with stable HD therapy over a three month period, using the same type of membrane and the same dialysis procedure. HD was performed with single-use hollow-fiber dialyzers equipped with modified cellulose-based or polysulfone membranes. Maintenance HD was carried out for 3-4 h each session, three times a week with a blood flow rate of 150-220 ml/min and with a dialysate flow rate of 500 ml/min. Dialysate contained Na^+ (140 mmol/l), K^+ (2.0 mmol/l), Ca^{2+} (1.5 mmol/l), and HCO_3^- (30 mmol/l). Ninety-one patients were treated with anti-hypertensive medications including angiotensin-converting enzyme inhibitors (16 patients), angiotensin receptor blockers (56 patients), calcium antagonists (51 patients), β -blockers (13 patients), α -blockers (7 patients), and diuretics (19 patients). $1\alpha(\text{OH})\text{D}_3$ was used in 52 participants. The study was approved by all local medical ethics committees and all patients gave informed consent before inclusion.

Data collection

Hematological and biochemical blood samples were collected after at least 10 h of fasting just prior to the first HD session of week and tested at the laboratory at our center. The levels of BAP, OC and NTx were measured by enzyme immunoassay, immunoradiometric assay and enzyme-linked immunosorbent assay, respectively, (SRL, Inc., Tokyo, Japan) [9-11]. Blood pressure (BP) was measured hourly during HD session using a mercury sphygmomanometer. Mean levels of systolic and diastolic BP were determined as the means of measurements obtained in eight different midweek HD sessions in which patients showed essentially the same increase in body weight [12].

Statistical Analysis

All the results are showed as mean $\pm$ standard deviation (SD). Two sided *t*-tests and Mann-Whitney U analyses were used to compare parametric and non-parametric data, respectively. The data were expressed as mean values $\pm$ SD. Analyses exploring

TABLE 1. Characteristics of patients undergoing dialysis treatment (n=143)

Age (year)	55.3±14.6
Gender (M:F)	59:84
Duration of treatment (month)	153.4±120.1
Hemoglobin (g/dl)	10.1±1.0
Albumin (g/dl)	3.7±0.3
Alkaline phosphatase (IU/l)	268.3±142.7
Blood urea nitrogen (mg/dl)	69.5±14.1
Creatinine (mg/dl)	12.9±9.4
Calcium (mg/dl)	9.1±0.8
Phosphorus (mg/dl)	5.5±1.4
Calcium-phosphorus product (mg ² /dl ²)	50.9±13.6
Cholesterol (mg/dl)	152.4±34.1
Triglyceride (mg/dl)	100.5±55.7
C reactive protein (mg/dl)	0.27±0.59
Hypertension (%)	62.9
Diabetes (%)	20.3
Vitamine D use (%)	36.4
Phosphorus binder use (%)	81.8
Cinacalcet use (%)	0
Statin use (%)	9.8
ACEI/ARB use (%)	11.2/39.2

ACEI:Angiotensin convert enzyme inhibitor, ARB:Angiotensin receptor blocker

the relationship between PDV levels and variables were performed using the Pearson correlation test. A *P*-value of <0.05 was considered statistically significant.

Results

Table 1 shows characteristics of the participants of the study. Serum levels of intact PTH showed no significant correlation with serum levels of Ca, P or Ca × P, indicating that the circulating level of iPTH is an independent factor from the three variants in the study (Fig. 1). Next, the degree of correlation between the four bone turnover markers, Ca, P, Ca × P and iPTH, and the four bone turnover markers was evaluated. Neither alkaline phosphatase (ALP) nor BAP showed a strong correlation with the bone turnover markers (Table 2). The serum levels of NTx and OC showed relatively good correlation with the serum levels of iPTH, but no correlation with the serum levels of Ca, P or the Ca × P (Fig. 2). These data suggest

FIGURE 1. Relationship between circulating intact parathyroid hormone (iPTH) and serum calcium (Ca), phosphorus (P) and calcium-phosphorus product (Ca × P) levels in uremic patients before dialysis treatment.

FIGURE 2. Relationship between circulating intact parathyroid hormone (iPTH) and serum amino-terminal telopeptides of type 1 collagen (NTx), osteocalcin (OC) and bone-specific alkaline phosphatase (BAP) levels in uremic patients before dialysis treatment.

that the circulating levels of NTx and OC could give additional information about SHPT in CKD population under HD therapy.

The new bone turnover markers were then evaluated to determine whether they changed independently. The levels of NTx and OC have a strong positive correlation, although they are markers of different processes: bone formation and resorption, respectively (Table 2). The levels of ALP show a positive correlation only with the serum levels of BAP but not with

TABLE 2. Correlation among four bone remodeling markers (*R*²)

	iPTH	ALP	BAP	NTx	OC
iPTH	1.000				
ALP	0.035	1.000			
BAP	0.073	0.309	1.000		
NTx	0.336	0.109	0.184	1.000	
OC	0.370	0.072	0.169	0.722	1.000

TABLE 3. Comparison of serum bone remodeling markers between two groups of dialysis patients with low or high levels of intact PTH

	low iPTH levels (n=100)	high iPTH levels (n=43)	p value
Age (year)	55.4±1.4	55.1±16.9	0.91
Gender (M:F)	58:42	26:17	0.78
Duration of treatment (month)	172.0±123.2	110.1±101.4	0.0044
Hemoglobin (g/dl)	10.0±0.9	10.3±1.1	0.05
Albumin (g/dl)	3.7±0.3	3.7±0.4	0.81
Alkaline phosphatase (IU/l)	69.2±137.8	205.9±148.1	0.038
Blood urea nitrogen (mg/dl)	69.2±1.4	70.4±2.2	0.65
Creatinine (mg/dl)	12.7±9.8	113.5±8.5	0.64
Calcium (mg/dl)	9.3±0.8	9.4±0.8	0.64
Phosphorus (mg/dl)	5.2±1.3	6.1±1.5	0.0004
Calcium-phosphorus product (mg ² /dl ²)	48.3±12.3	57.1±14.8	0.0003
Cholesterol (mg/dl)	151.2±34.4	155.3±33.7	0.51
Triglyceride (mg/dl)	99.4±52.7	103.2±62.6	0.71
C reactive protein (mg/dl)	0.27±0.66	0.27±0.39	0.97
Hypertension (%)	64	60.5	0.69
Diabetes (%)	20	20.9	0.9

Low iPTH levels: <300 pg/ml; high iPTH levels: >300 pg/ml

NTx or OC (Table 2). Serum BAP levels show weak, positive correlation with serum NTx and OC levels (Table 2). These results suggest that a combination of serum OC and NTx levels could give useful information about CKD-MBD in HD patients and that the imbalance of serum NTx and OC levels may reflect the pathological status of bone metabolism in HD patients even though the molecules are markers of different bone metabolic/catabolic processes.

The K/DOQI guidelines recommends that serum levels of iPTH should be controlled to within 150-300 pg/mL in HD patients [5]. To determine whether the three bone markers show significant differences between two groups, HD patients were divided into two groups according to their serum iPTH levels (Table 3). The high iPTH group shows significantly higher levels of the three bone markers compared with the low

or normal iPTH group (Table 4). Both bone formation and resorption markers show the same tendency: that the high iPTH group exhibits higher levels of two bone markers than the low iPTH group. This suggests that the three bone markers are as useful indicator of SHPT as the conventional markers such as ALP and iPTH.

Discussion

The correlation among four new mineral bone markers and conventionally used markers was examined in HD patients at a single center. A recent study indicated that therapeutic interventions associated with excessive lowering of parathyroid activity favor low bone turnover or adynamic bone disease, in turn leading to the progression of arterial calcification [13]. Serum levels of ALP usually increase in CKD-MBD, especially in high-turnover bone disease [14,15]. Although serum iPTH levels are frequently used as a marker for bone remodeling, serum ALP seems a more reliable marker of severity of the high-turnover bone disease than increased iPTH levels, because circulating serum ALP originates directly from pathologic bone; however, Consistent with this notion, Palmer *et al.* reported that treatment of CKD-MBD using vitamin D analogs can effectively decrease serum levels of ALP, even though such a treatment does not consistently decrease serum PTH levels [16]. In spite of the usefulness of ALP in high-turnover

TABLE 4. Comparison of serum bone turnover markers between HD patients with low or high serum iPTH levels

	low iPTH levels (n=100)	high iPTH levels (n=43)	p value
ALP (IU/l)	252±137.8	305.9±148.2	0.038
BAP (IU/l)	24.7±12.8	33.7±16.7	0.0006
NTx (nmolBCE/l)	76.5±58.4	183.1±121.8	<0.0001
OC (ng/ml)	63.8±52.0	166.7±111.6	<0.0001

Low iPTH levels: <300 pg/ml; high iPTH levels: >300 pg/ml

bone disease, there appears to be a requirement for novel bone markers to evaluate low-turnover or adynamic bone disease. Newly available bone markers have little information regarding their usefulness for evaluating CKD-MBD, even though there are many publications about the properties of each marker. There are also few reports examining the relationships among the various markers. Three compounds, BAP, NTx and OC, have been selected for this study because of their potential as markers of bone metabolism/disease. An added advantage to studying these particular compounds is that their measurement is currently covered under Japan's national health insurance system.

BAP is known as a stable molecule, not influenced by either renal dysfunction or the HD procedure because it is exclusively metabolized in the liver. Serum BAP concentration alone provides useful information about the rate of bone remodeling in HD patients [17]. Combining measurement of both serum iPTH and BAP levels improves the sensitivity, specificity, and predictive value in the diagnosis of the type of bone turnover [14]. With high values of serum BAP or iPTH, there is little correlation between these two markers even when serum iPTH levels are 100 pg/ml [8]. Our data suggest that circulating OC and NTx levels could be more strongly correlated with serum iPTH levels than with serum BAP levels. In other words, BAP may not be suitable for diagnosing SHPT. Our data suggest that serum OC and NTx levels could be more strongly correlated with serum iPTH even when iPTH levels are relatively high. One explanation is that BAP is more suitable marker for the adynamic status of bone; thus, a dissociation between serum iPTH and BAP levels becomes clear in high-turnover bone disease [18]. Our data are not totally consistent with a previous report from Takahashi *et al.* [7], in which serum BAP levels are more strongly correlated with NTx than serum PTH levels. Both studies were performed as cross-sectional studies, but characteristics of HD patients were quite different, especially with respect to the duration of HD and age of participants. The characteristics of HD patients may affect the status of CKD-MBD, leading to different levels of circulating bone markers. Bone mineral density is used to evaluate bone quality, but bone mineral density is unsuitable as an index for assessing the effect of therapeutic intervention to treat CKD-MBD because, although the gold standard for evaluating the state of bone metabolism is bone biopsy, repeated biopsies are inadvisable because they are invasive. A combination of these markers could be an adequate index for monitoring bone metabolism. The combination of OC and NTx could give precise information about bone metabolism because serum levels of these two markers showed more significant correlation with increase of

serum PTH in HD patients. Another new finding of this study is that many HD patients show good correlation between bone formation and resorption markers even when the patients were treated with $1\alpha(\text{OH})\text{D}_3$ or phosphorus binders. Imbalance of these two markers could be a suggestion of pathological state of bone metabolism and warrants further study.

The main limitation of the study is its cross-sectional design. Another important limitation of the study is the lack of bone biopsies, which are only performed in specialized centers. The strength of the study is the uniform laboratory measurements, with all laboratory data obtained from a single facility.

In conclusion, serum OC and NTx levels are here shown to be as reliable as iPTH or BAP levels for evaluation of bone turnover in HD patients. Serum OC and NTx levels show good, positive correlation even though they are markers for different aspects of bone metabolism (bone formation and bone resorption). Therapeutic interventions in CKD-MBD could alter the serum levels of these biomarkers, and such effects need to be shown in future clinical trials.

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