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Usefulness of bone resorption markers in hemodialysis patients

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ABSTRACT

Although bone biopsy is a golden standard in the diagnosis of renal osteodystrophy, it is invasive and repetitive evaluation of bone status by this method is practically impossible. Non-invasive evaluation by bone metabolic markers such as serum cross-linked N-telopeptide of type I collagen (NTX) might give us additional information which cannot be obtained only by measurements of parathyroid hormone (PTH). These days, bone resorption marker, serum tartrate-resistant acid phosphatase (TRAP5b) was reported to be correlated with histomorphometric parameters of bone resorption in a bone biopsy study enrolling hemodialysis (HD) patients. In the current study, we studied the correlation of sera NTX and TRAP5b with bone mineral density (BMD) of second metacarpal bone in 103 HD patients and serial changes of these markers just after parathyroidectomy (PTX). Sera NTX and TRAP5b showed a significant positive relationship ($R = 0.79$ $P < 0.0001$). Simple regression analyses showed that both markers were significantly associated with BMD *Z*-score, whereas 1-84 PTH showed no significant association at all. This result might be attributed to diverse difference among HD patients in skeletal sensitivity to endogenous PTH. In fact, the ratios of TRAP5b/PTH and NTX/PTH denoting PTH sensitivity were significantly higher in postmenopausal female than male patients. Female gender, hemodialysis vintage, and log(PTH) were found to be significant positive determinants of serum log(TRAP5b) by multiple linear regression analysis. Receiver operating curve analysis for young adult mean 70% revealed that sensitivity was more than 95% when the cut-off value was set at 56.5 nmolBCE/L and 340 mU/L, but the specificity was only 29.5% and 34.4% for NTX and TRAP5b, respectively. Multiple regression analysis revealed that both markers were independent determinants of *Z*-score in two models including respective marker. Serial changes of both markers after PTX in 2 HD patients were comparable with gradual decrease of these markers. However, the reduction was sooner and larger in serum NTX. This sooner reduction might be attributed to the difference in characteristics of these markers regarding the effect of single HD session. Whereas serum NTX decreased, TRAP5b increased significantly after single session. Taken together, both markers give us additional information that cannot be obtained just by using PTH, provided that we know the characteristics of these markers.

Introduction

Although bone biopsy is a golden standard of evaluating the bone remodeling status in renal osteodystrophy [1], bone biopsy is really invasive and repetitive evaluation by this method is impractical. From this point of view, some bone chemical markers to detect bone remodeling status in chronic kidney disease (CKD) has been required in clinical setting. Regarding bone formation markers, bone specific alkaline phosphatase (BSAP) is among established bone metabolic markers in CKD-mineral bone disorders (CKD-MBD) [2]. Serum BSAP is not interfered by renal function [3] and can be used as a surrogate for bone turnover in CKD-MBD. Some literature provided evidence that serum BSAP was correlated with bone formation rate than with either total alkaline phosphatase or intact parathyroid hormone (iPTH) in bone biopsy [4]. These days, it was found that elevations of normalized alkaline phosphatase were significantly associated with increased fractures, parathyroidectomy (PTX), risk of hospitalization due to major adverse cardiac events, higher all-cause cardiovascular, and infection-related mortality risk [5]. Moreover another report also confirmed that high levels of serum alkaline phosphatase especially >120 U/L was associated with associated with mortality [6].

However, there are few reports of the usefulness of bone resorption markers in CKD patients including hemodialysis (HD) patients. Maeno et al. revealed that serum cross-linked N-telopeptide of type I collagen (NTX), breakdown product of bone collagen was a useful resorption marker to predict annul decrease of bone mineral density in the radius in HD patients [7]. Recently, a bone biopsy study

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reported that serum NTX was one of the useful markers to detect adynamic bone disease (ABD) in 218 HD patients, of whom 74% had ABD [8]. They reported that sensitivity and specificity was 64% and 78%, respectively when the cut-off of NTX was set at 113 nmolBCE/L. Nevertheless, this study is still a preliminary study which needed to be confirmed. Moreover, NTX is secreted in urine, therefore, it is accumulated in CKD patients with decreased estimated glomerular filtration rate (GFR), leading to difficult interpretation of this marker in renal dysfunction. To eliminate the effect of the accumulation by impaired kidney function, we previously advocated the idea of *resorption index* to know the bone resorption status in CKD patients receiving glucocorticoid [9]. Resorption index decreased significantly by bisphosphonate therapy with no change of serum BSAP in early phase of this therapy.

In this context, some bone resorption marker which does not accumulate even in patients with renal dysfunction is required. Serum type 5b tartrate-resistant acid phosphatase (TRAP5b) is such a candidate to detect bone resorption status. It is secreted into the circulation from activated osteoclasts [10,11]. Yamada S, et al. revealed recently that this marker was not affected by kidney function just like serum BSAP [12]. There is a cross-sectional bone biopsy study confirming the correlation of this marker with bone resorption status in HD patients [13]. In this study they found that correlation between TRAP5b and histological parameters of osteoclasts were stronger than those of iPTH and other markers. However, there are few studies investigating longitudinal serial change of this marker after some intervention in HD patients.

In this cross-sectional and longitudinal study, we compared the usefulness of these bone resorption markers to detect bone loss and characteristics of these markers.

Methods and subjects

In cross-sectional study, we enrolled 103 HD patients. Blood samples were drawn before hemodialysis session from patients in Daini-Rokushima Clinic. We centrifuged the blood at room temperature at 3000 rpm for 5 min, with the serum then stored at −80 °C until analysis. Blood chemistry [serum creatinine, albumin (Alb), calcium (Ca), and phosphate] was measured using standard automated techniques. We also measured BSAP, 1-84 PTH, iPTH, osteocalcin (OC). Full length 1-84 PTH and iPTH were measured using a third-generation assay (whole PTH, Scantibodies, Santee, CA, USA) and second-generation Elecsys **PTH** assay (intact-PTH **ROCHE**). BSAP was assayed by using the Osteolinks-Bone ALP high-sensitivity diagnostic enzyme immunoassay (EIA) kit (Sumitomo Pharmaceuticals, Co., Osaka, Japan). The levels of serum NTX was measured by using an ELIZA kit (OSTEOMARK; Mochida pharmaceutical Co., Tokyo, Japan). TRAP5b activity was measured by a fragment absorbed immunocapture enzymatic assay (FAICEA) method using Osteolinks "TRAP-5b" (Nitto Boseki, Fukushima, Japan) [14]. The serum Ca level was corrected for Alb by the formula (S–Ca; serum corrected Ca = Ca + (4 − Alb), if Alb < 4.0 g/dL) [15].

Bone mineral density (BMD) of the second metacarpal bone was measured using a digital image processing (DIP) in every patient. This DIP method measures BMD and cortical thickness at the middle of the second metacarpal bone on a radiogram of the hand and an aluminum slope as a standard. We employed this method because the DIP method is inexpensive and prevalent method in Japan to detect BMD [16] and the data obtained by this method has a good correlation with data obtained by the dual-energy X-rays absorptiometry (DEXA) [17]. Receiver operating curve (ROC) analyses for YAM < 70% were performed for sera NTX and TRAP5b. We investigated the relationship between these bone turnover markers, percents of young adult mean (%YAM) and sex- and age-adjusted *Z*-score in percents. We also calculated normalized protein catabolic rate (nPCR) and dialysis dose in Kt/V in every patient.

In longitudinal study, we enrolled 2 female HD patients (63 and 59 years old), who underwent parathyroidectomy (PTX) with auto-transplantation of parathyroid in the upper arm. We measured sera NTX and TRAP5b longitudinally until 8 days after PTX. The timing of measurement included ones just before and after HD session. In observational period, serum ionized calcium was strictly controlled in normal ranges by adjusting the intravenous infusion rates of calcium gluconate after surgery including hemodialysis session. Serum PTH level was not detectable 2 days after surgery.

Statistical analyses

Variables with non-normal distribution (BSAP, iPTH, 1-84 PTH, NTX, TRAP5b and Osteocalcin) were logarithmically transformed prior to analyses. Multiple regression analyses were performed by setting BMD *Z*-score as a dependent variable. We forced potential variables into multivariate analysis that were significantly associated with *Z*-score in simple regression analyses. Independent variables where the multicollinearity was present were not included at the same time in a multivariate analysis, but to compare the usefulness of these bone resorption markers with that of 1-84 PTH, 1-84 PTH were included in potential explanatory variables. As a result, we created 2 models in multiple regression analyses. All models were adjusted by the presence of diabetes mellitus and vitamin D use. Multiple regression analyses for TRAP5b were also performed in the cohort excluding premenopausal patients. We employed Wilcoxon signed-ranks test to compare between pre-dialysis and post-dialysis parameters. Statistical tests were two-sided, and *P*-values less than 0.05 were considered statistically significant. All analyses were performed with JMP ver. 7.0.1J for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Cross-sectional study

Clinical characteristic including laboratory data of HD patients were depicted in Table 1. Fifty percent of total patients had intact PTH < 150 pg/mL and 40% of patients had 150 < iPTH < 300 pg/mL and 11% of patients had iPTH greater than 300 pg/mL. Sera NTX and TRAP5b showed a significant positive relationship ($r = 0.79$, $P < 0.0001$, Fig. 1A). Simple regression analysis showed that both markers were significantly associated with BMD (absolute value, % YAM and BMD *Z*-score, whereas neither 1-84 PTH nor intact PTH showed significant association with them (Table 2), despite the fact

Table 1
The characteristics of enrolled patients in cross-sectional study.

Gender	Male = 52, female = 51
Age (year)	68 [23–88]
BW (kg)	52.5 [29.8–70.1]
Hemodialysis vintage (year)	4.8 [0.5–29.6]
Percents of diabetes mellitus	39.8%
Percents of vitamin D users	60.2%
Alb (g/dL)	3.90 ± 0.390
corrected Ca (mg/dL)	9.38 ± 0.654
P (mg/dL)	5.50 ± 1.255
Intact-PTH (pg/mL)	159.0 [8.0–994]
1-84 PTH (pg/mL)	78.3 [3.78–637]
BSAP (U/L)	31.7 [10.9–85.6]
Osteocalcin (ng/mL)	39.0 [8.6–300]
Serum NTX (nmolBCE/L)	96.8 [16.0–1170]
TRAP5b (U/L)	5.2 [1.3–28.1]
Bone mineral density (mmAl)	2.16 ± 0.509
Young adult mean (%)	74.8 ± 17.3
Z-score (%)	82.6 ± 13.4