

Diagnostic Value of TRAP 5b Activity in Postmenopausal Osteoporosis

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Abstract

Objective: The aim of the study was to investigate whether tartrate resistant acid phosphatase 5b (TRAP 5b) could be used as a bone turnover marker in postmenopausal osteoporotic women.

Materials and Methods: The study population included 36 postmenopausal osteoporotic, 33 postmenopausal non-osteoporotic and 27 premenopausal non-osteoporotic subjects. TRAP 5b, total alkaline phosphatase (ALP), C-telopeptide, osteocalcin and urinary deoxypyridinoline levels were determined. TRAP 5b was measured by solid phase immunofixed enzyme activity assay.

Results: C-telopeptide and urinary deoxypyridinoline levels were significantly different in postmenopausal osteoporotic women compared with postmenopausal and premenopausal non-osteoporotic women. Total ALP and osteocalcin levels were significantly higher in postmenopausal osteoporotic patients than in premenopausal non-osteoporotic group, but there were no differences in total ALP and osteocalcin levels between postmenopausal osteoporotic and non-osteoporotic groups. TRAP 5b was not significantly different between the groups.

Discussion: Although TRAP 5b is a lysosomal enzyme secreted by activated osteoclasts, it has been evaluated that its enzymatic activity did not increase in postmenopausal osteoporotic women. The study suggested that it could not be used as a diagnostic test in slow turnover diseases like postmenopausal osteoporosis.

Keywords: postmenopausal osteoporosis, tartrate resistant acid phosphatase type 5, diagnostic techniques

Özet

Postmenopozal Osteoporozda TRAP 5b Aktivitesinin Tanısal Değeri

Amaç: Çalışmamızın amacı, tartrat rezistant asit fosfataz 5b (TRAP 5b) nin postmenopozal osteoporozlu hastalarda kemik yapım-yıkım markeri olarak kullanımını araştırmaktır.

Materyal ve Metot: 36 postmenopozal osteoporozlu, 33 postmenopozal osteoporozu olmayan ve 27 premenopozal osteoporozu olmayan olgu çalışma kapsamına alındı. TRAP 5b, total alkalin fosfataz (ALP), C-telopeptit, osteokalsin ve üriner deokspiridinolin seviyelerine bakıldı. TRAP 5b solid faz immunofiks enzim aktivitesi yöntemi ile ölçüldü.

Sonuç: C-telopeptit, ve üriner deokspiridinolin seviyeleri postmenopozal osteoporozlu kadınlarda postmenopozal ve premenopozal osteoporozu olmayan grupla karşılaştırıldığında istatistiksel olarak anlamlılık göstermekteydi. Total ALP, osteokalsin düzeyleri postmenopozal osteoporozu olan grupta premenopozal osteoporozu olmayan grupla karşılaştırıldığında istatistiksel olarak yüksek idi, ancak total alkalin fosfataz, osteokalsin düzeyleri postmenopozal osteoporozu olan ve postmenopozal osteoporozu olmayan grup arasında istatistiksel anlamlılığa sahip değildi. TRAP 5b seviyelerinde ise gruplar arasında istatistiksel olarak farklılık tespit edilmedi.

Tartışma: TRAP 5b aktive osteoklastlardan salgılanan lizozomal bir enzim olmasına rağmen postmenopozal osteoporozlu hastalarda herhangi bir yükseklik tespit edilememiştir. Postmenopozal osteoporoz gibi yavaş yapım-yıkımla seyreden hastalıklarda, diagnostik bir test olarak kullanılamayacağı düşünülmüştür.

Anahtar sözcükler: postmenopozal osteoporoz, tartrat rezistan asit fosfotaz 5b

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Introduction

A consensus has been reached about measurement of bone mineral density that is essential for the diagnosis of osteoporosis; however it does not help us to evaluate the rate of bone loss at a point in time (1). In clinical practice of osteoporosis, biochemical markers of bone turnover have proved to be of value in assessing bone metabolism. Classically, the activity of serum total alkaline phosphatase (ALP) and the measurement of urinary hydroxyproline have been used as markers of bone turnover. Nevertheless, in recent years, more sensitive and specific markers of bone metabolism have been developed, either by measuring bone matrix components released into the circulation during bone formation or resorption, such as osteocalcin or collagen components (2) or by measuring the activity of specific enzymes in the bone cells, such as bone alkaline phosphatase or tartrate-resistant acid phosphatase (TRAP).

Several factors may, however, make interpretation of the marker findings difficult. Some bone markers, especially those measured in urine, show great diurnal variation (3). Collecting urine samples is strenuous and precise timing is needed when 2-h morning samples are used. The day-to-day variation is approximately 10% for formation markers and 20% for resorption markers (3,4). Diet, age, sex, season of the year, phase of the menstrual cycle, exercise, bone mass, liver function, kidney clearance rates and anything that alters bone remodeling may confound the laboratory assays (5). Markers are only relatively specific for bone. ALP and TRAP are also derived from non-skeletal sources, and osteocalcin fragments may reflect both resorption and formation. Osteocalcin and bone alkaline phosphatase give discordant results in conditions such as Paget disease and renal osteodystrophy (4). To date no ideal marker has been established to meet the criteria, so much effort has therefore been allocated to the development of new, more sensitive, specific, and convenient assays.

Tartrate-resistant acid phosphatase has been widely used as a bone resorption marker for many years (6-8). In contrast to collagen-based markers, TRAP may reflect different aspects of osteoclast function and degradation of non-collagenous proteins (9). However, TRAP lacks of specificity and sensitivity (7,10-14), is not restricted to bone and is expressed in a wide variety of tissues and cell types, especially multinucleate cells with phagocytic activity like platelets, erythrocytes and placenta (9,15). It consists of two isoforms, 5a and 5b (7). Analysis of the two isoforms revealed that they were structurally and antigenically identical, but they had a different carbohydrate content, 5a containing sialic acid not found in 5b (16). Also, their pH optimum was different, being approximately 4.9 for 5a and 5.5-6.0 for 5b. The source of TRAP 5a is not entirely clear, but it may derive from macrophages or dendritic cells (17). TRAP 5b has been shown to correlate to the number of osteoclasts and its serum activity is practically unaffected by meals, and its diurnal variation is much smaller than that of serum and urinary telopeptides (3,9). It may be a useful biochemical marker for clinical assessment of many patients with various metabolic bone diseases (17).

The aim of the study was to investigate whether TRAP 5b had diagnostic value in postmenopausal osteoporosis comparing with other bone turnover markers.

Materials and Methods

Subjects

Three populations were studied. The first consisted of 36 postmenopausal osteoporotic women aged 40-75 yr (mean 57.05 ± 8.93 yr). The second group comprised 33 postmenopausal non-osteoporotic females aged 35-64 yr (mean 50.06 ± 6.68 yr). The women had no menstrual bleeding for at last one year since their last menstruation in the first two groups. The third group consisted of 27 premenopausal non-osteoporotic women aged 21-53 yr (mean 38.5 ± 7.59 yr) who had regular menstrual cycles.

None of the women had a history of metabolic bone disease, and none were taking any medication known to affect bone metabolism. None of the selected postmenopausal women had been treated with hormone therapy, bisphosphonates, or calcitonin before entry into the study. They had normal hepatorenal function and were free from endocrine disturbances, diabetes mellitus, and thyroid disorders. There were no significant abnormalities in urinary calcium excretion, no history of hypercalcaemia or urolithiasis in either group.

Measurement of bone markers

A blood sample and a spot urine sample were collected at 9:00 and 11:00 a.m. after an overnight fast and between days 2-6 of the menstrual cycle in premenopausal group. There were no strict controls over sleeping hours, diet or physical activities. All subjects continued their usual routine of diurnal activity and nocturnal rest.

Total ALP was determined by spectrophotometric (using p-nitrophenyl phosphate) method (Abbott, Aeroset, USA). Both the intra and interassay coefficients of variation were less than 3.9%.

Serum C-telopeptide was measured by chemiluminescent immunometric assay (ECLIA Elecsys 170, Roche, Germany). The intra and interassay coefficients of variation were 2.5% and 4.5%.

Urinary deoxypyridinoline was determined by chemiluminescent immunometric assay (Immulite 2000, USA). The intra and interassay coefficients of variation were 2.5% and 4.5%.

Urinary creatinine was measured by spectrophotometric Jaffe's method (alkaline picrate without deprotein, Abbott, Aeroset, USA), the intra and interassay coefficients of variation were less than 2% and 4% respectively.

Osteocalcin was assayed by chemiluminescent immunometric assay (Immulite 2000, USA). The intraassay coefficients of variation was 2.35% and interassay coefficients of variation was 2.55%.

TRAP 5b activity

TRAP 5b activity was measured using the Bone TRAP Assay (Suomen Bioanalytiikka Oy, Oulu, Finland) according to the manufacturer's instructions. Assay specificity is determined by the pH sensitivity of the kinetic activity of TRAP 5a and TRAP 5b isoforms as well as antibody specificity. TRAP immunocaptured using a mouse monoclonal antibody is assayed at pH 6.1 using para-nitrophenyl phosphate as chromogen. All samples were assayed in duplicate and samples from the same subject were analyzed in the same analytical batch. The intra and interassay coefficients of variation were less than 6%.

Bone density measurements

Bone mineral density (BMD) of lumbar spine and femoral neck were measured by Hologic QDR 4500 W (Hologic Inc, Bedford, MA) fan beam DXA scanner. The within subject coefficient of variations were 1% for both, the lumbar spine (LS) and femoral neck (FN).

Statistics

Values shown in the text and tables are mean \pm S.D. Analysis of covariance was used to measure age, bone turnover markers, BMD and TRAP 5b levels followed by Scheffe F test. The areas under the ROC curves were used to evaluate diagnostic values of analytes.

Optimal cut-off levels were selected based on ROC curves providing the maximum diagnostic efficiency (maximum value of specificity % plus sensitivity %). Significance was defined as $p < 0.05$. The entire statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) 11.0 for Windows.

Results

The patient characteristics, bone turnover markers, and TRAP 5b levels between the three groups were shown in Table 1. C-telopeptide and urinary deoxypyridinoline were significantly higher in postmenopausal osteoporotic women compared with postmenopausal non-osteoporotic group ($p < 0.0001$, for both), but there were no significant differences in total ALP and osteocalcin levels between the two groups. Total ALP, osteocalcin, C-telopeptide and urinary deoxypyridinoline in postmenopausal osteoporotic women were significantly higher than premenopausal non-osteoporotic women ($p < 0.0001$ for all of them). Similarly, these bone turnover markers (Total ALP, osteocalcin, C-telopeptide and urinary deoxypyridinoline) in postmenopausal non-osteoporotic group were also significantly higher than premenopausal non-osteoporotic group ($p < 0.01$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$ respectively). There was no significant change in TRAP 5b levels between the groups ($p > 0.05$).

The optimal cut off levels and specificity and sensitivity of bone markers in postmenopausal osteoporotic women were shown in Table II. The area under the ROC curve was 0.654 for total ALP, 0.764 for osteocalcin, 0.888 for C-telopeptide and 0.862 for urinary deoxypyridinoline (Figure 1).

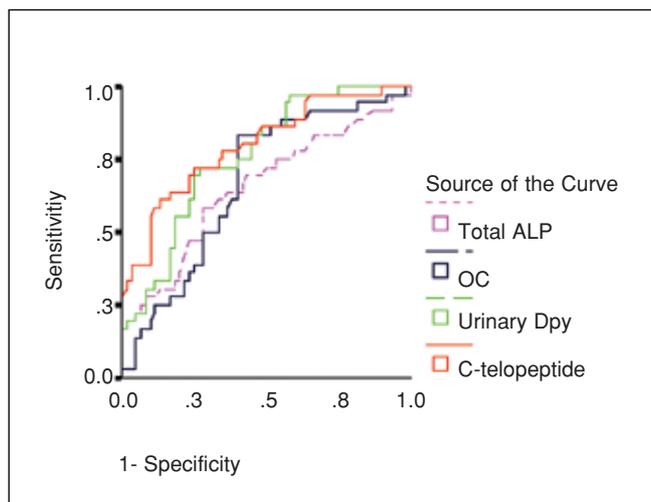


Figure 1. ROC curves of Total ALP, Osteocalcin (OC), Urinary Dpy (deoxypyridinoline), C-telopeptide.

Footnote: tartrate resistant acid phosphatase 5b (TRAP 5b), total alkaline phosphatase (ALP), osteocalcin (OC) and urinary deoxypyridinoline (Dpy).

Discussion

TRAP 5b is a lysosomal enzyme secreted by activated osteoclasts (18) and studies have demonstrated that elevated levels have been detected in high bone turnover diseases like Paget's disease, primary hyperparathyroidism, hemodialysis, osteomalacia, and breast and prostate cancer induced bone metastases (18,19).

To our knowledge, this was the first report in which the specific aim was to demonstrate the diagnostic activity of TRAP 5b in postmenopausal osteoporosis and we evaluated that the levels of this enzyme were not different from the postmenopausal non-osteoporotic women as well as the premenopausal non-osteoporotic controls.

Halleen et al. reported that serum TRAP 5b activity was significantly higher in postmenopausal women comparing with healthy premenopausal group ($p < 0.001$) (20). They also concluded that TRAP 5b activity was elevated in bone diseases like osteopenia, osteoporosis, Paget disease, and bone metastasis of breast cancer, but did not mention about postmenopausal osteoporosis specifically. It has been also evaluated that TRAP 5b activity was elevated in breast cancer metastasis and there was no difference in mean enzyme activity and range in patients of the reproductive age and postmenopausal women (19).

Some bone turnover markers were also investigated in this study. Total ALP and osteocalcin levels in postmenopausal osteoporotic women were significantly higher than in premenopausal non-osteoporotic group, but there were no significant differences between the postmenopausal non-osteoporotic groups. It has been evaluated that total ALP was increased in correlation with age and high bone turnover (21). Its specificity to bone is lower when compared with other

Table 1. Patient characteristics, bone turnover markers, BMD and TRAP 5b levels between the groups.

	Postmenopausal osteoporotic women n=36	Postmenopausal non-osteoporotic women n=33	Premenopausal non-osteoporotic women n=27
Clinical characteristics			
Age	57.05±8.93 ^{a,b}	50.06±6.68 ^b	38.55±7.59
Time since menopause	8.91±6.83 ^c	2.96±3.66	—
BMI (kg/m ²)	28.71±5.06	31.70±5.41	30.94±5.08
BMD (g/cm ²)			
FN BMD	0.64±0.07 ^{b,c}	0.76±0.08 ^b	0.88±0.09
LS BMD	0.72±0.09 ^{b,c}	0.93±0.09 ^b	1.11±0.12
Bone turnover markers			
Total ALP (U/L)	99.69±36.83 ^b	93.67±21.87 ^d	70.19±18.44
Osteocalcin (ng/ml)	27.11±5.64 ^b	25.31±4.15 ^b	18.62±5.42
C-telopeptide (ng/ml)	0.76±0.20 ^{b,c}	0.46±0.22 ^b	0.25±0.12
Urinary deoxypyridinoline (nmol/mmol creatinine)	13.67±4.48 ^{b,c}	8.52±3.68 ^b	3.30±4.67
TRAP 5b (U/L)	3.26±1.31	3.21±0.96	2.77±0.80
^a p<0.01 vs. postmenopausal non-osteoporotic women ^b p<0.0001 vs. premenopausal healthy women ^c p<0.0001 vs. postmenopausal non-osteoporotic women ^d p<0.01 vs. premenopausal healthy women			

bone turnover markers that reduce its reliability in postmenopausal osteoporosis. However, its level was reported to be elevated in postmenopausal women compared with premenopausal women in the literature that supported our findings (5,22-24). Osteocalcin was also found to be elevated in high bone turnover and its level in postmenopausal osteoporosis reported to be normal, high and low (5). This varied response has been attributed to the variability in bone formation rate. Studies showed that its level was higher in postmenopausal women than in premenopausal women (23,24).

C-telopeptide and urinary deoxypyridinoline levels in postmenopausal osteoporotic group were significantly higher than in postmenopausal and premenopausal non-osteoporotic groups. Our findings were supported by many studies since C-telopeptide and urinary deoxypyridinoline were considered as good bone turnover markers in postmenopausal osteoporosis (24-27).

In our study, cut-off levels based on ROC curve providing the maximum efficiency of the tests were selected. Our cut-off values for total ALP, osteocalcin, C-telopeptide and urinary

deoxypyridinoline were 99.7 U/L, 27.3 ng/ml, 0.76 ng/ml, and 13.8 nmol/mmol creatinine respectively. The cut-off values for these bone turnover markers were quite different from the reference upper limits recommended by the reagent kit manufacturers. These differences could be due to either the policy of the manufacturers to provide a high specificity for monitoring purposes or ethnic, behavioral, habitual and nutritional differences of the population (28).

According to our study, ROC curve based diagnostic values for total ALP, osteocalcin, C-telopeptide and urinary deoxypyridinoline are 0.654, 0.764, 0.888, and 0.862 respectively. Although total ALP and osteocalcin are the biochemical markers of bone formation, they are not sufficient in discriminating postmenopausal osteoporosis possibly because of osteoclastic nature of the disease (28). The most sensitive bone marker in our study was found to be C-telopeptide followed by urinary deoxypyridinoline. It has been evaluated that bone resorption markers especially serum C-telopeptide are better indicators than bone formation markers in postmenopausal osteoporosis (2,25,26). However, urinary deoxypyridinoline has been reported to have a wide intraindividual variation (1). Serum assessment of C-telopeptide offers an advantage when compared with urinary-based measurements in that the confounding errors in association with urinary creatinine measurement are eliminated (1,7).

TRAP 5b was measured by solid phase immunofixed enzyme activity assay in this study.

This assay has been reported to be precise, had low within-subject and diurnal variability, was not strongly influenced

Table 2. Optimal cut off levels and specificity and sensitivity of bone markers in postmenopausal osteoporosis.

Parameter	Sensitivity %	Specificity %	Cut off values
Total ALP	41	80	99.7 (U/L)
Osteocalcin	69	85	27.3 (ng/ml)
C-telopeptide	88	93	0.76 (ng/ml)
Urinary deoxypyridinoline	69	91	13.8 (nmol/mmol creatinine)

by feeding, responded to antiresorptive therapy and was not affected by renal failure (9). It has been pointed out that active serum TRAP 5b was quite stable and no instability problems were associated with the assay, therefore the assay was suggested to detect only active TRAP 5b molecules in the circulation. TRAP heterogeneity in serum has had a negative impact on the sensitivity and specificity of TRAP as a marker of bone resorption. TRAP 5a retains some activity at pH 6.1 which may compromise the absolute specificity of the assay for TRAP 5b. Previous studies suggested that it had little significance as an interfering substance in TRAP 5b measurement. One study implied that TRAP 5a may have practical, biological, and clinical significance of its own (7). TRAP 5a activity in the sera of elderly persons was evaluated to be three or more times higher than the 5b activity (6). Moreover, some TRAP activity derived from alveolar macrophages is contaminating the serum and need to be clarified to contribute to the total TRAP 5b in the serum.

In conclusion, TRAP is quite stable, reflects the intensity of bone resorption over the last 24 hours, isoform 5b-specific antibodies, substrates, or inhibitors are discovered to make TRAP immunoassays absolutely bone specific, however this strategy definitely improves the specificity of TRAP immunoassays for diseases of increased bone resorption. Since postmenopausal osteoporosis has a relatively low turnover state comparing with metabolic disorders and cancer metastasis, low concentrations in serum can be assessed accurately when a new highly specific anti-TRAP 5b antibody is found in future.

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