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ORIGINAL ARTICLE

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Reference intervals of serum tartrate-resistant acid phosphatase type 5b activity measured with a novel assay in Japanese subjects

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Abstract Among the isotypes of serum tartrate-resistant acid phosphatase (TRACP), only type 5b (TRACP-5b) is derived from osteoclasts, and it is necessary to develop an assay specific for this TRACP-5b for evaluation of osteoclastic activity. Recently, a novel assay system for TRACP-5b called the fragments absorbed immunocapture enzymatic assay (FAICEA) has been developed. With two unique monoclonal antibodies, one that is highly specific for TRACP-5b and another which absorbs inactive TRACP-5b fragments that interfere with measuring active TRACP-5b, this assay provides correct measurement of TRACP-5b activity in the serum without interference by the inactive fragments of TRACP-5b and other isotypes of TRACP, especially TRACP-5a. To study the reference data of Japanese subjects, we measured TRACP-5b activity in the serum of 320 men (age, 20–82 years) and 466 women [315 premenopausal (age, 18–55 years) and 151 postmenopausal (age, 45–77 years)] with this novel assay. In men, serum TRACP-5b activity did not vary significantly with age. The postmenopausal women had significantly higher serum TRACP-5b activity than the premenopausal women. The reference intervals (logarithmic mean ±1.96 SD) for men, premenopausal women, and postmenopausal women were 1.7–5.9 U/l, 1.2–4.4 U/l, and 2.5–7.6 U/l, respectively.

Key words tartrate-resistant acid phosphatase 5b (TRACP-5b) · osteoclastic activity · fragments absorbed immunocapture enzymatic assay (FAICEA) · reference interval

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Introduction

The biochemical markers of bone turnover play an important role in monitoring the therapeutic efficacy of osteoporosis. Currently, bone resorption markers in urine [1–3] are commonly used in Japan, but they are disadvantageous in that urine samples must be collected at the same time at each testing to accommodate circadian rhythm, and the results are corrected with urinary creatinine concentration because of individual differences in renal clearance. To overcome these disadvantages of urinary markers, serum markers, which have negligible circadian rhythm variation and are not influenced by renal clearance, were developed, and some of these are used routinely [4–6].

Serum tartrate-resistant acid phosphatase (TRACP) is also a well-known marker for bone resorption [7], but it is not widely used in clinical practice because of its minor specificity for bone and instability in serum. Further studies revealed that TRACP had isotypes and that only type 5b (TRACP-5b) was derived from osteoclasts. Accordingly, assay systems for TRACP-5b were developed, but their parameters, such as specificity, sensitivity, and measurable range, were not satisfactory for use in clinical practice [8,9].

Recently, Ohashi et al. have developed a novel assay system for TRACP-5b called fragments absorbed immunocapture enzymatic assay (FAICEA). They used a novel substrate, 2-chloro-4-nitrophenyl phosphate (CNPP), which is much more specific for TRACP-5b at the appropriate pH than *p*-nitrophenyl phosphate (PNPP), and a monoclonal

Table 1. The general characteristics of 320 men, 315 premenopausal women, and 151 postmenopausal women

	Men M ± SD (range)	Premenopausal women M ± SD (range)	Postmenopausal women M ± SD (range)
Age (years)	46.3 ± 15.4 (20–82)	33.4 ± 7.7 (18–55)	62.4 ± 6.0 (45–77)
Weight (kg)	66.3 ± 11.0 (40.8–133.1)	51.9 ± 7.7 (36.5–94.6)	51.9 ± 6.6 (36.9–74.1)
Height (cm)	168.4 ± 6.7 (140.5–186.1)	158.1 ± 4.8 (145.0–176.0)	152.6 ± 5.7 (135.1–167.3)
BMI (kg/m^2)	23.4 ± 3.4 (16.4–40.1)	20.8 ± 2.9 (15.9–38.9)	22.3 ± 2.6 (16.5–33.5)
YSM (years)	–	–	11.7 ± 6.8 (0–39)

BMI, body mass index; YSM, years since menopause

antibody raised against ultrapure TRACP-5b derived from bone. Furthermore, they used another monoclonal antibody for eliminating interference of the inactive TRACP-5b fragments that exist abundantly in serum and narrow the measurable range [10]. In this study, we examined healthy Japanese subjects with this novel assay to establish reference intervals of serum TRACP-5b activity.

Subjects and methods

Subjects and samples

For this study, healthy volunteers and patients undergoing medical examination were recruited from five sites in Japan. The patients having bone metabolism disorders (rheumatoid arthritis, osteoarthritis, thyroid disease, parathyroid disease, osteomyelitis, etc.) or a recent fracture were excluded. Subjects taking medications that affect bone metabolism and the lactating, pregnant, oophorectomized, or hysterectomized women were also excluded. In calculating the reference intervals for men and premenopausal women, subjects under 25 years of age were excluded to circumvent the possible effect of elevated bone metabolism at puberty. The young adult mean (YAM) was obtained from premenopausal women aged from 30 to 44 years.

A total of 320 male and 466 female healthy subjects were enrolled in this study; their general characteristics are shown in Table 1. Serum samples were taken by routine procedures at each facility and stored at –80°C until analysis.

This study was approved by the Institutional Review Board of the Osaka City University Graduate School of Medicine, and informed consent was obtained from all participants.

Measurement of TRACP-5b

TRACP-5b was measured by the novel assay system FAICEA developed by Ohashi et al. (Nitto Boseki, Fukushima, Japan). Briefly, 100 µl sample diluent and 50 µl serum were applied to the wells of an immunoplate onto which were adhered two kinds of monoclonal antibodies specific for TRACP-5b and its fragments and incubated for 1 h at room temperature with shaking. After incubation, the plate was washed three times with wash solution, 100 µl substrate solution was added to each well, and the plate was then incubated for 1 h at 37°C. Thereafter, 50 µl stop solution was added and the absorbance was measured at 405 nm. In this assay, the minimum detectable level was 0.1 U/l, and the intraassay and interassay variation were 2.15% and 2.95% at 3.4 U/l and 1.91% and 2.04% at 7.4 U/l, respectively.

Measurement of BMD

Bone mineral density (BMD) of the lumbar spine (L2–L4) was measured in 610 of the 786 subjects by dual-energy X-ray absorptiometry (DXA) with a QDR series instrument (Hologic, Bedford, MA, USA) at each facility.

Statistical analysis

The data of TRACP-5b in the men and premenopausal and postmenopausal women did not adhere to a standard distribution, and therefore logarithmic transformation was used to normalize the data. After the outliers were rejected by the rejection test, the reference intervals (logarithmic mean ±1.96 SD) were calculated.

The Z-score of each subject was calculated from the reference value of BMD (reference BMD) for the same age and sex established for Japanese subjects [11] using the following equation: Z-score = (BMD of a subject – mean of the reference BMD)/SD of the reference BMD [12].

Comparisons between the two groups were performed using the Mann–Whitney *U* test, and the Kruskal–Wallis test was utilized for comparison of more than three groups. If the Kruskal–Wallis test was significant, Dunn's multiple comparison test was used as a post hoc test. $P < 0.05$ was considered as statistically significant.

Results

Effects of age and gender on serum TRACP-5b activity and BMD

The enrolled men and premenopausal women were classified by age, and the postmenopausal women were divided into groups by years since menopause (YSM). Tables 2–4 show the mean (±SD) serum TRACP-5b activity and BMD in each group. Figure 1 shows the effects of age and gender on the serum TRACP-5b activity in all men and women. Figure 2 shows the effect of YSM in the postmenopausal women.

Table 2. Serum serum tartrate-resistant acid phosphatase type 5b (TRACP-5b) activity and bone mineral density (BMD) of each age group in men

Age (years)	TRACP-5b		BMD	
	n (cases)	Mean ± SD (U/l)	*n* (cases)	Mean ± SD (g/cm^2)
–24	11	3.6 ± 0.6	9	1.023 ± 0.057
25–29	45	3.4 ± 0.9	36	1.024 ± 0.117
30–39	72	3.2 ± 1.0	58	0.965 ± 0.136
40–49	55	3.1 ± 1.1	38	0.978 ± 0.117
50–59	56	3.3 ± 1.1	51	0.995 ± 0.168
60–69	63	3.5 ± 1.3	63	0.984 ± 0.143
70–	18	3.8 ± 1.2	17	0.935 ± 0.179
Total	320	3.3 ± 1.1	272	0.985 ± 0.141

Table 3. Serum TRACP-5b activity and BMD of each age group in the premenopausal women

Age (years)	TRACP-5b			BMD	
	n (cases)	Mean ± SD (U/l)		*n* (cases)	Mean ± SD (g/cm^2)
–19	4	2.7 ± 1.1		0	—
20–24	44	2.7 ± 0.9	*	9	1.000 ± 0.112
25–29	46	2.4 ± 0.8		19	1.025 ± 0.121
30–34	95	2.3 ± 0.8		78	1.015 ± 0.106
35–39	61	2.4 ± 0.8		47	1.012 ± 0.099
40–44	33	2.6 ± 0.9		23	1.054 ± 0.132
45–49	23	2.7 ± 1.0		15	1.019 ± 0.105
50–	9	3.0 ± 1.7		8	0.975 ± 0.139
Total	315	2.5 ± 0.9		199	1.018 ± 0.110

* $P < 0.05$, Dunn's multiple comparison test

Table 4. Serum TRACP-5b activity and BMD of each YSM group in the postmenopausal women

YSM (years)	TRACP-5b		BMD	
	n (cases)	Mean ± SD (U/l)	*n* (cases)	Mean ± SD (g/cm^2)
0–1	8	3.9 ± 2.1	6	0.954 ± 0.120
2–3	12	4.8 ± 1.0	9	0.942 ± 0.168
4–5	10	4.5 ± 2.2	8	0.879 ± 0.184
6–7	15	4.2 ± 0.9	12	0.882 ± 0.111
8–9	17	4.4 ± 1.2	16	0.850 ± 0.167
10–11	10	4.6 ± 1.9	10	0.804 ± 0.127
12–	79	4.5 ± 1.4	78	0.807 ± 0.115
Total	151	4.5 ± 1.4	139	0.837 ± 0.136

YSM, years since menopause

Serum TRACP-5b activity in men was the lowest at 40–49 years, but no significant differences were found among the groups listed in Table 2. The trend of BMD was similar to that of TRACP-5b in men.

In the premenopausal women, serum TRACP-5b activity was the lowest at 30–34 years. The level in this group was significantly different from that at 20–24 years, although their BMD was maintained virtually constant.

Serum TRACP-5b activity in the postmenopausal women was significantly higher than in the premenopausal women and men, but TRACP-5b activity did not increase with YSM.

Correlation between serum TRACP-5b activity and Z-score of BMD

The calculated correlation coefficients (r) between TRACP-5b and the Z-score of BMD were –0.213 ($P = 0.0004$) for men, –0.132 ($P = 0.063$) for premenopausal women, and –0.220 ($P = 0.009$) for postmenopausal women.

Reference intervals of serum TRACP-5b activity

The reference intervals were calculated from the logarithmic-transformed data because the serum TRACP-

Table 5. Calculated reference intervals of serum TRACP-5b activity

Group	*n*	Age	Reference interval (U/l)
Men	309	25–82	1.7–5.9
Premenopausal women	267	25–55	1.2–4.4
Postmenopausal women	151	45–77	2.5–7.6

Reference intervals set within the range of the logarithmic mean ±1.96 SD

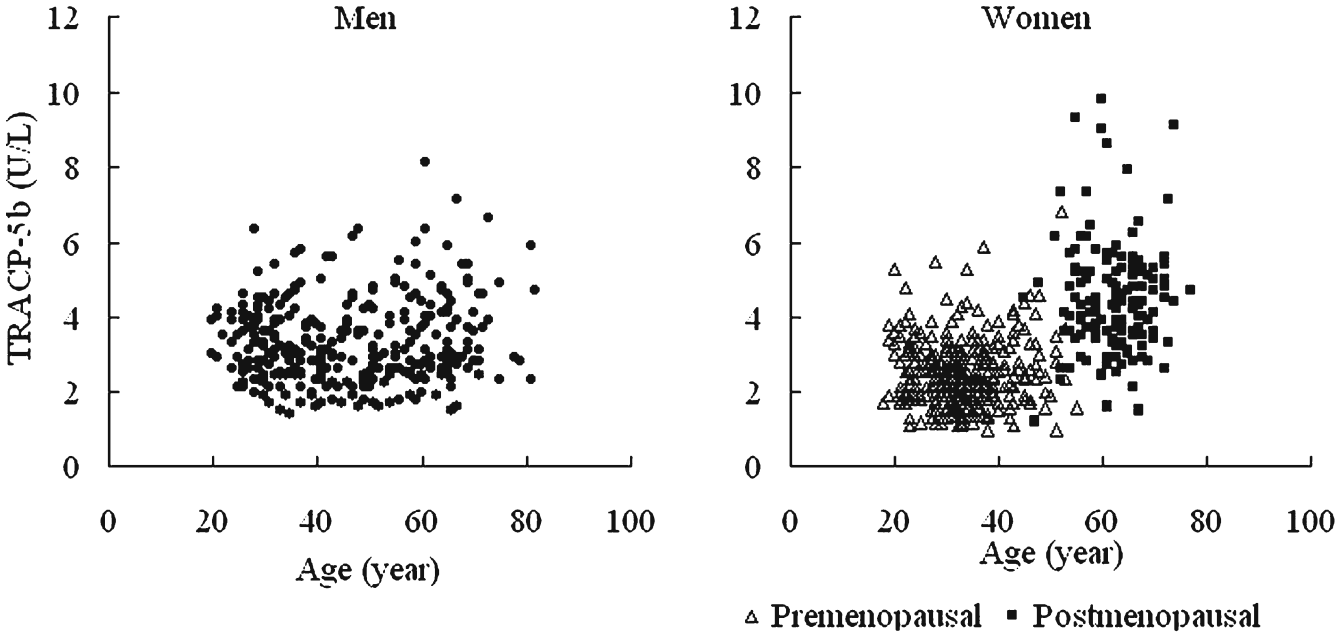

Fig. 1. Effects of age and gender on serum tartrate-resistant acid phosphatase type 5b (TRACP-5b) activity in men and women. Serum TRACP-5b activity in postmenopausal women was significantly higher than in premenopausal women and men

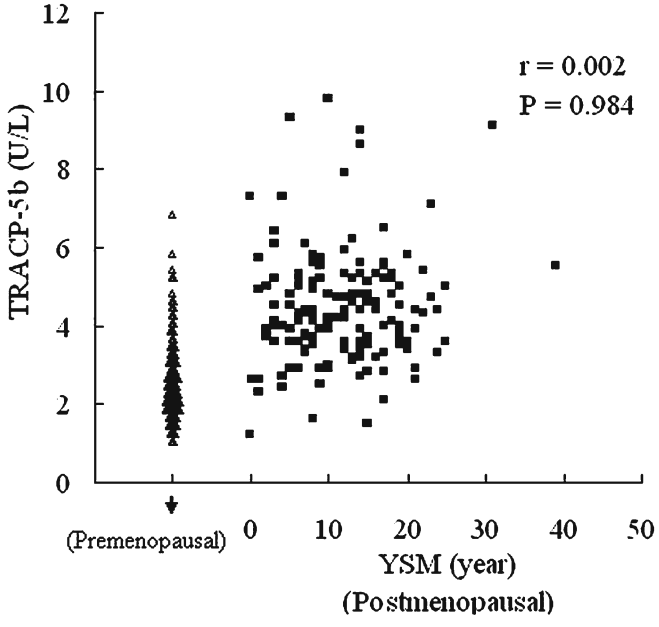

Fig. 2. Effect of years since menopause (YSM) on serum TRACP-5b activity in postmenopausal women. Serum TRACP-5b activity did not increase with YSM

5b activity in men, premenopausal women, and postmenopausal women were not normally distributed. The calculated reference intervals (logarithmic mean ±1.96 SD) in these groups were 1.7–5.9 U/l, 1.2–4.4 U/l, and 2.5–7.6 U/l, respectively (Table 5). The interval of YAM, which the Japan Osteoporosis Society (JOS) suggested to be used as reference when initiating treatment for osteoporosis in their guidelines for the use of biochemical markers of bone turnover in osteoporosis [13], was 1.2–4.2 U/l.

When the female subjects were restricted to those who had BMD greater than 0.809 g/cm^2 (the normal value according to the diagnostic criteria of osteoporosis established by the Japanese Society of Bone and Mineral Research [11]), the calculated reference intervals for the premenopausal and postmenopausal women were 1.2–4.2 U/l and 2.4–6.9 U/l, respectively.

Discussion

We measured serum TRACP-5b activity in healthy Japanese subjects with a newly developed assay. This new FAICEA is much more specific for TRACP-5b than the

existing assays [10], and its parameters (for example, sensitivity and measurable range) are satisfactory for measuring serum TRACP-5b activity [14]. Also, the diurnal variation and day-to-day variation of TRACP-5b studied with this assay were less than those of N-terminal crosslinking telopeptide of type I collagen (NTX) in the serum and urine, C-terminal crosslinking telopeptide of type I collagen (CTX) in urine, and deoxypyridinoline (DPD) in urine [15]. Therefore, TRACP-5b measured by this novel assay is a quite promising marker to evaluate systemic osteoclastic activity.

We studied the effects of age and gender on serum TRACP-5b activity. We found that menopause was the signal of elevation of the TRACP-5b activity, and that the activity remained at a high level after the menopause in women, whereas TRACP-5b varied little in elderly men. These observations are similar to those reported about other biochemical markers of bone turnover [16–25].

Regarding BMD, menopause is an important factor of bone loss in women. The decrease of BMD is modest before menopause, but the decrease is accelerated after menopause. In men, BMD at ages less than 69 years remained almost unchanged, but that at ages more than 70 years tended to decrease, which might be a result of the small numbers of subjects enrolled.

The correlation coefficients between serum TRACP-5b activity and the Z-score of BMD in men ($r = -0.213$), premenopausal women ($r = -0.132$), and postmenopausal women ($r = -0.220$) were modest. This finding is in consensus with previous reports on the correlation between BMD and other bone metabolic markers [26–31]. Even when the postmenopausal women were analyzed with the exception of those with YSM 0–3 years, the correlation coefficient did not improve ($r = -0.244$), but when the men were analyzed with ages more than 65 years, it improved a little ($r = -0.320$). This finding probably occurred because TRACP-5b is an index for the present systemic bone turnover, as Chu et al. showed by means of bone biopsy [32], and BMD is an integral for local bone turnover.

WHO showed that even cases with normal BMD had a risk of fracture because the diagnostic sensitivity of BMD was not sufficient, and the use of risk factors in addition to BMD would improve the predictive value for screening [33]. Accordingly, JOS established guidelines for initiating pharmacological treatment to prevent fragility fractures, which employed new risk factors (other than BMD) such as excessive alcohol intake, current habit of smoking, and family history of hip fracture [34].

Although not included in the diagnostic guidelines of JOS, the bone turnover markers are reportedly risk factors of fracture [35]. It is reasonable to presume that the enhanced bone markers reflect the condition of increased bone turnover, which leads to rapid bone loss and fragility fractures. Therefore, in identifying patients with high risk of fracture, we should pay great attention to the bone markers, especially in initiating treatment with antiresorptive agents. It would be necessary to measure the bone resorption markers for proper patient care.

As just mentioned, bone markers are expected to play an important role in treatment of osteoporosis. Serum TRACP-5b activity can sufficiently play the assigned role, with less diurnal variation than the urinary markers [15], and is not influenced by liver and/or renal impairment or ingested foods [36].

The novel FAICEA employed in this study may be sensitive enough to measure serum TRACP-5b activity. Further studies are necessary to validate the clinical usefulness of serum TRACP-5b activity with the reference intervals that we established in this study.

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