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Beta-2-Microglobulin in Diseases with High Bone Remodeling

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Abstract. β_2-microglobulin has been observed to behave as a biological marker of bone remodeling. We measured β_2-microglobulin and tartrate-resistant acid phosphatase (TRAP), a specific biological marker of bone remodeling, in 225 women: healthy premenopausal controls, healthy postmenopausal controls, and patients with diseases characterized by enhanced bone turnover (postmenopausal osteoporosis, primary hyperparathyroidism, primary hyperthyroidism, polyostotic Paget's bone disease), and in other Paget's group before and after calcitonin treatment. β_2-microglobulin levels differed significantly between the healthy premenopausal women (n = 38) and the women with hyperparathyroidism (n = 20) compared with all the other groups. However, β_2-microglobulin levels did not differ significantly between healthy postmenopausal women (n = 38) and patients with Paget's bone disease (n = 40) (P = 0.5095), or between women with postmenopausal osteoporosis (n = 30) and women with hyperthyroidism (n = 20) (P = 0.7890). TRAP concentrations differed significantly in all the groups paired except for women with Paget's bone disease and women with either hyperparathyroidism or hyperthyroidism (P = 0.5179 and 0.6993, respectively); likewise, TRAP levels did not differ significantly between the women with hyperparathyroidism and those with hyperthyroidism (P = 0.7804). After calcitonin treatment, there was a 22% increase in β_2-microglobulin, a 17% decrease in TRAP, and a 39% decrease in alkaline phosphatase, all of which were significant at $P < 0.0001$. Our findings indicate that serum β_2-microglobulin, like osteocalcin, behaves as a biological marker of remodeling in a number of diseases with enhanced bone remodeling but not in Paget's bone disease.

Key words: Serum β_2-microglobulin — Tartrate-resistant acid phosphatase — Paget's bone disease — Hyperparathyroidism — Hyperthyroidism — Postmenopausal osteoporosis — Normal women.

We recently found that β_2-microglobulin behaves as a biological marker of bone remodeling [1, 2], more specifically, of bone resorption. Paradoxically, Paget's bone disease [3], which is characterized by a high rate of bone remodeling [4], has normal β_2-microglobulin levels. We attribute this to the reutilization of β_2-microglobulin in the active new bone formation that occurs in this Paget's bone disease.

Aside from osteoporosis and Paget's bone disease, the course of hyperparathyroidism and hyperthyroidism is also with high rates of bone remodeling [5]. Our study examined the behavior of β_2-microglobulin concentrations in postmenopausal women with various diseases characterized by high bone remodeling (postmenopausal osteoporosis, Paget's bone disease, hyperparathyroidism, and hyperthyroidism) as compared with healthy postmenopausal women. We also studied the correlation between the levels of β_2-microglobulin and tartrate-resistant acid phosphatase (TRAP), quantitated using α-naphthyl phosphate as the substrate [6]. This substrate reacts specifically with isoenzyme 5b synthesized by osteoclasts [7], thus making TRAP a specific and sensitive biological marker of bone remodeling [8].

Bone remodeling in Paget's bone disease is intense [5, 9] but it decreases with calcitonin treatment [10], as do serum and urine levels of the biological markers of bone formation and resorption [11]. It is reasonable to think that if bone remodeling decreases in Paget's disease as a result of calcitonin treatment, these events should be accompanied by an increase in serum β_2-microglobulin and a decrease in the resorption markers that are not reutilized in new bone formation. In this second study, we proposed to investigate the effect on serum β_2-microglobulin levels of calcitonin treatment of Paget's bone disease. Presumably, if serum β_2-microglobulin increases with treatment of Paget's bone disease, it may be because it is reutilized in new bone formation.

Material and Methods

Subjects

A total of 225 subjects, all women, were studied: 38 healthy premenopausal women (mean age 35.5 ± 9.3 years); 77 healthy postmenopausal women (mean age 62.0 ± 8.0 years); 30 postmenopausal osteoporosis women (mean age 70.5 ± 4.0 years); 40 women with polyostotic Paget's bone disease, mixed form (mean age 65.9 ± 7.2 years); 20 primary hyperparathyroidism (HPTH) women (mean age 62.7 ± 6.0 years); and 20 women with primary hyperthyroidism (HT) (mean age 55.8 ± 5 years). All patients were postmenopausal.

In every case, the diagnosis of normality was corroborated by radiological studies of the dorsal and lumbar spine in which no vertebral deformity was detected. Vertebral deformity was defined as the loss of more than 25% of the anterior, middle, or posterior vertebral height in any vertebra. In the patients with Paget's bone disease, radiographic and scintigraphic studies were made to evaluate disease extension. Disease activity was assessed by measuring serum total alkaline phosphatase and TRAP; in the last 6 months, none of the patients had been treated for their disease. Seventeen patients with Paget's disease were diagnosed on the basis of a chance finding of increased alkaline phosphatase in a routine analysis. In four cases, the diagnosis was made after a urological radiographic study

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Table 1. Data on B_2-microglobulin and TRAP

Group	N	Age years	β_2-microglobulin mg/liter	TRAP U/liter
Premenopausal	38	35.5 ± 9.3	1.15 ± 0.22	2.2 ± 0.1
Postmenopausal	77	62.0 ± 8.0	1.41 ± 0.34	2.9 ± 0.6
PMOP	30	70.5 ± 4.0	1.73 ± 0.26	3.5 ± 0.7
Paget's disease	40	65.9 ± 7.2	1.45 ± 0.42	4.5 ± 1.2
HPTH	20	62.7 ± 6.0	2.46 ± 0.61	4.2 ± 0.6
HT	20	55.8 ± 15.4	1.71 ± 0.35	4.3 ± 0.6

All figures given as mean ± SD
Pre- and post-menopausal refers to healthy women; PMOP = postmenopausal osteoporosis; HPTH = hyperparathyroidism; HT = hyperthyroidism

for possible renal lithiasis. The diagnosis of hyperparathyroidism was corroborated by the measurement of whole-molecule serum parathyroid hormone (PTH) concentration. In only three cases was the diagnosis of hyperparathyroidism incidental, a result of hypercalcemia detection; in the other cases it was diagnosed as a result of renal lithiasis. All of the patients with hyperparathyroidism were treated with a calcium-free diet and none had been treated surgically. The diagnosis of hyperthyroidism was confirmed by hormonal studies (TSH, T_3, and T_4), although a clinical picture of hyperthyroidism was present in all. Tremor, palpitations, and weight loss were the most relevant findings. None of the patients had been treated for hyperthyroidism.

All the subjects were from the health district corresponding to the "Príncipe de Asturias" University Hospital of the Alcalá de Henares University (Madrid, Spain). The study was approved by the local Ethics Committee and all participants gave their written informed consent. The study subjects were not taking any medication that could interfere with calcium metabolism. All of them lived active lives but did not practice sports. Only 6% of the subjects smoked, but none smoked more than 10 cigarettes/day. Their alcohol intake was sporadic and coffee intake did not exceed 100 ml/day in any case.

A second study was made of 27 patients with Paget's disease: 14 were men and 13 women, with an average age of 65.7 ± 6.4 years. All patients were treated with 100 IU of salmon calcitonin I.M. followed by 500 mg of elemental calcium given 4 hours after calcitonin administration for 10 days a month for a period of 3 months. The same biochemical studies were made at baseline and after concluding treatment.

Analytical Studies

Coffee, tea, and alcohol intake, smoking, and exercise were not permitted for 24 hours before the day of investigation. The hematological and biochemical studies were performed on blood samples at 8.00 a.m. after an all-night fast. The biochemical studies included the usual blood parameters for metabolic bone studies: calcium, phosphorus, total alkaline phosphatase, TRAP, creatinine, and total proteins, all measured in serum using a Hitachi automated analyzer system 717 (Boehringer, Mannheim, Germany). In the same sample, β_2-microglobulin concentration was measured using microparticle enzyme immunoassay with a β_2-microglobulin commercial reagent from Abbott Laboratories (Abbott Park, IL, USA), and an IMX R automated analyzer from the same company. All samples from every subject were analyzed in the same assay to eliminate interassay variation. Assay reproducibility was determined by assaying four samples five times in five different runs at two laboratories. The coefficients of variation (CV) between runs and between laboratories were determined by components of variance [12], which give a statistical estimate of the variation of replicates of one in multiple assay runs. In every case, CV was less than 6%. TRAP was quantitated in serum in the Hitachi automated analyzer as the substrate a-naphthyl phosphate, using a reagent from Boehringer Laboratories (Boehringer) that reacts specifically with isoenzyme 5b synthesized by the osteoclast [7]. A 24-hour urinary calcium excretion was determined by atomic absorption spectroscopy using a Perkin Elmer model 5000 spectrophotometer (Perkin Elmer, Norfolk, CT, USA).

Table 2. Statistical differences in the serum concentrations of β_2-microglobulin and TRAP between the groups studied

Groups		*P* values*	
		β_2-microglobulin mg/liter	TRAP U/liter
Pre	Post	<0.0001	<0.0001
Pre	PMOP	<0.0001	<0.0001
Pre	Paget	0.0002	<0.0001
Pre	HPTH	<0.0001	<0.0001
Pre	HT	<0.0001	<0.0001
Post	PMOP	<0.0001	0.0010
Post	Paget	ns	<0.0001
Post	HPTH	<0.0001	<0.0001
Post	HT	0.0039	<0.0001
PMOP	Paget	0.0018	0.0009
PMOP	HPTH	<0.0001	0.0258
PMOP	HT	ns	0.0242
Paget's	HPTH	<0.0001	ns
Paget's	HT	0.0408	ns
HPTH	HT	0.0012	ns

Abbreviations same as in Table 1
* According to ANOVA

Statistical Studies

One-way analyses of variance (ANOVA) was used as appropriate to compare differences between patient groups for continuous variables. Single regression analyses were used as appropriate to examine relations between continuous variables. A simple linear regression study was made of serum β_2-microglobulin concentration against serum TRAP concentration in each group and against hormonal levels in the groups with hyperparathyroidism and hyperthyroidism. Pre- and posttreatment, the study parameters were compared using the Student's paired *t*-test. Pre- and posttreatment, a simple linear regression study was made of serum β_2-microglobulin concentration against serum TRAP concentration and serum alkaline phosphatase concentration. All studies were made with the StatView 4.02 program (Abacus Concepts, Inc., Berkeley, CA, USA) for Macintosh computers.

Results

β_2-microglobulin and TRAP measurements (mean ± SD) for each study group and the number and age of the subjects in each group are shown in Table 1. The differences in β_2-

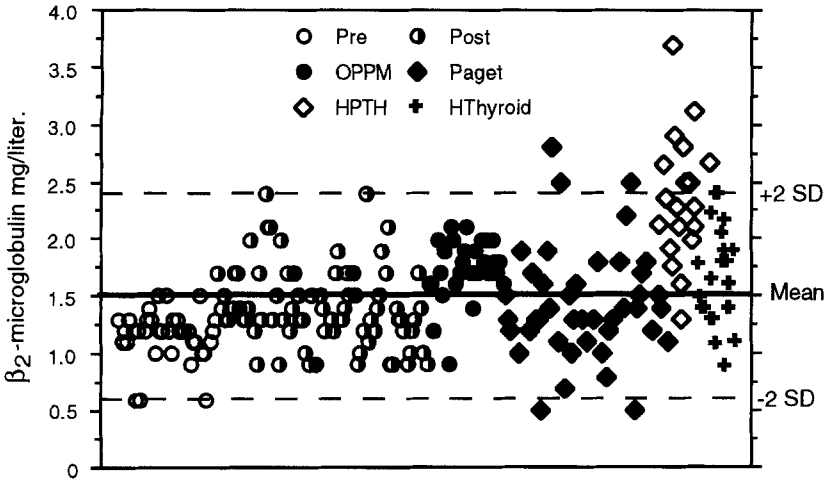

Fig. 1. Univariate scattergram of the serum levels of β_2-microglobulin in the groups studied. Abbreviations as in Tables 1 and 2.

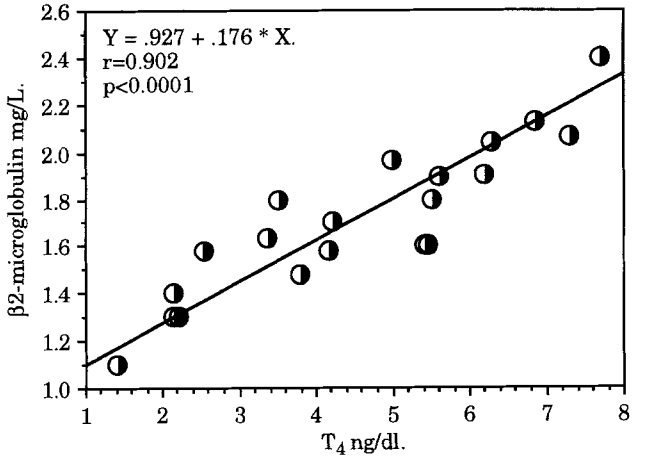

Fig. 2. Simple linear regression between serum β_2-microglobulin and T_4 in the women with hyperthyroidism that were studied.

microglobulin and TRAP levels between paired groups are shown in Table 2 (P values according to ANOVA). The differences in β_2-microglobulin levels between each pair of groups were significant (one-way ANOVA) with the exception of β_2-microglobulin levels between the healthy postmenopausal women and the women with Paget's bone disease ($P = 0.5095$) and between the women with postmenopausal osteoporosis and the women with hyperthyroidism ($P = 0.7890$). TRAP levels differed significantly between each pair of groups except between the women with Paget's bone disease and those with either hyperparathyroidism ($P = 0.5179$) or hyperthyroidism ($P = 0.6993$), and between the women with hyperparathyroidism and those with hyperthyroidism ($P = 0.7804$). The relative β_2-microglobulin levels for each group of women studied are shown in Figure 1 (univariate scattergram).

β_2-microglobulin and TRAP levels correlated significantly (linear regression: $P < 0.0001$ for all) in every group except the women with Paget's bone disease. In the women with hyperparathyroidism, β_2-microglobulin levels correlated marginally with PTH ($P < 0.05$). In contrast, β_2-microglobulin levels correlated well with T_4 levels ($r = 0.902$, $P < 0.0001$) in the women with hyperthyroidism (Fig. 2).

In the second study, the serum levels of β_2-microglobulin, TRAP, and alkaline phosphatase before and after calcitonin treatment (Table 3) showed a significant ($P < 0.0001$) posttreatment elevation of 22% for β_2-microglobulin and equally significant ($P < 0.0001$) decreases of 17% in TRAP and of 39% in alkaline phosphatase.

In baseline conditions we found that serum β_2-microglobulin concentration was related negatively and significantly ($r = -0.483$, $P = 0.016$) with serum total alkaline phosphatase concentration, but not with serum TRAP concentration (P = ns). After treatment, the lack of a significant relation between serum β_2-microglobulin and TRAP levels persisted but the significance of the relation between serum β_2-microglobulin and alkaline phosphatase levels increased ($r = -0.613$, $P = 0.004$) (Fig. 3).

Discussion

β_2-microglobulin, at first known as bone-derived growth factor (BDGF) [13], has been isolated recently from fetal and adult bone [14], and from bone culture medium [15]. It has been characterized as a polypeptide with a molecular weight of 11,000–12,000 d [16]. In bone cultures it stimulates DNA and osseous collagen synthesis [16], as well as osteoclastic activity [17]; it also regulates bone metabolism [18]. More recently, it was found to be synthesized by osteoblasts [19], such as osteocalcin (BGP) [20]. These findings suggest that it may be a biological marker of bone remodeling like BGP. The serum TRAP that we measured is highly specific for osteoclastic activity [8]. In postmenopausal osteoporosis and in healthy postmenopausal women, TRAP concentration correlates significantly and positively with serum β_2-microglobulin concentration [1, 2], a finding we confirmed in this study.

In hyperthyroidism, bone remodeling is increased [21] and β_2-microglobulin levels are significantly higher than in healthy women of a similar age. Other authors have reported an increase in the levels of the biological markers of bone remodeling in hyperthyroidism [22] and histological evidence of enhanced bone remodeling [23]. Macleod et al. [24] found that elevation of bone remodeling markers normalized after treatment of women with hyperthyroidism. We found significant correlations between TRAP and β_2-microglobulin levels and between β_2-microglobulin and T_4 levels (Fig. 2), which confirms the role of β_2-microglobulin as a biological marker of bone resorption.

Table 3. Serum values (mean ± SD) of β_2-microglobulin, TRAP, and total alkaline phosphatase in the 27 patients with Paget's bone disease with salmon calcitonin i.m., 100 U/day for 10 days each month for a period of 3 months and 500 mg of elemental calcium given 4 hours after each calcitonin dose

	Pre	Post	% Δ	*P*
β_2-microglobulin mg/liter	1.37 ± 0.33	1.53 ± 0.29	22	<0.0001
TRAP U/liter	4.65 ± 1.08	3.79 ± 0.64	−17	<0.0001
Alkaline phosp. U/liter	906 ± 641	556 ± 342	−39	<0.0001

% Δ = percentage change; *P* according to Student's *t* test for paired data

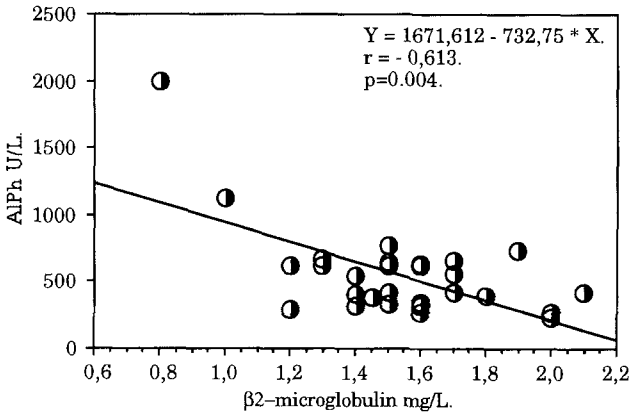

Fig. 3. Simple linear correlation between serum β_2-microglobulin and total alkaline phosphatase concentrations in 27 patients with Paget's bone disease after treatment with salmon calcitonin i.m., 100 U/day for 10 days each month for 3 months and 500 mg of elemental calcium given after each calcitonin dose.

In hyperparathyroidism, TRAP and β_2-microglobulin levels are increased and correlate significantly with each other, as in hyperthyroidism. Increased TRAP also was observed in patients with hyperparathyroidism by Scarnecchia et al. [25]. On the other hand, Cosman et al. [26] found that PTH administration caused TRAP to increase in healthy postmenopausal women not receiving any treatment.

In Paget's bone disease we found normal β_2-microglobulin values, which confirms our opinion that β_2-microglobulin is not an adequate biological marker of bone remodeling in this disease [3]. In contrast, TRAP levels were significantly higher in Paget's bone disease than in women with postmenopausal osteoporosis but did not differ from TRAP levels in hyperparathyroidism and hyperthyroidism. As there is more bone remodelling in Paget's bone disease than in either hyperparathyroidism or hyperthyroidism, the absence of any difference in TRAP levels between these groups probably is due to the localized nature of the bone lesion in Paget's disease as opposed to the systemic bone disorder in hyperparathyroidism and hyperthyroidism. Paget's disease of the bone is characterized by enhanced bone remodelling [5, 9]. Calcitonin reduces bone remodelling significantly [10], as indicated by the attendant decrease in levels of biological markers [11]. This decrease in levels of biological markers reflects decreased bone resorption and formation as a result of cellular coupling, which explains why β_2-microglobulin, used in new bone formation [3], increases after calcitonin treatment and TRAP and alkaline phosphatase decrease reciprocally.

To conclude, we found that β_2-microglobulin was a biological marker of bone remodelling in various diseases. It is characterized by high bone turnover in postmenopausal osteoporosis, primary hyperparathyroidism, and primary hyperthyroidism. The increase in serum β_2-microglobulin concentration observed after calcitonin treatment of Paget's bone disease supports the hypothesis [3] that this polypeptide may be reutilized in new bone formation in this disease, as occurs with BGP [27, 28], and it was not a useful biological marker of bone remodelling in polyostotic Paget's bone disease.

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