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Relationship between Bone Formation Markers Bone Alkaline Phosphatase, Osteocalcin and Amino-terminal Propeptide of Type I Collagen and Bone Mineral Density in Elderly Men. Preliminary Results

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Abstract. *Bone remodeling is altered in all metabolic bone diseases, especially in post-menopausal women and in the elderly. Predicting changes in bone mineral density (BMD) is useful to manage the progression of such diseases and to potentially provide interventions in reducing fracture risk. Continuous bone formation and resorption processes can be monitored by measuring biochemical markers of bone turnover (BTMs) and a relationship between BMD and BTMs has been known for long. The aim of this study was to evaluate the relationship between BMD and serum BTMs bone alkaline phosphatase (BAP), osteocalcin and amino-terminal propeptide of type I collegen (PINP) in elderly (>65 years) men. We prospectively studied 18 elderly men (median age=69, range=65-77 years) with no history of fractures, angina, stroke, myocardial infarction or diabetes mellitus. Patients who had undergone corticosteroid, calcitonin, androgen or bisphosphonate therapy were excluded from the study, as well as those who were vitamin D and calcium supplementation users. All the patients underwent lumbar-spine (L2-L4) dual-energy x-ray absorbtiometry and BMD, BAP, osteocalcin and PINP measurements. The mean BMD and body mass index (BMI) were 0.963±0.04 g/cm^2 and 24.4±1.2 kg/m^2, respectively. BAP, osteocalcin and PINP were 27.8±11.3 U/l, 25.6±7.1 ng/ml and 36.0±7.5 ng/ml, respectively. No correlation was found between BMD and BAP (R=−0.28, p=0.25), osteocalcin (R=−0.18, p=0.48) and PINP (R=−0.21, p=0.39), nor between BMI and both age (R=0.05, p=0.83) and BMD (R=0.10, p=0.67). In conclusion, we did not find any relationship between bone formation markers BAP, osteocalcin and PINP and bone density. Thus, our preliminary data suggest that BTMs are not useful in monitoring the bone mineral status of elderly men.*

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Osteoporosis is a systemic skeletal disease characterized by deterioration of bone microarchitecture, leading to mechanical fragility and increased risk of fracture (1). Osteoporosis is usually diagnosed based on the T-score of bone mineral density (BMD) ≤2.5 standard deviations (SD) lower than peak bone mass, according to World Health Organization guidelines (2).

Bone remodeling is altered in all metabolic bone diseases, especially in post-menopausal women and in the elderly. Usually, bone loss is lower in men than in women, but osteoporosis in men represents a severe condition, probably more frequent than generally expected (1, 3). Predicting changes in BMD is useful to manage the progression of the disease and potentially allow interventions in reducing fracture risk. Continuous bone formation and resorption processes can be monitored through measuring biochemical markers of bone turnover (BTMs) and a relationship between BMD and BTMs has been known for long (4, 5).

The aim of this study was to evaluate the relationship between BMD and serum BTMs bone alkaline phosphatase (BAP), osteocalcin and amino-terminal pro-peptide of type I collegen (PINP) in elderly (>65 years) men.

Patients and Methods

We prospectively studied 18 elderly men (median age=69, range=65-77 years) with no history of fractures, angina, stroke, myocardial infarction or diabetes mellitus. Patients who had

undergone corticosteroid, calcitonin, androgen or bisphosphonate therapy were excluded from the study, as well as those who were vitamin D and calcium supplementation users. Patients with hyperparathyroidism, hypogonadism, renal or liver failure, or cancer were also excluded. Causes of secondary osteoporosis were excluded by physical examination and routine laboratory tests, including serum calcium, creatinine and parathyroid hormone (PTH) assays. Written informed consent was obtained from all participants.

All the patients underwent lumbar-spine (L2-L4) dual-energy x-ray absorbtiometry (DEXA) and BMD measurement (g/cm^2), as previously reported (6, 7). Blood samples obtained after overnight fasting were assayed in duplicate and the average was compared with the manufacturers' standard curves. BAP and osteocalcin were measured by immunoradiometric assay (IRMA), while PINP by radioimmunoassay (8). Serum intact PTH was measured through two-site chemiluminescent immunometric assay, using two goat monoclonal antibodies against human PTH, while both serum calcium and creatinine were measured spectrophotometrically, by standard laboratory methods (9).

The reported data are expressed as the mean±SD. Pearson's correlation coefficient (R) calculation was used to evaluate the linear relationship between pairs of variables. The differences were considered significant at a p-value <0.01.

Results

The mean BMD and body mass index (BMI) were 0.963±0.04 g/cm^2 and 24.4±1.2 kg/m^2, respectively. BAP, osteocalcin and PINP serum levels were 27.8±11.3 U/l, 25.6±7.1 ng/ml and 36.0±7.5 ng/ml, respectively (Figure 1).

No correlation was found between BMD and BAP (R=−0.28, p=0.25), osteocalcin (R=−0.18, p=0.48) or PINP (R=−0.21, p=0.39) (Figure 2). There was also no correlation between BMI and both age (R=0.05, p=0.83) and BMD (R=0.10, p=0.67). Only a weak inverse relationship between age and PINP (R=−0.52, p=0.02) was observed.

Discussion

With the aim of monitoring continuous bone remodeling and its mechanisms, a number of BTMs have been studied. The most common serum markers of bone formation are BAP, osteocalcin and PINP, while the most common markers of bone resorption are urinary markers, such as deoxypyridinoline, cross-linked-*N*-telopeptide of type I collagen, *C*-terminal telopeptide of type I collagen and serum telopeptide of type I collagen (1, 3). Measurements of BTMs in the serum are usually considered more reliable and accurate than urinary markers (5). Lower levels of BTMs appear to be associated with a lower risk of fracture in patients treated with bisphosphonates (10).

BAP is the bone-specific isoenzyme of total alkaline phosphatase, widely used in studying metastatic bone disease, while osteocalcin is useful whenever resorption and formation are uncoupled (11). Osteocalcin is a 5.8-kDa hydroxyapatite-binding bone-specific protein solely produced by osteoblasts, odontoblasts and hypertrophic chondrocytes (11, 12). This protein is considered a specific indicator of osteoblast activity, involved in bone remodeling (13, 14).

More than 90% of organic bone matrix consists of type I collagen, formed from precursor molecules (15). During normal bone catabolism, before fibril formation, type I collagen is degraded and small fragments pass into the bloodstream (16). PINP is one of the two pro-peptides of type I protocollagen (17). Several pre-analytical confounder variables regarding the use of BMTs have been reported. Prolonged use of corticosteroids and testosterone replacement therapy may reduce bone resorption marker levels (18, 19). Similar effects are described in users of alendronate and risedronate and in patients who underwent selective estrogen receptor modulator (SERM) therapy for prostatic cancer (20-22).

In women, the relationship between BTMs and BMD ranges from non-significant to moderate, but bone loss is usually more pronounced than in men (5, 23). Older men who are current smokers have poor trabecular microarchitecture, but no decrease in BMD (24). There are also regional differences in BMD and serum BTMs between patients who are residents of mountainous and seaside areas and the site (*i.e.* forearm, femoral neck, lumbar spine) in which DEXA is performed, may influence the accuracy of BTMs measurements (2). Scariano *et al.* (25) showed that in elderly women aged 60-90 years, PINP measurement had a diagnostic sensitivity of 83% and specificity of 64% for identifying patients with decreased BMD.

In men, there are several risk factors for fractures due to low BMD, including age, weight loss, physical inactivity, prolonged corticosteroid use and androgen deprivation therapy (26). Low BMD or high bone resorption may also increase the risk of myocardial infarction and stroke, in addition to fracture (27). Men classified as osteopenic by T-score criterion may have higher serum BAP and PINP levels compared with controls (28). In recent studies, measurement of BMD and osteocalcin was considered of value in estimating bone turnover rates and BAP was useful to predict the BMD reduction in patients with diabetes mellitus undergoing hemodialysis (29, 30). Unfortunately, we did not have similar results and no significant relationship was found between serum formation markers and BMD. However, other studies confirmed that serum PINP was inversely related to changes in BMD, but the sensitivity was low and not able to predict the rate of change (5).

Conclusion

In this group of elderly men, we did not find any relationship between bone formation markers BAP, osteocalcin and PINP, and bone density. Moreover, BTMs seem to be independent parameters. Thus, our preliminary data suggest that they are not useful in monitoring the bone mineral status of elderly men.

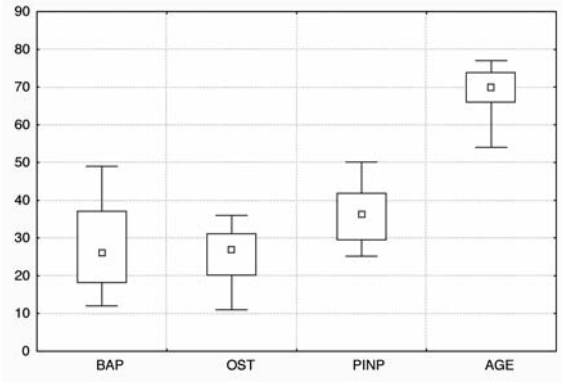

Figure 1. *Serum concentration of bone alkaline phosphatase (BAP, U/l), osteocalcin (OST, ng/ml) and amino-terminal propeptide of type I collegen (PINP, ng/ml), and age of the patients (AGE, years). Bars=range, boxes=mean±SD, □=median values.*

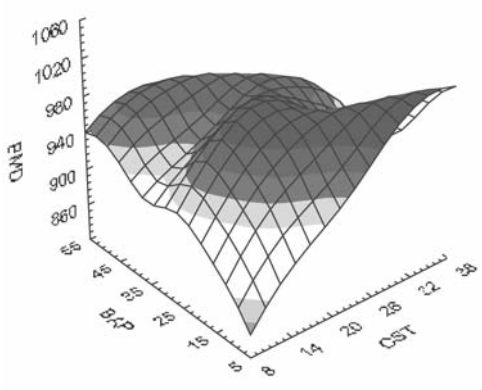

Figure 2. *Relationship between bone mineral density (BMD, g/cm^2), osteocalcin (OST, ng/ml) and bone alkaline phosphatase (BAP, U/l).*

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