Bone alkaline phosphatase isoenzyme in renal osteodystrophy

C. Jarava1, J. R. Armas2, M. Salgueira1 and A. Palma1

1Nephrology and 2Pathology Services, Hospital Universitario Virgen Macarena, 41071 Seville, Spain

Abstract. Serum total alkaline phosphatase is the most commonly used biochemical marker of bone disease in renal patients, but alkaline phosphatase originates from different organs and sometimes lacks specificity. Bone isoenzyme measurement is considered superior to total alkaline phosphatase for the assessment of bone metabolism. We have studied the value of bone isoenzyme, determined by a new IRMA (Tandem-R-Ostase), in haemodialysis patients with secondary hyperparathyroidism and renal osteodystrophy. Fifty-six haemodialysis patients were studied. Intact parathyroid hormone (PTH), osteocalcin, total alkaline phosphatase and bone alkaline phosphatase were determined. A transiliac bone biopsy was performed in 20 of the 56 patients after double tetracycline labelling. There was a significant correlation between bone alkaline phosphatase and PTH (r=0.79, P<0.001) and between bone and total alkaline phosphatase (r=0.84, P<0.001) in all patients. The patients who underwent a bone biopsy showed osteitis fibrosa in 17, mixed lesion in one, adynamic bone disease in one and normal bone in one. Bone alkaline phosphatase showed a significant correlation with static and dynamic histomorphometric indices similar to that obtained with PTH and better than those of total alkaline phosphatase and osteocalcin. It is concluded that bone alkaline phosphatase (ostase) seems to be a useful non-invasive marker of bone metabolism in patients on haemodialysis with high turnover bone disease. More studies are necessary to know its value in low turnover bone disease.

Key words: bone alkaline phosphatase; renal osteodystrophy

Introduction

Bone histomorphometry is accepted as the best method for the study of renal osteodystrophy. However bone biopsy is an invasive procedure accompanied by technical difficulties in the processing and studying of the specimens. This explains why bone biopsy is not widely performed and why numerous biochemical markers have been proposed for examining bone disease.

Serum alkaline phosphatase has been used as a biochemical marker of bone disease for many years. But total alkaline phosphatase originates from several organs (liver, bone, intestine, placenta etc.) and its measurement lacks specificity [1]. Recently a new method for serum bone alkaline phosphatase measurement has been developed. This method, Tandem-R-Ostase (Hybritech, San Diego, CA), is a two site IRMA that involves specific monoclonal antibodies to measure bone alkaline phosphatase in serum, and has been previously validated [1-4].

The aims of the present study were: (1) to determine the correlations between serum bone alkaline phosphatase and other biochemical parameters of bone metabolism in haemodialysis patients; and (2) to analyse the relationship between bone alkaline phosphatase and the histomorphometric parameters in a group of dialysis patients.

Subjects and methods

The study was carried out in 56 chronic renal failure patients being treated with chronic haemodialysis. They were 32 males and 34 females, aged 48±15 years (mean ±SD) and on haemodialysis treatment for 71±53 months. They were dialysed with a standard membrane and had been under treatment with aluminium hydroxide or calcium carbonate in different doses. None of the patients had clinical or biochemical evidence of liver disease. At least half of the patients were receiving oral calcitriol at a dose not greater than 1.5 μg/week, but they had stopped treatment no less than 15 days before the study was performed. Immediately before a dialysis session, blood samples were drawn from the arterial port for the assay of intact parathyroid hormone (PTH), osteocalcin, total alkaline phosphatase and bone alkaline phosphatase.

Bone biopsy was performed in 20 of the 56 patients. Transiliac bone biopsy was obtained with a 7 mm Bordier trephine after double tetracycline labelling which was separated by an interval of 10 days. Bone samples were fixed in 70% ethanol and then embedded in methylmethacrylate. Bone sections were stained with Masson-Goldner stain and the aurintricarboxylic acid method for the identification of aluminium at the mineral-osteoid interface. For histo-
dynamic evaluation ultraviolet light was used for the identification of the fluorescent tetracycline labels. The following histological parameters were measured: osteoblast surface (OcS/BS, %), osteoclast number per mm² tissue section (NOc/TA, mm²), osteoclastic surface (OcS/BS, %), volume of fibrosis (FbV/TV %), osteoid surface (Os/BS %), osteoid volume (OV/BV, %) and osteoid seam thickness (OTh, μm). The dynamic parameters evaluated were: mineral appositional rate (MAR, μm/day) and bone formation rate (BFR/BS, μm²/μm²/day). Bone biopsies were classified according to the method of Sherrard et al. [5,6].

**Analytical methods**

Intact PTH was measured by immunoradiometric assay (Allegro, Nichols Institute, San Juan Capistrano, CA, USA); the normal range for this assay is 10–65 pg/ml. Serum osteocalcin was measured by radioimmunoassay (INCSTAR Corporation, Rochester, Minnesota); the normal range is 5–35 ng/ml for males and 8–55 ng/ml for females. Total alkaline phosphatase (normal range 30–115 U/l) was determined by autoanalyser (SMAC, Technicon Instruments Corp., Tarrytown, New York, USA). Bone alkaline phosphatase was measured by an IRMA using two monoclonal antibodies directed against the human bone isoenzyme and bone alkaline phosphatase purified from human SAOS-2 osteosarcoma cells as a standard (Ostase, Hybritech, San Diego, CA, USA). Intra- and interassay coefficients of variation are less than 7% and 9%, respectively. The assay has shown a cross-reactivity of only 16% with the circulating liver isoenzyme [3]. The sensitivity of the assay is 0.2 ng/ml. The normal range for bone alkaline phosphatase is 8–16 ng/ml.

**Statistics**

Statistical analysis included the evaluation of correlation matrix, linear regression analysis and analysis of variance. Data are expressed as the mean ± SD.

**Results**

Mean humoral values of the patients under study are shown in Table 1, together with the normal ranges. Table 2 shows a correlation matrix. Mean values above the normal range suggest a secondary hyperparathyroidism in most of our patients. Bone alkaline phosphatase correlated significantly with the other humoral parameters, with a correlation coefficient of 0.79 (P < 0.001) with PTH and 0.84 (P < 0.001) with total alkaline phosphatase (Table 2).

The histopathological diagnoses in the 20 patients who had a bone biopsy were: severe osteitis fibrosa in 15, mild osteitis fibrosa in two, mixed lesion in one, adynamic bone disease in one and normal bone in one. Aluminium staining was negative in the 20 patients. Table 3 shows the correlation between the four humoral indices and the histomorphometric parameters. Humoral markers correlated significantly with most of the histomorphometric indices. Bone alkaline phosphatase and PTH showed the best correlation coefficients with both static and dynamic parameters. Figure 1 shows the relationship between bone alkaline phosphatase and several histomorphometric indices (OBS/BS, Fb/TV, NOc/mm², BFR).

**Discussion**

This study analyses the value of serum bone alkaline phosphatase, determined by a new IRMA (Ostase), as a marker of bone turnover in haemodialysis patients. Serum bone alkaline phosphatase is considered superior to the determination of total alkaline phosphatase activity for assessing bone metabolism [7]. Several methods have been developed to measure serum bone alkaline phosphatase, including heat inactivation [8], agarose gel electrophoresis [9], wheat germ lectin precipitation [10] and HPLC [11]. However, these methods have been considered to be cumbersome and not always specific and they have not received wide acceptance [1,3,7,12]. More recently, immunoassays
with monoclonal antibodies that preferentially distinguish between bone and liver isoenzymes have been developed [2,13,14]. We have used the new immunoradiometric assay (Tandem-R Ostase) [2] to measure the serum bone alkaline phosphatase in our patients. This assay has been used to study bone metabolism in patients with Paget disease of bone, postmenopausal osteoporosis, malignancies, primary hyperparathyroidism, and secondary hyperparathyroidism in patients with chronic renal failure [1,3,4,15,16]. Van Hoofer et al. compared the results of this IRMA with those of agarose electrophoresis for a group of patients including patients on haemodialysis [16]. They found a good correlation between the two methods except for low values of bone alkaline phosphatase and in some patients with increased concentrations of total alkaline phosphatase, both due to cross-reactivity of anti-bone alkaline phosphatase antibodies with liver isoenzyme. A similar study by Garnero et al. reported a cross-reactivity of the bone alkaline phosphatase (ostase) for the liver alkaline phosphate of 16% [3]. They found that this IRMA is a sensitive method to measure bone alkaline phosphatase and that, even in patients with liver disease, can be used to assess bone turnover when total alkaline phosphatase does not exceed 2.6-fold of the upper limit of the normal range. Our patients had no clinical or biochemical evidence of liver disease and total alkaline phosphatase never exceeded those limits. In our group of haemodialysis patients serum bone alkaline phosphatase correlated positively and significantly with other biochemical markers of renal osteodystrophy. This is in agreement with other previous studies that include haemodialysis and CAPD patients and also predialysis patients [17,18]. Serum bone alkaline phosphatase also showed a significant correlation to both static and dynamic histomorphometric parameters, with $r$ values similar to those of PTH and greater than those of total alkaline phosphatase and osteocalcin, as has been previously reported [18].

Bone alkaline phosphatase is a tetrameric glycoprotein found on the cell surface of active osteoblasts lining the osteoid tissue undergoing the mineralization process [19,20]. Consequently it is commonly considered a marker of bone formation [20]. However, in some pathological states both events, bone formation and bone resorption, may be coupled and in balance and the biochemical markers will reflect the overall rate of bone turnover [20]. This explains why bone alkaline phosphatase shows a similar correlation to histomorphometric parameters of formation and resorption.

Most of our patients had osteitis fibrosa. As a
consequence our study can only suggest that bone alkaline phosphatase (ostase) may be a useful marker of high bone turnover in patients on haemodialysis, as has been previously reported [16,18]. More studies are necessary to know the usefulness of this IRMA in patients with low bone turnover (osteomalacia, adynamic bone disease).

References