Renal Osteodystrophy: α-Heremans Schmid Glycoprotein/Fetuin-A, Matrix GLA Protein Serum Levels, and Bone Histomorphometry

Giorgio Coen, MD, Paola Ballanti, PhD, Alessandro Balducci, MD, Fabio Grandi, PhD, Micaela Manni, MD, Daniela Mantella, MD, Andrea Pierantozzi, PhD, Maria Ruggeri, PhD, Daniela Sardella, PhD, Giovanni Sorbo, MD, and Ermanno Bonucci, MD

• ***Background:*** **Fetuin-A of hepatic origin circulates in large amounts in serum, but also is expressed in bone, where it is an inhibitor of transforming growth factor β (TGF-β)/bone morphogenetic protein (BMP) proteins. Together with matrix GLA protein (MGP), fetuin-A is able to make up a complex with calcium and phosphate that is more soluble than calcium and phosphate alone, preventing its deposition in extraskeletal tissues. Experimental results suggested that this complex is made at bone tissue level. The aim of this study is to evaluate whether serum fetuin-A and MGP are influenced by type of renal osteodystrophy, they correlate with bone histomorphometric and histodynamic parameters, and/or serum levels may influence bone turnover.** ***Methods:*** **Thirty-eight hemodialysis patients who volunteered to undergo a bone biopsy were studied. Patients (27 men, 11 women) had a mean age of 55.2 ± 11.8 years and dialysis vintage of 75.7 ± 57.4 months. They were not administered vitamin D or drugs connected with mineral metabolism. They underwent transiliac bone biopsy after tetracycline labeling. Biopsies were performed for histological, histomorphometric, and histodynamic evaluation and aluminum histochemistry. Serum fetuin-A and MGP were measured by using enzyme-linked immunosorbent assay kits.** ***Results:*** **Serum fetuin-A levels were significantly less than normal, whereas MGP levels were less than the normal average. Fetuin-A levels in patients with hyperparathyroidism, mixed osteodystrophy, and low-turnover osteodystrophy were 0.219 ± 0.1, 0.27 ± 0.1, and 0.197 ± 0.1 ng/mL, respectively (*P* = not significant). Fetuin-A level significantly correlated inversely with values for several histomorphometric parameters, such as osteoid volume (OV/BV), osteoblastic surface (Ob.S/BS), osteoid surface (OS/BS), and osteoclastic surface (Oc.S/BS). Logistic regression showed odds ratios of 5.3 and 4.9 for the association of high fetuin-A levels with low values for OS/BS and Ob.S/BS, respectively. Results of multiple regression analysis with intact parathyroid hormone and fetuin-A levels as independent variables and OV/BV and Ob.S/BS as dependent variables showed that independent variables correlated significantly with dependent variables, positively for intact parathyroid hormone levels and inversely for fetuin-A levels. MGP levels in patients with hyperparathyroidism, mixed osteodystrophy, and low-turnover osteodystrophy were not significantly different (3.94 ± 0.86, 3.40 ± 0.99, and 5.64 ± 2.4 nmol/L, respectively). By dividing MGP serum values into tertiles, mean values for OV/BV were different (analysis of variance, *P* < 0.04), with a greater value in the higher MGP tertile. By exclusion of 3 extravariant cases (>3 SDs greater than the mean), 1 case for each type of osteodystrophy, a significant correlation between bone formation rate and MGP serum level was found (*P* < 0.05). In addition, a significant correlation was found between MGP level and trabecular thickness.** ***Conclusion:*** **Fetuin-A and MGP levels correlated with bone formation parameters. This association could be caused by an effect of these proteins on bone formation, presumably mediated by the TGF-β/BMP system. Fetuin-A, as opposed to MGP, is known to inhibit the TGF-β/BMP complex, a protein-cytokine system that appears to be an important regulator of bone formation and probably a factor with an important role in renal osteodystrophy.** ***Am J Kidney Dis*** **48:106-113.**

INDEX WORDS: Renal osteodystrophy; fetuin-A; matrix GLA protein; bone biopsy; histomorphometry; transforming growth factor β/bone morphogenetic protein.

FETUIN-A, or α-Heremans Schmid glycoprotein, is a protein synthesized by the liver and circulating in large amounts in serum.[1,2] It is a negative acute-phase reactant protein with decreased levels after an inflammatory insult, and it accumulates in bone, where it has not-yet well-

From the Ospedale Israelitico; Department of Experimental Medicine and Pathology, La Sapienza University; Departments of Nephrology and Laboratory Medicine, S. Giovanni-Addolorata Hospital; Department of Nephrology, San Camillo Hospital; Department of Laboratory Medicine, Policlinico TorVergata, Rome; and Hospal SpA, Bologna, Italy.

Received January 10, 2006; accepted in revised form March 31, 2006.

Originally published online as doi:10.1053/j.ajkd.2006.03.083 on May 30, 2006.

Support: This study has been made possible by funds of Ospedale Israelitico, Rome, and of Hospal SpA, Bologna. Potential conflicts of interest: None.

Address reprint requests to Giorgio Coen, MD, Via Dandolo 75, 00153 Rome, Italy. E-mail: giorgio.coen@fastwebnet.it

0272-6386/06/4801-0012$32.00/0

doi:10.1053/j.ajkd.2006.03.083

defined functions. In addition, it can be expressed in cartilage[3] and is considered an inhibitor of the transforming growth factor β (TGF-β)/bone morphogenetic protein (BMP) family.[4] Experimental studies of fetuin-A knockout mice showed impairment of growth plate chondrocyte maturation and retardation in the longitudinal growth of femurs.[5] There also was accelerated trabecular bone remodeling with an increase in number of osteoblastic cells. In the absence of fetuin-A, results were in favor of increased activity of the TGF-β/BMP system, a protein system with osteoinductive properties.

Matrix GLA protein (MGP) is a vitamin K–dependent protein containing carboxyglutamic acid[6] that is like osteocalcin and some coagulation factors, namely prothrombin. It is a 10-kd molecular-weight secreted protein expressed by a wide variety of tissues and cells. Cells shown to express MGP messenger RNA include chondrocytes, vascular smooth muscle cells, kidney cells, and fibroblasts, but also osteoblasts.[7] MGP seems to have a role in the regulation of the process of cartilage calcification.[8]

In experimental conditions, fetuin-A, together with MGP, is able to make up a complex with calcium and phosphate that is more soluble than calcium and phosphate alone, preventing deposition of the salt in extraskeletal tissues. Price and Lim[9] showed that the fetuin-A–MGP–calcium–phosphate complex was formed experimentally in animals treated with etidronate, a bisphosphonate able to block the mineralization of osteoid. The overflow of calcium-phosphate, caused by mineralization impairment, could be buffered, and extraskeletal calcium deposition could be prevented by formation of the complex. Therefore, circulating fetuin-A and MGP levels, although derived from other organs and cell systems, could be influenced by bone turnover in patients with renal osteodystrophy.

The aim of this study is to evaluate whether levels of these proteins are linked to histological type of renal osteodystrophy, they correlate with histomorphometric and histodynamic parameters, and/or serum levels may influence bone turnover.

METHODS

The study was carried out with 38 hemodialysis patients with a wide range of serum intact parathyroid hormone (iPTH) levels who volunteered to undergo bone biopsy. Patients (27 men, 11 women) had a mean age of 55.2 $\pm$ 11.8 years and a mean dialysis age of 75.7 $\pm$ 57.4 months. Causes of terminal renal failure were chronic glomerulonephritis in 9 patients, hypertension/ischemic nephropathy in 6 patients, tubulointerstitial nephropathy in 2 patients, polycystic kidney disease in 5 patients, diabetic nephropathy in 3 patients, obstructive nephropathy in 3 patients, and unknown in 10 patients.

Patients were not administered vitamin D compound. However, most patients had been treated previously with relatively limited doses of calcitriol administered orally or intravenously (1.5 to 3.5 μg/wk), but had discontinued this treatment at least 3 months before bone biopsy in 2 patients and more than 5 months in the other patients. They were not administered anticoagulants, corticosteroids, nonsteroidal anti-inflammatory drugs, antiepileptic drugs, or estrogens. Phosphate binders were mainly calcium salts and sevelamer. In the majority of patients, regular erythropoietin intravenous treatment was underway. All patients were treated with standard hemodialysis, 12 hours a week, divided into 3 sessions.

Patients underwent transiliac bone biopsy with a Bordier trocar after a double-labeling course with oral tetracycline, with a 12-day interval. The biopsy, performed 3 to 5 days after the end of tetracycline administration, was for histological, histomorphometric, and histodynamic evaluation and aluminum histochemistry. At the same time, a blood sample was drawn for the following assays: calcium, phosphate, iPTH, total alkaline phosphatase, serum fetuin-A, and MGP.

Serum fetuin-A was measured by using an enzyme-linked immunosorbent assay kit (Epitope Diagnostics Inc, San Diego, CA); normal values are 0.519 $\pm$ 0.15 g/L (25 healthy control subjects of the same age as the hemodialysis cohort). Intra-assay and interassay coefficients of variation were 6.8% and 8.2%, respectively. MGP was measured by using an enzyme-linked immunosorbent assay provided by Biomedica (Wien, Austria); normal values for 25 subjects were 6.2 $\pm$ 3.5 nmol/L. Intra-assay and interassay coefficients of variation were 5.5% and 8%, respectively.

Serum PTH was measured by means of a commercial (Nichols Institute Diagnostics, San Juan Capistrano, CA) immunoradiometric assay, directed to the "intact" molecule, based on a double-antibody technique. Both 1-84 and "7-84" PTH species are measured together. Normal range of values is 10 to 65 pg/mL.

Serum total calcium was determined by means of a spectrophotometric assay using cresolphthalein as substrate. Serum phosphate and alkaline phosphatase measurements were performed spectrophotometrically (DU-65; Beckman, Fullerton, CA), using molybdate and *p*-nitrophenyl-phosphate as respective substrates. Normal ranges for the adult population are 2.5 to 4.5 mg/dL and 60 to 170 U/L, respectively.

Bone specimens were fixed by using 4% paraformaldehyde in 0.1 mol/L of phosphate buffer (pH 7.2). They then were longitudinally halved, dehydrated in acetone, and embedded without decalcification in glycol-methacrylate, as previously described.[10] Sections were cut with a Reichert-Jung Autocut microtome (Leica Microsystems, Wetzlar, Germany) equipped with a tungsten carbide