A Case of Renal Osteodystrophy with Unexpected Serum Intact Parathyroid Hormone Concentrations

Danni L. Meany,1 Suzanne M. Jan de Beur,2 Mary Jo Bill,1 and Lori J. Sokoll1*

CASE

A 64-year-old female with long-standing end-stage renal disease (ESRD), status post 2 failed renal transplants, was evaluated for management of renal osteodystrophy with particular concern for adynamic bone disease (ABD). ABD was suspected because of low normal serum intact parathyroid hormone (PTH) concentrations (range 2.5–54 ng/L, reference range 10 – 65 ng/L), intermittently increased serum calcium concentrations (range 88 –107 mg/L, reference range 84 –105 mg/L), and severe osteoporosis. However, her mildly increased serum alkaline phosphatase activities (range 149 –196 U/L, reference range 30 –120 U/L) were inconsistent with the low bone turnover observed in ABD. This discrepant clinical profile prompted investigation into the PTH assay used at our institution. Simultaneous samples were analyzed for intact PTH on our Roche Elecsys 2010 immunoassay analyzer and at a reference laboratory (Quest Diagnostics) on the Siemens Immulite 2000 immunoassay analyzer. Discrepant values of 48 and 786 ng/L were obtained, respectively.

DISCUSSION

Parathyroid hormone functions to maintain serum calcium concentrations within a tight physiologic range. Patients with chronic renal failure develop secondary hyperparathyroidism owing to decreased renal production of 1,25-dihydroxyvitamin D and hyperphosphatemia, both of which result in hypocalcemia. These derangements in mineral metabolism stimulate PTH production to raise serum calcium and promote phosphorus excretion. Increased serum PTH leads to excessive bone resorption through stimulation of osteoblasts and osteoclasts. The combination of secondary hyperparathyroidism and mineralization defects (osteomalacia) represents the most common form of renal osteodystrophy (ROD). In contrast, a subtype of ROD known as adynamic bone disease can be observed in the setting of prolonged peritoneal or hemodialysis, oversuppression of PTH with calcitriol or calcium-based phosphate binders, or the use of bisphosphonates for osteoporosis treatment (1). ABD is characterized by marked suppression of bone remodeling that leads to fracture. Common biochemical hallmarks of ABD include hypercalcemia, low or inappropriately normal PTH concentrations, and reduced markers of bone turnover (e.g., alkaline phosphatase) (2).

In this case, the increased intact PTH result from the reference laboratory (786 ng/L) was more consistent with the patient’s clinical picture, suggesting that the 48 ng/L value obtained in our clinical laboratory was falsely low. The magnitude of the difference between the 2 results suggested an assay interference as opposed to method differences or specificities (3), and therefore the following studies were performed. Dilution studies confirmed the presence of a negative interference, since PTH concentrations were higher in the diluted samples than in the undiluted sample (undi-luted, 48 ng/L; diluted 1:20, 567 ng/L). Treatment with heterophilic blocking reagent (Scantibodies Laboratory) had no effect (intact PTH posttreatment 68 ng/L), suggesting that heterophile antibodies or human antianimal antibodies (HAAAs) were not likely to be the cause of the interference.

Further review of the patient’s medical history revealed that she was ingesting 10 mg biotin per day for restless leg syndrome and had been doing so for at least the past 2 years. In humans, biotin is a coenzyme for 4 important carboxylases in fatty acid synthesis, branched-chain amino acid catabolism, and gluconeogenesis (4, 5). Although not a widely used regimen, high doses of biotin (10 mg/day) have been reported to improve symptoms of encephalopathy and peripheral neuropathy in patients with renal failure and undergoing chronic hemodialysis (6). In addition, it has been used for patients with diabetic peripheral neuropathy (7, 8).

Biotin is recognized as a potential interferent in PTH and other assays on the Elecsys platform, and it is recommended in the product insert that samples from
patients receiving high biotin doses of $>5$ mg/day be collected at least 8 h after biotin administration (9). In the Elecsys intact PTH assay, a biotinylated anti-PTH monoclonal antibody and a ruthenium-labeled anti-PTH monoclonal antibody form a sandwich complex with PTH, after which streptavidin-coated microparticles are added to magnetically separate out the sandwich complex via biotin and streptavidin interaction. Specimens with high concentrations of biotin may prevent the binding of the sandwich complex to the streptavidin-coated microparticles, thus giving falsely low signals. In contrast, the Immulite 2000 intact PTH assay does not use biotin–streptavidin interaction. Despite the fact that the sample in question was collected >8 h after biotin administration, as recommended by the manufacturer, we hypothesized that biotin was in fact the cause of the falsely low result. Due to insufficient volume of our clinical laboratory specimen (serum), the plasma (EDTA) specimen sent to the reference laboratory was obtained and used for subsequent investigational experiments. When this plasma sample was analyzed by the Roche Elecsys intact PTH assay (suitable for both serum and EDTA plasma), undiluted and diluted results from this plasma sample were similar to our previous results.

An aliquot of the specimen was sent to Cambridge Biomedical Research Group for determination of the biotin concentration using a bioassay. The biotin concentration in the specimen was 4.8 $\mu$g/L, approximately 10-fold higher than the reference range upper limit (200–500 ng/L). Two different approaches were subsequently used to examine the interfering role of biotin: (1) studying the effect of added biotin on the Roche assay and (2) removing the effect of biotin (10), in this case using streptavidin-coated microparticles. The first approach was carried out by adding various amounts of free biotin (0, 5, 10, 20, 40, 80, 160 $\mu$g/L) (Sigma-Aldrich) into sera with normal and increased intact PTH concentrations (33 and 487 ng/L, respectively). The recovery curves shown in Fig. 1 illustrate that biotin did inhibit the measured recovery of PTH. Surprisingly, addition of 5 $\mu$g/L of biotin, a concentration similar to the concentration in the patient’s specimen, caused a $<3\%$ decrease in PTH concentration compared with an untreated serum specimen with a normal PTH concentration, and no decrease at all in a specimen with an increased PTH concentration. Only when biotin concentrations were increased to 160 $\mu$g/L did PTH results decrease $>50\%$. Per the manufacturer, intact PTH results are purportedly unaffected (recovery within 10% of the target) when biotin concentrations are $<50$ $\mu$g/L (9). In contrast, the second approach using streptavidin microparticles clearly identified biotin as the interference. In this approach, 50 $\mu$L of the patient’s specimen was incubated with the streptavidin microparticles (magnetically extracted from 250 $\mu$L of reagent M from the intact PTH immunoassay) for 1 h at room temperature with shaking. Because the microparticles were isolated out of solution, this treatment did not dilute the patient’s specimen. In this experiment, the intact PTH concentration in the patient’s specimen increased from 32 ng/L pretreatment to 419 ng/L posttreatment. The PTH recovery was $>1000\%$ compared to recoveries of approximately 80% in 3 control specimens from patients not taking large doses of biotin.

The discordant results from these 2 investigations may be due to differences between the free biotin we spiked in and biotinylated molecules such as enzymes and metabolites endogenously present in this patient’s specimens. As described above, biotin is a coenzyme for 4 carboxylases. In its physiologically active form, biotin is covalently attached to these carboxylases via an amide linkage to a specific lysine residue (4). In addition, catabolism of biotin leads to formation of many metabolites such as bisnorbiotin and biocytin. We measured the biotin concentration in the patient’s specimen using a microbial growth assay that underestimates biotinylated molecules (11), suggesting that the actual concentration of biotinylated molecules present may be substantially higher than 4.8 $\mu$g/L. Hence, the first approach by addition of free biotin into

![Fig. 1. Effect of biotin on serum intact PTH concentrations determined using the Roche Elecsys 2010 analyzer.](image)

Percent recovery was calculated as the ratio of PTH concentration after the addition of biotin at various concentrations (5, 10, 20, 40, 80, 160 $\mu$g/L) to the PTH concentration without biotin addition. The solid diamond and solid squares indicate specimens with normal and increased concentrations of intact PTH (33 and 487 ng/L, respectively).
Upon Authors’ Disclosures of Potential Conflicts of Interest: the published article.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Employment or Leadership: None declared.
Consultant or Advisory Role: S.M. Jan de Beur, Kirin Pharma.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.

The PTH sera did not appear to mimic the interference of endogenously biotinylated molecules present, whereas the second approach, treating the specimen with streptavidin microparticles, eliminated the interfering biotin.

To further confirm the interfering role of biotin, serum intact PTH concentrations were measured in both laboratories after the patient stopped taking biotin for 2 weeks. The Elecsys 2010 result, 158 ng/L, was consistent with the reference laboratory PTH concentration measured on the Immulite 2000 (223 ng/L) using the same specimen. Interestingly, although the biotin concentration was still increased at 1.3 μg/L, this increase did not result in assay interference on the Elecsys 2010 analyzer, as indicated by similar PTH concentrations before and after streptavidin microparticle treatment (data not shown).

To summarize, using the streptavidin microparticle treatment, we found that the biotinylated molecules present in specimens from patients with renal impairment who take high doses of biotin may interfere with immunoassays that use biotin streptavidin mechanisms in their assay designs. An in vitro interference study using exogenous biotin failed to mimic the interference, which may be due to the discrepancy between exogenous biotin and endogenous biotinylated molecules. As in the case of this patient, the delay in initiating appropriate therapy for severe secondary hyperparathyroidism in ESRD has important clinical consequences that can include tertiary hyperparathyroidism and progressive hyperparathyroid bone disease.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Acknowledgments: The authors gratefully acknowledge the assistance of Roger Frye, Quest Diagnostics, Baltimore, MD, in the investigation of this case.

References

Commentary

Tom Cantor

ABD is a serious complication of ESRD that leads to soft tissue/vascular calcification and ultimately to myocardial infarction, the leading cause of death in ESRD patients. Proper diagnosis of ABD is important because administration of potent vitamin D analogs to a patient with ABD (as would be appropriate for a patient with high bone turnover) must be avoided. The gold standard for diagnosing ABD is the invasive bone biopsy. Measurements of serum calcium and alkaline phosphatase are not reliable for ABD. However, bone-specific alkaline phosphatase can be used as a marker of ABD. Bone biopsy studies have demonstrated that iPTH is nondiagnostic for ABD when concentrations are $>10.6 \text{ pmol/L}$; however, if iPTH is $<10.6 \text{ pmol/L}$, there is a $>85\%$ probability of ABD (1). The Roche Elecsys iPTH assay was calibrated to the former Nichols Allegro iPTH IRMA (the assay on which treatment guidelines are based), whereas the Siemens DPC iPTH assay generates values that are on average 50% higher than the Roche assay. Therefore, the case study patient can be reliably concluded to have ABD, and the DPC iPTH should not be used to guide therapy.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: T. Cantor, Scantibodies Laboratory.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Reference


Commentary

Keith Hruska

Meany et al. present a novel cause of a current major problem in clinical medicine, difficulty in accurately diagnosing chronic kidney disease–mineral bone disorder (CKD-MBD), a term coined by the Kidney Disease: Improving Global Outcomes foundation to replace ROD. The basis for the change in terminology stems from the realization that the skeletal and mineral disorders complicating kidney disease are critical in the pathogenesis of the excess cardiovascular mortality risk associated with CKD. The cardiovascular risk for the presented 64-year-old woman, who was on dialysis after 2 failed kidney transplants and had severe osteoporosis, was extreme. In this patient’s CKD-MBD, diagnosis of the skeletal remodeling disorder was going to guide critical therapy. The case presentation portrays the weakness in the current standard practice, which is in the process of being replaced. The current practice is that iPTH assays are used to relate to past bone biopsy studies and to predict the state of bone remodeling. A bone biopsy would have been indicated in the patient presented, but this is not the standard practice in 2009. Multiple studies have defined the weakness of the standard practice employed here, and the various assays for iPTH used to estimate bone remodeling have numerous problems in addition to the novel biotin interference discovered in this case.

The finding of the adynamic bone disorder would have been associated with attempts to increase bone remodeling by correcting the hypercalcemia and in-
creasing the actions of PTH. The increased PTH concentration results from the reference laboratory and the high alkaline phosphatase concentration confirmed that the patient actually had high-turnover CKD-MBD contributing to her osteoporosis. This finding diametrically changes the treatment strategy to controlling the effects of CKD on the cardiovascular system by decreasing the serum phosphorus and on the PTH concentration by using a calcimimetic agent, because the presence of hypercalcemia contraindicates increasing the dose of vitamin D analogs.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: K. Hruska, Genzyme, Shire, Fresenius, and Stryker.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: K. Hruska, Shire, and Genzyme.
Expert Testimony: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.