Hypophosphatemia Associated with Paraproteinemia: A Case Report and Review of the Literature

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ABSTRACT

The differential diagnosis for hypophosphatemia is long, and involves complex, overlapping physiological systems. Practitioners are often guilty, however, of simply supplementing phosphate without fully investigating the etiology of the problem. The purpose of this case presentation is to illustrate a case of spurious hypophosphatemia that initially led to unnecessary phosphate replacement in a woman with undiagnosed multiple myeloma.

An 85-year-old African American woman was admitted to the hospital for congestive heart failure exacerbation. The patient was incidentally found to be profoundly hypophosphatemic and was also diagnosed with multiple myeloma at this hospitalization. Normal phosphorus levels were difficult to maintain despite aggressive replacement. A serum sample initially reported to have an abnormally low phosphorus concentration on the Beckman CX7 analyzer was reanalyzed with the Kodak Ektachem 700 system, revealing the phosphorus concentration to be towards the higher limit of the normal range on the same sample.

We conclude that clinicians should proceed with caution before aggressively treating abnormal phosphorus levels in patients with known paraproteinemia. Conversely, unexplained phosphorus abnormalities should bring disorders associated with paraproteinemia, such as multiple myeloma, into the differential diagnosis. Knowledge of how various phosphorus assays are affected by paraproteins is essential to guiding diagnosis and treatment. We also review mechanisms of reported interference with common assays.

INTRODUCTION

Phosphate level is a commonly ordered test in hospitalized patients. Between 5% and 10% of hospitalized patients have hypophosphatemia.[1] Normal phosphate level in adults is approximately 2.5-4.8 mg/dL.[2] The cause of the hypophosphatemia is sought and found in only a few of these patients. In others, the etiology remains elusive.

Extracellular phosphate is in flux with 4 major systems: the intestines, the kidneys, bone, and cells. The typical adult takes in about 1 gram of phosphorus per day. Phosphate absorption occurs very easily; except for the portion bound to calcium in the feces, almost all dietary phosphate is absorbed into the blood. Absorption of calcium and phosphate are greatly enhanced by 1, 25-dihydroxycholecalciferol produced in the kidneys under the influence of parathyroid hormone (PTH). Renal phosphate excretion is thought to occur by an overflow mechanism. When plasma phosphate is below a critical value, almost all of the renal phosphate is absorbed. When levels are above the critical value, the extra phosphate is excreted at a rate proportional to the increase. Having an independent effect, PTH greatly increases phosphate excretion in the kidneys by reducing its proximal tubular reabsorption. In bones, hydroxyapatite (a calcium and phosphate salt) is a major storage pool for phosphate. Osteoclasts, under the influence of active vitamin D and PTH, not only release phosphate from bones, but also proliferate, leading to further increase in extracellular phosphate levels.[3]

Approximately 85% of total body phosphate is stored in bones, 14%-15% in cells, and only <1% is found in extracellular fluid. In cells, phosphorus is a component of ATP and 2, 3 DPG, both essential to cell survival. Phosphorus in the plasma is mainly found in 2 forms: HPO_4^{2-} and $H_2PO_4^-$, in a ratio of about 4:1 respectively. Because it is difficult to measure serum

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phosphate levels, assays measure total inorganic phosphorus, typically reported in milligrams per deciliter.[2,3]

Detailed explanations of commonly used phosphorus assays have been given elsewhere.[2,4-6] Briefly, most commercially available systems use a variation of the Fiske and Subbarow acid filtrate method.[4-6] In this method, serum proteins are precipitated by trichloroacetic acid and centrifuged from the serum; acid molybdate is then added to the supernatant, to convert phosphate to a phosphomolybdate complex. The addition of a reducing agent develops the phosphomolybdate complex into a blue-colored complex, the measured absorbance of which is proportional to the concentration of inorganic phosphorus in the solution. Variations on this method, used in many automated systems, rely on either serial dilution of the serum sample or a multilayered slide system, rather than trichloroacetic acid precipitation of proteins, prior to the formation of the phosphomolybdate complex. Some assays do not utilize a reducing agent prior to measurement of absorbance. We present the case of an 85-year-old woman in whom low serum phosphate levels were found to be secondary to laboratory assay interference from an unrelated disease process.

CASE HISTORY

An 85-year-old woman with type 2 diabetes mellitus, hypertension, and longstanding heart failure presented to the emergency department with acute shortness of breath, chest tightness, and weakness. She also complained of increased leg swelling over the past 2 weeks and progressive weight loss with poor appetite over the last 6-8 months. Her heart failure had been controlled with diuretics. Due to recent renal insufficiency, the patient's furosemide had been held. Prior to the current episode, she had never been hospitalized for heart failure. Her medical history was also significant for gout, monoclonal gammopathy, and anemia of unclear etiology. Medications included ferrous sulfate, metoprolol, lisinopril, rosiglitazone, pantoprazole, colchicine, and epoetin alfa. The patient was alert and appeared mildly short of breath. Physical examination was significant for a blood pressure of 179/91, jugular venous distension, and an increased pulmonic component of the second heart sound. Pulmonary examination revealed right basal rales with decreased breath sounds at the left base. She also had pitting edema up to the midcalf. Pertinent laboratory results included a white blood cell count of 2.3 x 103/uL (normal range=4 x 103/uL-10 x 103/uL), hemoglobin 8.7 g/dL (normal range=13-17 g/dL), hematocrit 27.1% (normal range=39%-51%), mean corpuscular volume 103.9 fL (normal range=80-99 fL), platelet count 210 x 103/uL (normal range=150 x 103/uL-400 x 103/uL), B-type natriuretic peptide >5000 pg/mL (normal range=0-99 pg/mL), and a creatinine of 1.8 mg/dL (baseline creatinine of 1.2 mg/dL about 6 months prior to this examination). Three sets of cardiac enzymes drawn at 8 hourly intervals were within normal limits. Serum phosphate was 2.7 mg/dL. Chest X-ray showed a moderate-sized left pleural effusion.

The patient was admitted for exacerbation of CHF and acute renal failure. The patient responded well to nesiritide infusion and furosemide. In addition to metoprolol, the patient was started on digoxin, hydralazine, and isosorbide dinitrate. Echocardiogram revealed a very low ejection fraction (25%-30%), moderately severe pulmonary hypertension (right ventricular systolic pressure of 51 mmHg), global hypokinesis, and mild left ventricular hypertrophy.

On the second hospital day, the patient was incidentally found to have a critically low phosphate level of 1.2 mg/dL. She was given aggressive intravenous and oral phosphate supplementation. The patient's phosphate was kept within normal limits using 2 packets of 7.125/7.125-mEq potassium phosphate/sodium phosphate powder orally 3 times a day. This, however, resulted in significant abdominal discomfort, bloating, and flatulence. When the oral supplementation was stopped, the patient's phosphorus level dipped to 1.4mg/dL in less than 24 hours. A combination of intravenous and oral supplementation was continued.

Further investigation of the hypophosphatemia showed intact PTH levels of 93 pg/mL and 136 pg/mL on 2 different occasions (normal 10-65 pg/mL); 25-hydroxy vitamin D was 4.6 ng/mL (normal 10-60 ng/mL).

The patient's anemia was originally thought to be multifactorial. Colchicine was held due to concerns about bone marrow toxicity. The patient was also vitamin B_{12} deficient (171 pg/mL, normal range 300-900 pg/mL). Given the patient's anemia and leukopenia, however, along with a history of monoclonal gammopathy, an investigation for possible plasma cell dyscrasia was undertaken. Serum protein electrophoresis revealed a serum total protein of 10.6 g/dL (normal range=6.2-8 g/dL) with an IgG fraction of 7470 mg/dL and immunofixation revealed a monoclonal IgG spike. Urine protein electrophoresis revealed a monoclonal IgG spike and free Kappa light chains. β2-microglobulin level was 15.3 mg/L and reticulocytes were 0.9%. There was Rouleaux formation on peripheral smear. Bone marrow biopsy revealed extensive infiltration by plasma cells (47%). Bone survey showed no lytic lesions.

Given the new diagnosis of multiple myeloma, the question was raised whether the patient's disease might be contributing to hypophosphatemia. Fractional excretion of phosphate was estimated to be 82.9%. Urine glucose was <20mg/dL and urine bicarbonate was <10 mmol/L. A serum sample initially shown to have a phosphate level of 1.4mg/dL on the Beckman CX7 autoanalyzer (Beckman Instruments, Brea, Calif) was sent for analysis at an outside lab to evaluate whether the low phosphorus levels were spurious. A Kodak Ektachem 700 system (Kodak, Rochester, NY) revealed the phosphorus level to be 4.6 mg/dL on the same sample.

DISCUSSION

The differential diagnosis for hypophosphatemia is broad.[7] The consequences of hypophosphatemia can be devastating. Reported clinical manifestations include rhabdomyolysis, osteomalacia, cardiomyopathy and arrythmia, respiratory insufficiency, delirium, seizures, neuropathy, hemolysis, and leukocyte dysfunction.[7] In the above case, the patient did present with cardiac, respiratory, and hematologic symptoms that could have been attributed to hypophosphatemia. The etiology could have been attributed to elevated PTH (though mild) or vitamin D deficiency. Cases of proximal tubular dysfunction, causing isolated hyperphosphaturia, have also been reported.[8] There are other case reports of more severe proximal tubular dysfunction (Fanconi's syndrome) associated with multiple myeloma.[9] Our patient, by the original laboratory measurements, had evidence of renal phosphate wasting, without evidence of glycosuria or bicarbonaturia to suggest Fanconi's syndrome. This phenomenon was likely due to unnecessary supplementation, but increased PTH and vitamin D deficiency were also present. The increased PTH and decreased vitamin D levels were likely the result of poor nutrition and chronic kidney disease. Ultimately it was found that the low phosphorus levels were all likely spurious.

Several cases in the literature report spurious phosphate abnormalities associated with paraproteinemia. Weisbord and colleagues[10] report a case of MGUS (monoclonal gammopathy of undetermined significance, characterized by the production of an abnormal M-protein by non-cancerous plasma cells in the absence of other manifestations typical of multiple myeloma) and a case of multiple myeloma, both of which presented with unexplained hypophosphatemia <1 mg/dL. In both cases, the spurious value was given by a Synchron LX20 system (Beckman Coulter, Fullerton, Calif). Samples were re-analyzed using a different instrument (Vitros Chemistry System; Ortho-Clinical Diagnostics, Rochester, NY), giving phosphorus values well within normal limits. Similar spurious cases are reported on the Beckman Synchron CX7[11] (Beckman Coulter, Fullerton, Calif), and a Beckman CX7 autoanalyzer[12] (Beckman Instruments, Brea, Calif). In both cases the spurious values were overcome by manual protein precipitation and assay, or by using a completely different automated system,[12] (Kodak Ektachem 700 system, Kodak, Rochester, NY).

Spurious hyperphosphatemia has also been reported in association with paraproteins. Marcu and Hotchkiss report a case of a patient with multiple myeloma who presented with a phosphorus level of 12.5mg/dL in the setting of normal renal function.[13] A deproteinized sample showed the phosphorus to be within the normal range. Barutçuoğlu and colleagues report an even more striking case (levels 20.2-40.7 mg/dL), with levels normalized by deproteinization prior to analysis.[14]

Serum from patients with paraproteinemia seems to result in spurious values with some assays, but not with others. Most problems have been attributed to methods that do not precipitate proteins prior to determination of phosphorus, thereby attributing interference directly to paraproteins in the sample. Loghman-Adham and colleagues, however, were able to achieve similar results using a manual acid filtrate determination and with an automated assay that does not use prior precipitation of proteins, but does additionally utilize a reductant to stablize the phosphomolybdate complex prior to measurement of absorbance.[12] They speculate that IgG from their patient interfered with the stablization of the phosphomolybdate complex; the Kodak system's addition of a reductant (p-methylaminophenol sulfate) cancelled this effect. The Vitros system used by Weisbord and colleagues to reanalyze samples uses the same "stabilizing" reductant.[10] It seems that either prior deproteinization or reductant stabilization of the phosphomolybdate complex may be effective for accurately analyzing samples containing paraproteins.

CONCLUSION

Despite previous case reports demonstrating paraprotein interference with commonly used phosphate assays, this relationship is not well known to clinicians. In cases with abnormal phosphate levels, where the etiology remains unclear, clinicians must question whether the abnormalities are spurious. Unexplained phosphate abnormalities must be investigated further to rule out significant gammopathy, especially in patients at risk for multiple myeloma. Finally, in patients with known

paraproteinemia, samples should be deproteinized prior to analysis (manually or otherwise), or alternatively, should be run on an automated assay known to be accurate for samples containing paraproteins. In order to ensure quality measurements, a study analyzing serum from a larger number of patients using a variety of phosphorus assays needs to be done to further elucidate the mechanism of interference in this special population of patients.

REFERENCES

1. Halevy J, Bulvik S. Severe hypophosphatemia in hospitalized patients. *Arch Intern Med.* 1988;148(1):153-155.
2. Bauer JD. *Clinical Laboratory Methods.* Saint Louis: The C.V. Mosby Company; 1982.
3. Guyton A, Hall J. *Textbook of Medical Physiology.* Philadelphia: W.B.Saunders; 2000.
4. Fiske C, Subbarow Y. The colorimetric determination of phosphorus. *J Biol Chem.* 1925;66:375-400.
5. Gomori A. A modification of the colorimetric phosphorus determination for use with a photoelectric photometer. *J Lab Clin Med.* 1942;27:955-960.
6. Bartlett GR. Phosphorus assay in column chromatography. *J Biol Chem.* 1959;234:466-468.
7. Subramanian R, Khardori R. Severe hypophosphatemia: pathophysiologic implications, clinical presentations, and treatment. *Medicine.* 2000;79(1):1-8.
8. Dash T, Parker M, Lafayette R. Profound hypophosphatemia and isolated hyperphosphaturia in two cases of multiple myeloma. *Am J Kidney Dis.* 1997;29(3):445-448.
9. Lacy MQ, Gertz MA. Acquired Fanconi's syndrome associated with monoclonal gammopathies. *Hematol Oncol Clin North Am.* 1999;13(6):1273-1280.
10. Weisbord S, Chaudhuri A, Blauth K, Derubertis F. Monoclonal gammopathy and spurious hypophosphatemia. *Am J Med Sci.* 2003;325(2):98-100.
11. Malhotra A, Koduri P. Polyclonal hyperglobulinemia and spurious hypophosphatemia. *Ann Intern Med.* 1999;131(4):314.
12. Loghman-Adham M, Walton D, Iverius P, Deiss A, Knight J, Cheung A. Spurious hypophosphatemia in a patient with multiple myeloma. *Am J Kidney Dis.* 1997;30(4):571-575.
13. Marcu CB, Hotchkiss M. Pseudohyperphosphatemia in a patient with multiple myeloma. *Conn Med.* 2004;68(2):71-72.
14. Barutçuoğlu B, Parildar Z, Mutaf I, Habif S, Bayinder O. Spuriously elevated inorganic phosphate level in a multiple myeloma patient. *Clin Lab Haematol.* 2003;25:271-274.

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