

Pseudohyperphosphatemia in a Patient with Multiple Myeloma, Measurement Dependent Interference

Short title: Pseudohyperphosphatemia in Multiple Myeloma

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ABSTRACT

Background: Pseudohyperphosphatemia in patients with multiple myeloma is described in literature as analytical interference or phosphates binding to monoclonal protein. We suspected pseudohyperphosphatemia in 69 years-old patient with IgG kappa type of multiple myeloma (Phosphates >3 mmol/L, normal concentrations of creatinine, parathormon and vitamin D). The aim was to prove pseudohyperphosphatemia and to determine its origin.

Methods: Inorganic phosphate was measured by routine molybdate method in untreated serum and after deproteination by sulfosalicylic acid. Correlation coefficient of phosphate in untreated serum and monoclonal immunoglobulin concentrations between September 2016 and February 2018 was calculated. The reaction was manually simulated and the detailed record of measurement from the analyzer was scrutinized retrospectively. Total phosphorus concentration was measured by mass spectrometry in untreated and deproteinated serum of this patient, as well as in control group of 10 samples.

Results: Inorganic phosphate concentrations were 3.38 mmol/L in untreated and 1.17 mmol/L in deproteinated serum. Correlation coefficient $R_2=0.8948$ between phosphate and monoclonal immunoglobulin was measured. Formation of turbidity during manual simulation and gradual increase in absorbance in measurement record were observed.

Total phosphorus concentrations were 3.60 mmol/L before and 1.20 mmol/L after deproteination, within 95% confidence interval of control group (3.01-4.12 mmol/L untreated; 0.79-1.28 mmol/L deproteinated samples).

Conclusion: Noticeable difference in phosphates in untreated and deproteinated serum together with the normal results of total phosphorus confirm pseudohyperphosphatemia caused by analytical interference rather than binding to monoclonal protein. Rising numbers of patients with myeloma, shows necessity to check for possible interferences and adopt appropriate measures.

Keywords: Pseudohyperphosphatemia; Multiple myeloma; Interference; Phosphate assay; Mass spectrometry

1. Introduction

A 69 year old male suffered from disturbance of cardiac rhythm. In the course of examination, anemia, high serum protein and immunoglobulin G levels were discovered. Subjectively, he didn't have any medical problems and felt healthy. Sternum bone marrow aspiration biopsy confirmed 26 % of pathological plasmatic cells. X-ray image of entire skeleton surprisingly did not show any deposit, only on calva, there were visible changes assessed only as suspicious brightening. Patient had lower level of hemoglobin, 101 g/L even after receiving two transfusions of erythrocyte concentrate one week earlier. His original hemoglobin level was significantly under 100 g/L. The concentration of total protein was 101 g/L, concentration of monoclonal immunoglobulin class G was 32.4 g/L. Patient had normal creatinine level and normal glomerular filtration (estimated as CKD-EPI, 1.01 mL-s-1.73m²). Calcium concentration, as well as parathormon were within reference range. There was surprisingly high concentration of inorganic phosphate, 3.36 mmol/L (RI 0.87-1.45 mmol/L). Patient met criterion for establishing the diagnosis of multiple myeloma by relatively massive infiltration of marrow by pathological plasmocytes and by the presence of monoclonal immunoglobulin of high concentration. The diagnosis was set up as symptomatic IgG kappa type monoclonal myeloma, II A, ISS I. The symptom leading to the starting of the treatment was anemia.

The first course of treatment was started in September 2016 and ended in March 2017. The concentration of monoclonal immunoglobulin lowered from 32.4 g/L to 11.0 g/L, which was more than 50%. Considering that the plato phase was reached, the concentration of monoclonal immunoglobulin was stable during final three months and did not lower any further, it was not beneficial to continue in this treatment. The patient was offered maintenance treatment. This treatment was ended in August 2017 as the illness was not sensitive to it, the concentration of monoclonal immunoglobulin was slightly increasing and was 20 g/L at the time of discontinuation of the treatment. Subsequently, the illness was slowly progressing and in December 2017 the next course of treatment was started. The last visit and last application of the treatment was in March 2018. During the last three months of the treatment the concentration of monoclonal immunoglobulin lowered from 20 g/L to 9.1 g/L. Regrettably, the patient died of complications related to existing disease in March 2018.

The laboratory results corresponded to the diagnosis, except from phosphate concentration, which was unusually increased.

Hyperphosphatemia can be caused by lowered phosphate excretion during chronic renal insufficiency or acute kidney failure, hypoparathyroidism or vitamin D intoxication. Other reasons are increased phosphate intake, increased release from bones (metabolic or respiratory acidosis, osteolytic metastases) or phosphate release from cells during tissue ischemia, tumor lysis syndrom or massive infection¹.

None of these seemed to be the case, as the patient did not suffer from renal insufficiency, calcium and parathormon levels were within reference range and all of the other laboratory values were common given the hematologic malignity. Therefore spurious increase (pseudohyperphosphatemia) was suspected.

Rare cases of pseudohyperphosphatemia in patients with myeloma are already described in literature and are assigned to either of two possibilities. Firstly, the interference with molybdate UV assay used for the measurement was described²⁻⁸

caused by the reaction with monoclonal protein during the measurement leading to increased turbidity of the sample. The other possible cause is the binding of serum phosphate to the monoclonal protein and therefore its increased levels, which, at the same time, does not affect its biologically available amounts in patients serum and presumably does not have any clinical significance^{2-4,9}, even though Mandry⁹ remarks, that the clinical insignificance cannot be globally confirmed. Most of the described cases talk about IgG kappa type^{4,6-8,10-14} less about IgG lambda type^{3,10} or IgA type^{2,12} of multiple myeloma. There is also a description of correlation of phosphate concentration on monoclonal protein concentration^{6,7,10,14,15}. The decrease of pseudohyperphosphatemia/phosphatemia caused by plasmapheresis or chemotherapy was also described in some cases^{2,10-12,16}.

Regardless the suspected cause of hyperphosphatemia, either different method of measurement or measurement of protein-depleted serum^{2-8,13-16} is recommended to exclude the phenomena, as the interference is dependent on the method used for phosphate measurement. While it was often proven in determination with ammonium molybdate^{3-10,12-16} which is nowadays the most used method for phosphate determination in practice, no interference was proven neither with the methods, where the proteins are separated prior to measurement^{5,10,13-15}, nor with atomic spectrometry^{4,5,10}.

The aim of this study was to prove pseudohyperphosphatemia in this patient and to specify its origin by determination of phosphorus concentration by absolute method, mass spectrophotometry and comparison of results with phosphorus concentrations found in serum of healthy control group.

2. Methods

As a routine method for inorganic phosphate measurement, 2-point end molybdate UV method, Phos2 (Hoffman-La Roche, Basel, Switzerland; further as Roche)¹⁷ was used, on c702 module of cobas 8000 system (Roche). Inorganic phosphate forms an ammonium phosphomolybdate complex with ammonium molybdate in the presence of sulfuric acid. The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration and is measured photometrically, using main wavelength of 340 nm and subsidiary wavelength of 700 nm¹⁷.

As a method for total phosphorus measurement, inductively coupled plasma mass spectrometry (ICP-MS) Agilent 7500ce (Agilent Technologies, Tokyo, Japan) was used. The collision reaction cell was used for suppressing of spectral interferences on the mass 31P. For the quantification purpose and suppressing of matrix effect, the method of standard addition was used. The samples were 100 times diluted by deionized water, and a known amount of P was added before the analysis. For the blank and deproteinated samples, the addition was 0, 500 and 1000 ng of P, for the native sample 0, 1500 and 2500 ng of P. ICP-MS measure a total amount of phosphorus (inorganic phosphates, organic phosphorus bound in phospholipid structures, inorganic phosphorus bound to the proteins).

The deproteination of samples were done by sulfosalicylic acid as was previously used in literature^{2-4,6,7,14,16}. The 20% sulfosalicylic acid was added to serum, usually in 1:10 ratio. In case of high residual protein concentration (more than 10 % of the total protein levels in untreated serum), the 1:5 ratio was used. Then the samples were mixed for one minute on vortex and then centrifuged for 5 min at 2500 RCF. After the deproteination, only inorganic phosphate is still present in the supernatant.

The untreated patient sample was measured by UV method and the measurement was repeated after deproteination. The phosphate concentration in untreated serum and monoclonal protein concentrations were measured 16 times between September 2016 and February 2018 and the correlation coefficient between them was counted.

We also simulated the automatic molybdate UV method manually in our laboratory by following the protocol described in instructions for use with the original reagents from the reagent pack, only in volume 10 times higher, to actually see the process and to compare any potential visible abnormalities with normal sample behavior.

To determine the origin of the interference in this case, the patient's samples collected over time were pooled, divided into two aliquots and the second aliquot was deproteinated. The concentrations of phosphates before and after the deproteination were measured by cobas 8000 and the concentrations of total phosphorus before and after deproteination were measured by ICP-MS. The concentration of phosphorus would be higher than concentrations of phosphates, so simple comparison of the values not be enough to assess the cause of the interference. Because of this, we also measured the phosphorus concentration in 10 samples from healthy control group with the same procedure. We worked on the assumption that in case of interference by increased turbidity the total phosphorus levels measured by ICP-MS would not show significant difference between the concentrations measured in a sample from our patient and samples from healthy control, which would not be the case if the pseudohyperphosphatemia was caused by biologically unavailable phosphates bound to monoclonal protein.

3. Results

We measured the untreated and deproteinated serum of patient with myeloma. While the inorganic phosphate concentration in untreated serum reached 3.38 mmol/l, the value in deproteinated serum was 1.17 mmol/L and well within the reference interval (RI 0,87-1,45 mmol/L). We repeated the measurement between September 16 and February 2018 and summarized the results in (Figure 1).

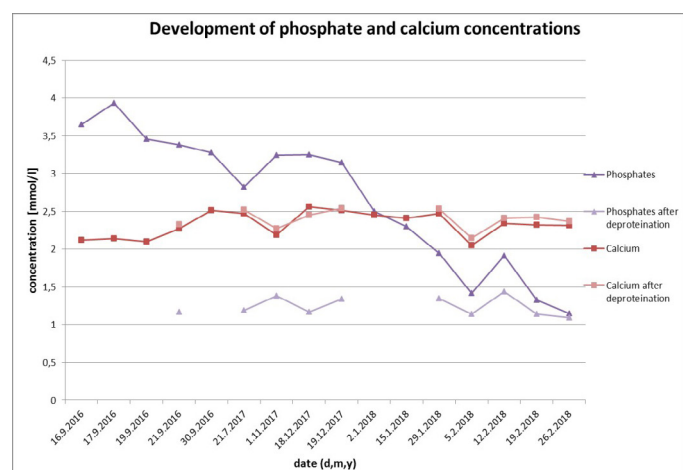


Figure 1: The phosphate and calcium concentrations before and after deproteination. While the calcium concentration remains the same after the deproteination, the concentrations of phosphate decrease after the deproteination. There is a significant drop in untreated serum phosphate concentrations during the time caused by treatment of monoclonal gammopathy.

We simulated the automatic process of the colorimetric method in our laboratory manually, by following the instructions

for use, modified by multiplying the volumes by 10, to visualize the reaction in reaction tubes. After addition of R1, a very strong turbidity formed in the test tube with our patient sample, contrary to the test tube with healthy control which remained clear. We also scrutinized the measuring record from c702. In the diagram from reaction monitor option, there was a visible rise in absorbance continually increasing during all measurement (Figure 2).

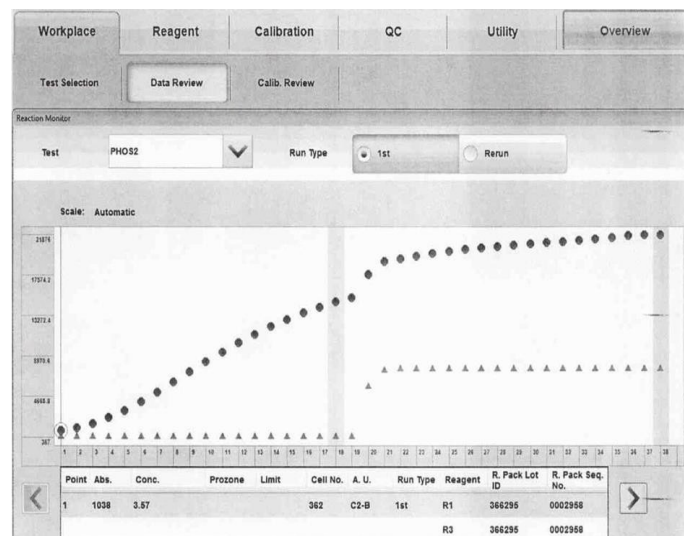


Figure 2: Measuring record from c702, dots represent measured absorbance of patient samples in times, triangles represent normal course of measurement, based on quality control. The absorbency of the sample continually increases during the measurement, even before the addition of reagent R2 in time point 17.

To understand the nature of interference, we measured the phosphates in pooled serum from the patient with myeloma before and after deproteination by molybdate UV method. The concentration in untreated pooled serum was 3.13 mmol/L, after the deproteination, the concentration decreased to 1.19 mmol/L. In the same samples, we measured the concentration of total phosphorus before and after deproteination by ICP-MS. The concentration of phosphorus in untreated pooled serum was 3.6 mmol/L, after deproteination it lowered to 1.2 mmol/L. There were no significant differences in concentration of phosphate and total phosphorus in deproteinated pooled serum.

As reference interval of total phosphorus in serum is not well defined, we determined phosphate and phosphorus concentrations in 10 samples from healthy control, in untreated and deproteinated sera. The phosphorus concentrations varied between 3.00 and 4.49 mmol/L, with the mean of 3.50 mmol/L (95% CI 3.16-3.84 mmol/L) and median of 3.40 mmol/L (95% CI 3.01-4.12 mmol/L). The measured concentration of phosphorus of our patient (3.60 mmol/L) lies within these concentrations. Phosphorus concentrations in deproteinated sera varied between 0.6 mmol/L and 1.32 mmol/L, with the mean of 1.01 (95% CI 0.85-1.17) mmol/L and median of 1.01 (95% CI 0.787-1.276) mmol/L.

4. Discussion

The significant difference between the phosphate concentrations measured in untreated and deproteinated serum (3.38 and 1.17 mmol/L, respectively) from patient with myeloma confirmed the assumption of spuriously increased concentrations of inorganic phosphates, which lowered during the time as a result of the treatment of monoclonal gammopathy (Figure 1). The spuriously increased phosphate concentrations showed positive

linear correlation to the monoclonal protein concentrations with $R^2=0.8948$. The phosphorus concentration measured by ICP-MS in the sample from patient with myeloma and in 10 samples from healthy control group showed no difference between the patient sample and the control group. That led us to believe that the spurious increase of phosphates is caused by interference with molybdate UV assay rather than by biologically unavailable phosphates bound to the monoclonal protein. This was confirmed by observing unusual turbidity in the tube with patient sample, when we simulated the automatic process manually, in opposition to the tubes with healthy control samples, which remained clear. Also, the retrospective inspection of the diagram gained from reaction monitor option of the analyzer showed a visible rise in absorbance, which was gradually increasing during the measurement, which assured us of our conclusion. We assume that it is the turbidity forming continuously during the 10 minute measurement and thus creating the interference. Similar finding had been observed in reaction curve from Advia 1650 (Siemens Healthineers, Erlangen, Germany) with the same method principle⁸. The number of patients diagnosed with multiple myeloma is growing, which can be among other reasons related to the rising median of life expectancy¹⁸. The database of Brno University Hospital comprise of 200 patients with diagnosed multiple myeloma. Retrospectively, we discovered other two more patients with pseudohyperphosphatemia. These cases with other rare cases described in literature^{2-4,6-12,14,16} show, that it is necessary to keep this interference in mind when evaluating the results. In case of any suspicious result, we suggest to use the procedure with deproteination by sulfosalicylic acid, as used in literature^{2-4,6,7,14,16}.

5. Conflict of Interest

None declared.

6. References

- Jabor A. Vnitřní prostředí. 1st ed. Praha: Grada, 2008:530.
- Maden M, Pamuk GE, Asoglu V, et al. The rapid resolution of pseudohyperphosphatemia in an IGAκ multiple myeloma patient after therapy with a bortezomib-containing regimen: Report of the first case. *J Cancer Res Ther* 2015;11(4):1043.
- Vaidya GN, Bhattad VB, Aggarwal A. Pseudohyperphosphatemia in multiple myeloma: a commonly misdiagnosed phenomenon. *Science Postprint* 2014;1:e00039.
- BouchtliE, BelkhouAB, YounsiR, et al. Pseudohyperphosphatemia in multiple myeloma. *Joint Bone Spine* 2007;74(2):206-207.
- Larner J. Pseudohyperphosphatemia. *Clin Biochem* 1995;28(4):391-393.
- Lee Y, Koo T, Yi JH, et al. Pseudohyperphosphatemia in a patient with multiple myeloma. *Electrolyte Blood Press* 2007;5(2):131-135.
- Barutçuoğlu B, Parildar Z, Mutaf I, et al. Spuriously elevated inorganic phosphate level in a multiple myeloma patient. *Clinical & Laboratory Haematology* 2003;25(4):271-274.
- Stratta P, Canavese C, Quaglia M, et al. A patient with unexplained hyperphosphataemia. *Nephrol Dial Transplant* 2006;21(9):2664-2666.
- Mandry JM, Posner MR, Tucci JR, et al. Hyperphosphatemia in multiple myeloma due to a phosphate-binding immunoglobulin. *Cancer* 1991;68:1092-1094.
- Sonnenblick M, Eylath U, Brisk R, et al. Paraprotein interference with colorimetry of phosphate in serum of some patients with multiple myeloma. *Clin Chem* 1986;32(8):1537-1539.
- Oren S, Feldman A, Turkot S, et al. Hyperphosphatemia in multiple myeloma. *Ann Hematol* 1994;69:41-43.
- Mavrikakis M, Vaiopoulos G, Athanassiades P, et al. Pseudohyperphosphatemia in multiple myeloma. *Am J Hematol* 1996;51:178-179.
- Chakraborty S, Sen S, Gupta D, et al. Spurious hyperphosphatemia in a case of multiple myeloma. *Indian J Clin Biochem* 2014;29(2):250-252.
- Izzedine H, Camous L, Bourry E, et al. The Case | The case presentation. *Kidney Int* 2007;72:1035-1036.
- McClure D, Lai LC, Cornell C. Pseudohyperphosphataemia in patients with multiple myeloma. *J Clin Pathol* 1992;45:731-732.
- Kiki I, Gundogdu M, Kaya H. Spuriously high phosphate level which is promptly resolved after plasmapheresis in a patient with multiple myeloma. *Transfus Apher Sci* 2007;37(2):157-159.
- Phosphate (Inorganic) ver.2 (package insert). Basel: Roche Diagnostics Documentation; 2017.
- Hájek R, Jarkovsky J, Maisnar V, et al. Real-world Outcomes of Multiple Myeloma: Retrospective Analysis of the Czech Registry of Monoclonal Gammopathies. *Clin Lymphoma Myeloma Leuk* 2018;18:219-240.