



Asymptomatic elevated parathyroid hormone level due to immunoassay interference

M.B. Zanchetta¹ · E. Giacoia¹ · F. Jerkovich¹ · E. Fradinger¹

Received: 5 November 2020 / Accepted: 5 April 2021 / Published online: 24 April 2021
© International Osteoporosis Foundation and National Osteoporosis Foundation 2021

Abstract

Background Parathyroid hormone (PTH) measurement using immunoassays is inherently vulnerable to interferences due to the presence of different proteins such as heterophile antibodies, human anti-animal antibodies, auto-analyte antibodies, the rheumatoid factor, and others. The frequency of immunoassay interference can be as high as 6%. We report the case of a patient showing persistent high levels of PTH without impact on calcium and bone metabolism.

Case presentation The patient was a 59-year-old asymptomatic woman who consistently showed elevated PTH levels (385–482 pg/ml) using the Roche Elecsys (Cobas e-411) and ADVIA Centaur assays, with normal calcium, phosphorus, vitamin D, and renal function parameters. She had no history of fractures, nephrolithiasis, gastrointestinal complaints, renal insufficiency, or autoimmune diseases. Her physical examination revealed no abnormalities. Biomarkers of bone metabolism were within the reference range. To rule out falsely elevated PTH levels, we initially performed serial dilutions using both assays, which revealed nonlinearity. After a polyethylene glycol precipitation test, less than 10% of PTH was recovered from the supernatant. These results suggested the presence of heterophile antibodies as the cause of the falsely elevated PTH levels.

Conclusion Physicians should be aware of this issue in order to avoid unnecessary clinical investigations and inappropriate treatments.

Keywords Parathyroid hormone · Immunoassays · Heterophile antibodies · Polyethylene glycol precipitation test

Introduction

Immunoassays for measuring parathyroid hormone (PTH) use a “sandwich” technique with a solid phase antibody targeting one epitope of the analyte and the signal antibody targeting a different epitope. Despite its sensitivity, these immunoassays are inherently vulnerable to immunoglobulin-related interferences [1]. These interferences might be due to the presence of heterophile antibodies, human anti-animal antibodies, auto-analyte antibodies, the rheumatoid factor, and other proteins [2]. The frequency of immunoassay interference can be as high as 6%, depending on the type of antibody interference [3]. Physicians should be aware of this issue so as to avoid unnecessary clinical investigations and inappropriate treatments.

Here, we report the case of persistently high levels of PTH in an asymptomatic woman with no impact on calcium and bone metabolism, which was finally demonstrated to be an immunoassay interference, most probably due to the presence of heterophile antibodies.

Case presentation

A 59-year-old woman consistently showed elevated PTH levels (385–482 pg/ml) with normal calcium, phosphorus, vitamin D, and renal function parameters (Table 1). She was asymptomatic. The PTH measurement was ordered by a physician from another institution as part of a screening for osteoporosis and then was referred to us. She had osteopenia and was receiving cholecalciferol 2400 IU per day and had a normal calcium diet. She had no history of fractures, nephrolithiasis, gastrointestinal complaints, renal insufficiency, or autoimmune diseases. Her family history was unremarkable. Her weight was 64.8 kg (body mass index: 24 kg/m²), and the physical examination revealed no abnormalities.

✉ M.B. Zanchetta
mbzanchetta@idim.com.ar

¹ IDIM, Universidad del Salvador, Libertad 836, C1012 AAR Buenos Aires, Argentina

Table 1 Laboratory results

	At presentation	After 12 mo	After 24 mo	Reference range
PTH (pg/ml)	482	385	432	10–65
Calcium (mg/dl)	9.5	9.8	9.5	8.5–10.5
Phosphorus (mg/dl)	3.9	4.2	4.3	2.5–4.5
Creatinine (mg/dl)	0.8	0.8	0.7	0.6–1.2
β -CTx (ng/ml)	0.193	0.250	0.461	<0.550
25OHD (ng/ml)	31.6	26.5	25.8	
ALP (IU/L)		186	66	30–120
Bone-specific ALP (μ l)		7.9	8	<21.3
Osteocalcin (ng/ml)	21.5	26.9	26.2	11.0–43.0
TSH (μ U/ml)	1.59	1.56	1.32	0.4–5.6
24-hour urinary calcium (mg/24 hs)	154			<220

PTH parathyroid hormone, *β -CTx* C-terminal cross-linked telopeptide of type I collagen, *25OHD* 25-hydroxyvitamin D, *ALP* alkaline phosphatase, *TSH* thyroid stimulating hormone. PTH, β -CTx, and osteocalcin values were measured by the Elecsys Roche (Cobas e-411), electrochemiluminescence assay (Roche Diagnostics GmbH, Mannheim, Germany). Calcium, phosphorus, and creatinine were measured using Cobas Integra/cobas c systems (Roche Diagnostics GmbH, Mannheim, Germany). Bone-specific ALP and 25-hydroxyvitamin D were measured using Liaison XL Assay (Diasorin Inc., Stillwater, MN, USA). ALP was measured by Beckman Coulter AU chemistry analyzers, Brea, CA, USA. TSH was measured by Beckman Coulter ACCESS (3rd IS).

Biomarkers for bone metabolism (β -cross laps, alkaline phosphatase, and osteocalcin) were within the reference range, and the 24-h urine calcium excretion was normal.

Initially, PTH had been measured using the Elecsys Roche (Cobas e-411), electrochemiluminescence assay (Roche Diagnostics GmbH, Mannheim, Germany). As falsely elevated PTH level was suspected, the measurement was repeated with the same patient sample using the ADVIA Centaur, chemiluminescence assay (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The PTH value with this analytical platform was 540 pg/ml. Subsequently, we performed serial dilutions using both assays which showed nonlinearity (Table 2). A non-linear pattern is seen when samples do not return consistent dilution-adjusted concentration measurements across multiple dilutions. Therefore, 250 μ l of the patient serum was subjected to precipitation with 250 μ l of polyethylene glycol (PEG) 6000. After centrifugation, PTH was measured using Roche Elecsys assay in the supernatant, and the result was corrected for dilution. After the PEG precipitation test, less than 10% of PTH was recovered from the supernatant (PTH value 42.1, normal values: 10–65 pg/ml). These

results suggested the presence of macroimmunocomplex as the cause of the falsely elevated PTH levels.

Discussion

There are few case reports in the literature of asymptomatic elevated PTH level as a result of immunoassay interference [4–8], mostly due to the presence of heterophile or anti-animal antibodies.

Heterophile antibodies are the most common cause of interference in two-site immunoassays [3]. Although they are found in 30–40% of all serum samples, they lead to interference in only 0.5–3% of the cases [9]. Cavalier et al. [10] showed that in a series of 743 patients with an elevated PTH level, 3.4% presented interference due to heterophile antibodies and 1.2% due to the rheumatoid factor.

The source of these antibodies in the serum of patients has been associated with the use of therapeutic monoclonal antibodies [4] or a monoclonal gammopathy [5]. The patient reported in our case, however, had not received therapeutic monoclonal antibodies, and she had no clinical evidence of gammopathy in a serum protein electrophoresis.

There is no universal way to detect assay interference. The presence of laboratory values that are inconsistent with the clinical picture is key and relies on close communication between physicians and laboratorians.

Our initial approach for identifying interference was to make serial dilutions of the sample. We found nonlinearity, which is expected in the presence of interfering antibodies. Our second step was to use PEG precipitation. In clinical practice, PEG precipitation is routinely used to assess

Table 2 Serial dilutions of the patient serum sample

PTH assay	Without dilution	1/2 dilution	1/4 dilution	1/10 dilution
Elecsys Roche (pg/mL)	482	380	315	300
ADVIA Centaur (pg/mL)	540	660	420	380

PTH parathyroid hormone

macroprolactinemia. PEG, however, precipitates not only macrocomplexes but also soluble immunoglobulins and may also have precipitated heterophile antibodies in our patient. One of the problems with PEG is that there are no clear diagnostic cutoff limits for postprecipitation recoveries. A recovery rate of less than 40% is used for the diagnosis of macroprolactinemia. However, in our patient, we obtained a recovery of less than 10% which made the presence of a macromolecular interference undeniable.

Once an assay interference is suspected, another option might be to analyze the sample using alternate assays that employ antiserum raised in different species [3]. As in other reports [4–6], our patient had falsely elevated PTH when measured with Roche and ADVIA Centaur immunoassays. Both platforms use monoclonal anti-PTH antibody of mouse origin, so we concluded that the interference must have been caused by anti-murine antibodies. In the case of van der Doelen et al. [5], however, the interference was detected using the Abbott Architect PTH assay but not when using the Roche Elecsys assay. Interestingly, in the case of Prodan et al. [6], falsely elevated PTH levels were detected in both Abbott and Roche immunoassays. Antibodies should be called heterophiles when there is no history of medicinal treatment with animal immunoglobulins or other well-defined immunogens, and the interfering antibodies can be shown to be multispecific [11], as in our patient. Gulbahar et al. [10] reported a case in which immunoassay interference was found for several hormones simultaneously, including prolactin, TSH, ACTH, PTH, FSH, and b-human chorionic gonadotropin. Prolactin and TSH levels in our patient were within the reference level. Since the presence of heterophile antibodies was the strongest hypothesis in our case, we decided not to assess the patient sample on another different analytical platform as it would have been of no clinical relevance.

PTH assays use different antibodies against PTH and recognize not only biologically active full-length PTH but also PTH fragments. This explains the large variations between results with different assays. Therefore, the comparability between assays must be improved. Standardization of PTH is mandatory. The International Federation of Clinical Chemistry (IFCC) proposed the use of an international reference standard (IS 95/646) so that all assays could be calibrated against it, improving the between-method agreement [12]. To our knowledge, none of the second-generation assays are calibrated against IS 95/646. In addition, a PTH reference measurement procedure is needed. Liquid chromatography coupled with tandem mass spectrometry was proposed by the IFCC as a promising candidate, but the analytical sensitivity must be improved.

In the case reports mentioned above, interference in PTH real values can vary from slightly elevated to more than 5000 pg/ml [4–8], reflecting different mechanisms in which these antibodies can exert their interference.

PTH assessment is indicated to exclude secondary causes of osteoporosis [13]. However, primary hyperparathyroidism is now commonly discovered in the form of normocalcemic primary hyperparathyroidism [14]. This entity is defined by an elevated PTH, normal serum calcium concentration, and a 25-hydroxyvitamin D level > 20 ng/ml in the absence of renal or liver disease, malabsorption, hypercalciuria, uncontrolled thyroid disease, or another metabolic bone disease [15]. Bone density in these patients is, on average, within the osteopenic range, and these subjects should be monitored regularly for progression of their disease [14, 15]. Taking this into consideration, it is not clear if PTH measurements should be included in an initial bone mass evaluation, as occurred in our patient. Anyway, differently from our case, patients with normocalcemic primary hyperparathyroidism have slightly inappropriately elevated PTH (no more than twice as high as the upper normal values), and PTH values progress over time.

Conclusion

This case highlights the importance of good communication between clinicians and laboratory staff when a laboratory result does not match the clinical picture. Although falsely high PTH levels due to immunoassay interference are rare, clinicians need to keep in mind its existence to avoid unnecessary diagnostic or therapeutic procedures.

Declarations

Ethics approval The study was in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication Written informed consent was obtained from the patient.

Conflict of interest None.

References

1. Klee GG (2004) Interferences in hormone immunoassays. *Clin Lab Med* 1:1–18
2. Bolstad N, Warren DJ, Nustad K (2013) Heterophilic antibody interference in immunometric assays. *Best Pract Res Clin Endocrinol Metab* 27:647–661
3. Tate J, Ward G (2004) Interferences in immunoassay. *Clin Biochem Rev* 24:105–120
4. Levin O, Morris LF, Wah DT, Butch AW, Yeh MW (2011) Falsely elevated plasma parathyroid hormone level mimicking tertiary hyperparathyroidism. *Endocr Pract* 17:e8–e11
5. van der Doelen RHA, Nijhuis P, van der Velde R, Janssen MJW (2018) Normocalcemia but still elevated parathyroid hormone levels after parathyroidectomy. *Clin Case Rep* 6:1577–1581

6. Prodan P, Nandoshvilli E, Webster C, Shakher J (2016) Asymptomatic elevated PTH due to immunoassay interference resulting from Macro-PTH: a case report. *Endocr Abstr* 44:CC4
7. Laudes M, Frohnert J, Ivanova K, Wandinger KP (2019) PTH immunoassay interference due to human anti-mouse antibodies in a subject with obesity with normal parathyroid function. *J Clin Endocrinol Metab* 104:5840–5842
8. Gulbahar O, Konca Degertekin C, Akturk M, Yalcin MM, Kalan I, Atikeler GF, Altinova AE, Yetkin I, Arslan M, Toruner F (2015) A case with immunoassay interferences in the measurement of multiple hormones. *J Clin Endocrinol Metab* 100:2147–2153
9. Preissner CM, O’Kane DJ, Singh RJ, Morris JC, Grebe SK (2003) Phantoms in the assay tube: heterophile antibody interferences in serum thyroglobulin assays. *J Clin Endocrinol Metab* 88:3069–3074
10. Cavalier E, Carlisi A, Chapelle JP, Delanaye P (2008) False positive PTH results: an easy strategy to test and detect analytical interferences in routine practice. *Clin Chim Acta* 387:150–152
11. Jones AM, Honour JW (2006) Unusual results from immunoassays and the role of the clinical endocrinologist. *Clin Endocrinol* 64: 234–244
12. Sturgeon CM, Sprague S, Almond A, Cavalier E, Fraser WD, Algeciras-Schimmich A, Singh R, Souberbielle JC, Vesper HW, IFCC Working Group for PTH (2017) Perspective and priorities for improvement of parathyroid hormone (PTH) measurement: a view from the IFCC Working Group for PTH. *Clin Chim Acta* 467: 42–47
13. Cosman F, de Beur SJ, LeBoff MS, Lewiecki EM, Tanner B, Randall S, Lindsay R, National Osteoporosis Foundation (2014) Clinician’s guide to prevention and treatment of osteoporosis [published correction appears in *Osteoporos Int*. 2015 Jul;26(7):2045–7]. *Osteoporos Int* 25:2359–2381
14. Silverberg SJ, Clarke BL, Peacock M, Bandeira F, Boutroy S, Cusano NE, Dempster D, Lewiecki EM, Liu JM, Minisola S, Rejnmark L, Silva BC, Walker MD, Bilezikian JP (2014) Current issues in the presentation of asymptomatic primary hyperparathyroidism: proceedings of the Fourth International Workshop. *J Clin Endocrinol Metab* 99:3580–3594
15. Bilezikian JP, Silverberg SJ (2010) Normocalcemic primary hyperparathyroidism. *Arq Bras Endocrinol Metabol* 54:106–109

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.