Open Access http://www.jparathyroid.com

Journal of Parathyroid Disease

Journal of Parathyroid Disease 2022,10, e01

Case Report

Interference caused by anti-streptavidin antibodies in a parathyroid hormone assay; a case report

Benjamin Siegfried Trautz[1*], Thomas Negele[1]

Abstract

Endogenous antibodies can lead to test interference in streptavidin-biotin based laboratory assays, such as thyroid function tests or parathyroid hormone (PTH) levels. We report about a patient, who presented for thyroid surgery, showing undetectable PTH levels despite normocalcaemia in internally assessed preoperative laboratory work. Previously, anti-streptavidin antibodies were detected in the patient's blood and normal PTH levels could be measured in a streptavidin-free assay. To our knowledge this is the first reported case of a clinically relevant PTH test interference due to endogenous anti-streptavidin antibodies, thus complicating the evaluation of postoperative hypoparathyroidism.

Keywords: PTH, Anti-streptavidin, Thyroid surgery, Hypoparathyroidism, Interference, TSH

Please cite this paper as: Trautz BS, Negele T. Interference caused by anti-streptavidin antibodies in a parathyroid hormone assay; a case report. J Parathyr Dis. 2022;10:e01.

Introduction

Parathyroid hormone (PTH) is secreted by the parathyroid glands as a response to low-extracellular calcium concentrations. PTH regulates calcium and phosphate levels by facilitating the synthesis of active vitamin D in the kidneys (calcitriol). Primary hyperparathyroidism is the most frequent cause of hypercalcaemia commonly found in postmenopausal women (1). Moreover, PTH serum levels are altered in patients with secondary hyperparathyroidism due to chronic renal failure (2). Hypocalcaemia caused by postoperative hypoparathyroidism is the most common complication after bilateral thyroidectomy (3). Therefore, postoperative assessment of PTH levels facilitates early supplementation of calcium and calcitriol preventing symptomatic hypocalcaemia. Furthermore, intraoperative PTH testing is necessary when performing parathyroid surgery.

Case Presentation

A 52-year-old female was referred to our institution with a toxic bilateral nodular goitre causing symptomatic dysphagia for total thyroidectomy. Thyroid stimulating hormone (TSH, also known as thyrotropin), free T4 (FT4; free thyroxine) and free T3 (FT3; free tri-iodothyronine) serum levels, assessed by her general physician were within the normal range under medication with 5 mg of thiamazole daily. Furthermore, a previous treatment with L-thyroxine 25 µg due to hypothyroidism was reported. Ultrasound examination of the thyroid gland revealed an increased size of 35 ml with bilateral nodules (ACR TI-RADS 4). The thyroid hormones revealed a thyroid function at the upper limit with a TSH of 0.44 µU/mL (normal range 0.27–4.20 µU/mL), fT3 of 3.46 pg/mL (normal range 1.80–4.20 pg/mL), and fT4 of 1.22 ng/mL (normal range 0.89–1.76 ng/mL). Vitamin D levels were in the lower normal range with 37.00 ng/mL (normal range 30.00–100.00 ng/mL), calcitonin levels ranged below the detection limit of 0.500 pg/mL (normal range 5.17–9.82 pg/mL). PTH levels ranged below the detection limit of 1.20 pg/mL (normal range 15.00–65.00 pg/mL). However, serum calcium and albumin-corrected calcium levels showed a normal range of 2.41 mmol/L and 2.33 mmol/L, respectively (normal range 2.20–2.65 mmol/L). A second blood sample tested for PTH and electrolyte levels confirmed the unusual constellation. Therefore, we sent a third blood sample to our external laboratory.

The surgery was performed without complications, intraoperative frozen sections of the thyroid gland showed no malignancy. The caudal right parathyroid gland with a parathyroid cyst and a haemorrhage was excised first, since it was not possible to preserve its blood supply and replanted in the right sternocleidomastoid muscle. The

Received: 2 July 2021, Accepted: 31 July 2021, ePublished: 8 August 2021

[1]Martha-Maria Hospital, Munich, Germany.

***Corresponding author:** Benjamin Siegfried Trautz, Email: b.trautz@web.de

Implication for health policy/practice/research/medical education

In this case report it is demonstrated how the presence of one inconsistent laboratory value lead to the detection of an antibody test interference, caused by endogenous anti-streptavidin antibodies. However, it turned out that much more values were affected by the test interference, though less obvious. Therefore, clinical findings should always be carefully reviewed and scrutinized.

remaining three parathyroid glands could be identified clearly and preserved in situ.

On the first postoperative day, routine laboratory examinations were performed to check for postoperative hypoparathyroidism. PTH levels were still below the detection limit. However, calcium and albumin-corrected calcium were within the normal range of 2.29 mmol/L and 2.23 mmol/L, respectively (normal range 2.20–2.65 mmol/L). In the meantime, we received the results of the preoperative blood sample sent to the external laboratory which revealed a PTH level of 62.0 ng/L (normal range 15–68 ng/L), suggesting a test interference in our laboratory system. We therefore sent a postoperative blood sample to the external laboratory to analyse postoperative PTH levels. These results, which we received on the second postoperative day, showed a normal PTH level of 31.6 ng/L (normal range 15–68 ng/L) though slightly lower serum calcium levels of 2.15 mmol/L were measured internally on the second postoperative day. We discharged the patient on the second postoperative day without any postoperative complications.

Since the discrepancy between the low PTH levels measured by our laboratory and the PTH levels measured by the external laboratory remained unclear, a more detailed medical history was recorded. This revealed that the patient had been employed as a laboratory assistant at a pharmaceutical company until 2013, working with streptavidin powder. Security precautions included wearing gloves and masks. The patient did not report any exposure to streptavidin powder exceeding the prescribed limits. In 2015, the company performed a screening test for anti-streptavidin antibodies, since these had been detected in blood samples of other employees. The presence of anti-streptavidin antibodies was confirmed in our patient's blood sample.

In our laboratory, PTH is assessed using a Roche Cobas e411 system, based on a biotin-streptavidin electrochemiluminescence immunoassay (ECLIA). Moreover, TSH, fT3, fT4, calcitonin and vitamin D are measured the same way. The external laboratory uses a streptavidin-free chemiluminescent microparticle immunoassay (CMIA). We subsequently ordered an external measurement of TSH, fT3, fT4 and vitamin D levels in the preoperative blood sample. The discrepancy of the measurements of the external laboratory and our laboratory measurements of the preoperative blood sample are shown in Table 1. Calcitonin levels could not be determined by the external laboratory since this required frozen plasma. While we measured a low TSH, fT3 in the upper normal range and fT4 in the middle range, suggesting a thyroid function at the upper limit, the external TSH was far higher with 3.08 mIU/L, fT3 was found in the middle range with 2.75 ng/L and fT4 in the lower range with 0.91 ng/dL, suggesting a thyroid function at a lower limit. Interestingly, vitamin D was only 1.2 units lower compared to our own results.

Discussion

At our institution PTH, levels are determined regularly before surgery to screen for hyperparathyroidism and obtain a reference value in case of postoperative hypoparathyroidism, which is a frequent complication after bilateral thyroidectomy (3). When screening for postoperative hypoparathyroidism, serum calcium levels are potentially misleading since calcium levels react slower compared to PTH levels. Sometimes calcium serum levels are within the normal range on the first postoperative day, while PTH levels have already decreased, with calcium levels then dropping significantly on the second postoperative day. Furthermore, a mild postoperative hypocalcaemia may occur despite normal PTH levels, normally requiring no further treatment. Hence, reliable PTH level measurements are important to prevent symptomatic hypocalcaemia.

To the best of our knowledge, this is the first reported case of discrepant PTH level measurements caused by anti-streptavidin antibody interference. In our case

Table 1. Preoperative values assessed internally by ECLIA and externally by CIMA assay

	Internal laboratory examination			External laboratory examination		
	Result	Range	Unit	Result	Range	Unit
PTH	<1.20	15.00–65.00	pg/mL	62.0	15 - 68	ng/L
TSH	0.44	0.27–4.20	µU/ml	3.08	0.27–4.2	mlU/L
fT3	3.46	1.80–4.20	pg/mL	2.75	1.71–3.71	ng/L
fT4	1.22	0.89–1.76	ng/dL	0.91	0.70–1.48	ng/dl
Vit. D	37.00	30.00–100.00	ng/mL	35.8	40.00–100.00	µg/L

PTH: parathyroid hormone, TSH: thyroid stimulating hormone, fT3: free triiodothyronine, fT4: free thyroxine, Vit. D: Vitamin D, ECLIA: electrochemiluminescence immunoassay, CIMA: chemiluminescent microparticle immunoassay.

endogenous anti-streptavidin antibodies led to an interference with our laboratory examinations, resulting in undetectable PTH values despite normocalcaemia. Furthermore, thyroid hormone levels were also altered mimicking a thyroid function at the upper normal limit.

Previous reports have been published of falsely low PTH levels in patients with secondary hyperparathyroidism, caused by end-stage renal disease due to a regular biotin ingestion (4). Biotin has been reported to cause similar patterns of interference as anti-streptavidin antibodies in streptavidin-biotin based assays (5). Moreover, a case of falsely high PTH levels due to endogenous anti-mouse antibodies was reported recently (6).

So far, the reason for the formation of endogenous anti-streptavidin antibodies is unclear (7). Generally, streptavidin, produced by *Streptomyces avidinii*, is widely used in molecular science for protein purification and in many tests including immunoassays and immunohistochemistry (8,9). In our case, the formation of anti-streptavidin antibodies is plausible since the patient was in direct contact with streptavidin powder likely causing the antibody formation. However, for the majority of the reported cases it remains unclear which mechanisms triggered the formation of the antibodies (7,10).

To identify a test interference caused by e.g. anti-streptavidin antibodies, different methods including pre-treating blood samples with streptavidin microparticles or beads (11-13), heterophilic blocking, Protein-A sepharose (13-15) and polyethylene glycol (PEG) precipitation (15) have been described. However, it is challenging to implement these approaches into daily clinical routine and neither can one of them guarantee for a correct PTH value. Therefore, it seems most feasible to use a biotin-streptavidin free test, for example in an external laboratory, when suspecting laboratory measurement interference. However, this may not be suitable if parathyroid surgery is performed and intraoperative PTH measurement is required. A dilution test, which can be performed in most laboratories, is another easy way to assess the presence of interfering antibodies. Thereby a nonlinear relation indicates the presence of interfering agents (13,14).

Besides interfering with PTH measurements, streptavidin also interferes with the results of thyroid function tests. In 2013, the first case of interference by endogenous anti-streptavidin antibodies was reported, leading to a misdiagnosis of hyperthyroidism (14). More recently other reports have been published describing a similar phenomenon (7,11,13,15). Mostly, the discrepancy of clinical appearance and laboratory test values led to the final revelation of test interference, however, often after a certain time of mistreatment (e.g. with thiamazole). Though a similar trend was visible in our patient's thyroid parameters, alterations were weaker compared to other published cases. Nevertheless, she also had received alternating mistreatment with L-thyroxine and thiamazole due to incoherent thyroid function tests.

There are two main biotin-streptavidin assays: the so-called sandwich assay and the competitive assay. In both techniques, biotinylated substrates are bound to streptavidin-coated microparticles. In the sandwich-assay, higher concentrations of the target analyte lead to an increased detection signal, while in competitive assays higher analyte concentrations lead to a decreased detection signal (10). Endogenous streptavidin-antibodies will bind the streptavidin-coated microparticles and block the binding site for biotin, finally leading to a falsely lower result in the sandwich assays and a falsely higher result in competitive assays (10,11). In the Cobas e411 system by Roche which we use in our laboratory, PTH, calcitonin and TSH are determined by a sandwich assay, whereas fT3, fT4 and vitamin D are detected by a competitive assay.

It has been reported that competitive assays are more susceptible to interference by anti-streptavidin antibodies compared to sandwich-assays (10). However, in our case vitamin D, fT3, and fT4 levels were only changed slightly, while TSH and PTH measurements were significantly altered. For biotin interference, evidence suggests that the grade of interference is dependent on the amount of biotin ingestion (10). Since the streptavidin antibody-titre differs between individuals, this will likely lead to varying results between patients (11). Moreover, it was shown that the degree of interference varied between different test-platforms (12). Interestingly Lam et al. reported no relevant interference of anti-streptavidin antibodies when assessing vitamin D levels measured with competitive assays (15). This might be due to the fact, that vitamin D has a high natural concentration (about 1000-fold higher than fT3 and PTH). It was shown, that a high pre-test sample dilution reduced the amount of antibody-interference (12,16). Therefore, interfering antibodies likely have a weaker effect if the analyte has a higher concentration, while analytes of low natural concentration will be more susceptible to test interference.

Conclusion

In conclusion the formation of anti-streptavidin antibodies in our patient was most likely triggered by occupational exposure to streptavidin powder. However, for the majority of reported cases, the reason for antibody formation remains unclear. Test interference should be suspected in cases of inconsistent laboratory constellations or mismatch to clinical appearance in order to avoid mistreatment of patients.

Limitations of the study

In the case described, we had not the possibilities to detect the presence of the anti-streptavidin antibodies in the patient's blood directly by ELISA or immunofluorescence. However, the patient stated that a direct antibody detection was conducted in the past.

In view of the patient's short hospital stay, no detailed

experimental plan could be developed in advance. Therefore, no additional method like a dilution test was performed to show an antibody test interference.

Acknowledgements

The authors would like to thank Sarah Foreman MD, Johanna Negele and Kathrin Negele for their valuable review on language and writing.

Authors' contribution

TN initiated and supervised the project. BST carried out the research work and wrote the initial manuscript. Both authors discussed the results and contributed substantially to the final version of the manuscript.

Conflicts of interest

The author declares no conflicts of interest to disclose.

Ethical issues

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors. The patient gave the consent to publish as a case report.

Funding/Support

Benjamin S. Trautz did not receive any funding or financial support for the project. Thomas Negele did not receive any funding or financial support for the project.

References

1. Silva BC, Cusano NE, Bilezikian JP. Primary hyperparathyroidism. Best Pract Res Clin Endocrinol Metab. 2018;32:593-607.
2. Mizobuchi M, Ogata H, Koiwa F. Secondary Hyperparathyroidism: Pathogenesis and Latest Treatment. Ther Apher Dial. 2019;23:309-18.
3. Orloff LA, Wiseman SM, Bernet VJ, Fahey TJ, 3rd, Shaha AR, Shindo ML, et al. American Thyroid Association Statement on Postoperative Hypoparathyroidism: Diagnosis, Prevention, and Management in Adults. Thyroid. 2018;28:830-41.
4. Meany DL, Jan de Beur SM, Bill MJ, Sokoll LJ. A case of renal osteodystrophy with unexpected serum intact parathyroid hormone concentrations. Clin Chem. 2009;55:1737-9.
5. Colon PJ, Greene DN. Biotin Interference in Clinical Immunoassays. The Journal of Applied Laboratory Medicine. 2019;2:941-51.
6. Laudes M, Frohnert J, Ivanova K, Wandinger KP. PTH Immunoassay interference due to human anti-mouse antibodies in a subject with obesity with normal parathyroid function. J Clin Endocrinol Metab. 2019;104:5840-2.
7. Robier C, Kolbus N, Demel U. Anti-streptavidin antibodies as a cause of false-positive results of streptavidin-based autoantibody assays. Clin Chem Lab Med. 2019;58:e5-e7.
8. Dundas CM, Demonte D, Park S. Streptavidin-biotin technology: improvements and innovations in chemical and biological applications. Appl Microbiol Biotechnol. 2013;97:9343-53.
9. Desarnaud F, Marie J, Larguier R, Lombard C, Jard S, Bonnafous JC. Protein purification using combined streptavidin (or avidin)-Sepharose and thiopropyl-Sepharose affinity chromatography. J Chromatogr. 1992;603:95-104.
10. Piketty ML, Polak M, Flechtner I, Gonzales-Briceno L, Souberbielle JC. False biochemical diagnosis of hyperthyroidism in streptavidin-biotin-based immunoassays: the problem of biotin intake and related interferences. Clin Chem Lab Med. 2017;55:780-8.
11. Lam L, Bagg W, Smith G, Chiu WW, Middleditch MJ, Lim JC, et al. Apparent hyperthyroidism caused by biotin-like interference from IgM anti-streptavidin antibodies. Thyroid. 2018;28:1063-7.
12. Berth M, Willaert S, De Ridder C. Anti-streptavidin IgG antibody interference in anti-cyclic citrullinated peptide (CCP) IgG antibody assays is a rare but important cause of false-positive anti-CCP results. Clin Chem Lab Med. 2018;56:1263-8.
13. Favresse J, Lardinois B, Nassogne MC, Preumont V, Maiter D, Gruson D. Anti-streptavidin antibodies mimicking heterophilic antibodies in thyroid function tests. Clin Chem Lab Med. 2018;56:e160-e3.
14. Rulander NJ, Cardamone D, Senior M, Snyder PJ, Master SR. Interference from anti-streptavidin antibody. Arch Pathol Lab Med. 2013;137:1141-6.
15. Peltier L, Massart C, Moineau MP, Delhostal A, Roudaut N. Anti-streptavidin interferences in Roche thyroid immunoassays: a case report. Clin Chem Lab Med. 2016;54:e11-4.
16. Tate J, Ward G. Interferences in immunoassay. Clin Biochem Rev. 2004;25:105-20.