Biotin interference and biotin-depletion protocol

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Introduction

Biotin, also called vitamin B7 or vitamin H, is one of the B vitamins and is involved in a wide range of metabolic processes in our body. Biotin is also an essential reagent in certain immunoassay designs. In recent years, supplements containing high dose of biotin have gained commercial popularity for their claimed benefits on healthy hair and nail growth, and there were increasing published reports of high-dose biotin causing erroneous results, especially on thyroid function tests. We report here a case of hypercalcaemia mimicking vitamin D intoxication, and subsequent workup confirmed the presence of positive biotin interference on vitamin D result using biotin-depletion protocol.

Case Description and Initial Investigations

A 62-year-old female patient presented with generalised discomfort. Initial blood tests revealed severe hypercalcaemia (total calcium 3.14 mmol/L, ionized calcium 1.79 mmol/L), suppressed parathyroid hormone level (< 0.64 pmol/L), normal phosphate (0.95 mmol/L) and very high serum total 25-hydroxyvitamin D (25-OHD) levels on two consecutive days (> 250 nmol/L on 14/11/2020 and 163 nmol/L on 15/11/2020 by Roche Elecsys Vitamin D Total II assay). A review on other immunoassay results of the patient also found discordant thyroid function tests with high free thyroxine (T4), free triiodothyronine (T3) and thyroid stimulating hormone (TSH) using Beckman Access on 13/11/2020. In view of suspected interference, the serum specimens were sent to other platforms for analysis.

	Beckman	Roche	Abbott
TSH (mIU/L)	Access TSH	Elecsys TSH*	Alinity i TSH
	8.84 (0.17 – 4.37)	**0.23 (0.27 – 4.20)**	8.03 (0.35 – 4.94)
Free T4 (pmol/L)	Access Free T4*	Elecsys FT4 III*	Alinity i Free T4
	60.8 (7.7 – 16.2)	15.1 (12.0 – 22.0)	13.6 (9.0 – 19.1)
Free T3 (pmol/L)	Access Free T3*	Elecsys FT3 III*	Architect Free T3
	12.6 (2.5 – 6.3)	2.1 (3.1 – 6.8)	1.7 (2.6 – 5.7)

Table 1: Thyroid function test results on three different analytical platforms.
Remarks: *Biotin/streptavidin-based assay

	Roche Elecsys Vitamin D Total II*	LC-MS/MS
Total 25-OHD (nmol/L) on 14/11/20	**> 250**	49
Total 25-OHD (nmol/L) on 15/11/20	**163**	50

Table 2: Total 25-OHD test results on two different analytical platforms.
Remarks: *Biotin/streptavidin-based assay

Discrepancies in thyroid function test and total 25-OHD results among the different platforms were observed and the presence of immunoassay interference was confirmed. Among the various causes of interferences, the possibility of biotin interference was high in this case as the Roche Elecsys Vitamin D Total II assay, Beckman Access Free T4 and Free T3 assays, and Roche Elecsys TSH assay all employ biotin/streptavidin technology.

Subsequently, the patient reported a history of using a supplement called “Hair Gummy Haircarebear”, which contains 5 mg of biotin per serving and she has been taking two servings per day (in total 10 mg per day). In addition, she was also taking Caltrate and calcium carbonate supplements which could possibly explain the severe hypercalcaemia.

Methods

The presence of biotin interference can be confirmed using the biotin-depletion protocol. Serum samples were incubated with streptavidin-coated magnetic microparticles that were retrieved from reagent packs of Roche Elecsys immunoassay kits. The microparticles were then immobilized using magnetic separation, and the biotin-depleted supernatants were aspirated for relevant analyses.

Fig. 1 Reagent packs of Roche Elecsys immunoassay kits that contain streptavidin-coated magnetic microparticles.

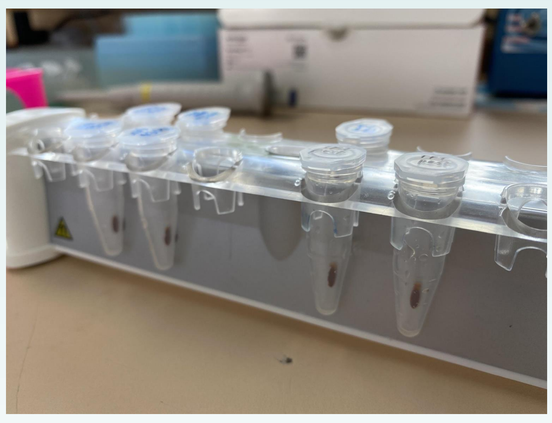

Fig. 2 Immobilization of magnetic microparticles by magnetic separation.

Results

After biotin depletion procedures, the measured values of TSH and 25-OHD, which are two of the Roche Elecsys assays with the lowest threshold to biotin interference, were restored to levels similar to those of the non-biotin-based platforms. While for assays with higher biotin tolerance levels (PTH, free T3 and free T4), there were no significant changes in the post-biotin depletion results, signifying these tests were not interfered by the biotin usage in this patient.

Roche Elecsys assays		Pre-biotin depletion result	Post-biotin depletion result
Assays	Claimed biotin threshold (ng/mL)		
TSH (mIU/L)	25	**0.23 (L)**	8.79 (H)
25-OHD (nmol/L)	30	**> 250 (H)**	39*
		163 (H)	37*
PTH (pmol/L)	50	< 0.64 (L)	< 0.64 (L)
Free T3 III (pmol/L)	70	2.1 (L)	2.2 (L)
Free T4 III (pmol/L)	100	15.1	16.8

Table 3: Patient’s result on selected analytes performed on the Roche Elecsys platform (pre- and post-biotin depletion).
Remarks: H, High tag; L, Low tag; *Mild deficiency

Discussion

Initial investigations with high serum total 25-OHD results and hypercalcaemia point towards a diagnosis of vitamin D toxicity. However, the serum concentration of total 25-OHD is considered an indicator of vitamin D status with long half-life of 15–35 days, and therefore the discrepancy of the total 25-OHD results in two consecutive days raised the suspicion of interference. The workup for suspected immunoassay interference includes serial dilution, heterophilic antibody blocking tests and reanalyzing samples in assays that adopt a different technology. In this patient, the suspicion for biotin interference is the highest as the erroneous results came from immunoassays that employ biotin/streptavidin technology. Taking into account of the types of assays affected and the manufacturer’s claimed upper limit of biotin concentration tolerated in individual assays, the serum biotin concentration in our patient was estimated to be between 30 and 50 ng/mL.

According to various pharmacokinetic studies, oral doses of 10 mg biotin a day could result in peak blood concentrations ranging from 55 to 140 ng/mL at 1 to 2 hours post-dose. Roche also recommended an 8-hour washout period for individuals taking 5-10 mg biotin per day. In addition to the biotin dosage regimen, clinicians need to be aware of other patient factors such as renal function and concurrent drug intake that may influence biotin pharmacokinetics when considering the timing for blood collection. The prolonged biotin effect observed in this case could possibly be explained by the impaired renal clearance as the patient was suffering from end-stage renal failure, and thus a longer washout period is expected. Clinicians may consider informing the laboratory in advance if the patient is known to have consumed high-dose biotin, or should contact the laboratory when laboratory results do not fit the patient’s clinical picture. To verify suspected biotin interference, laboratory could arrange sample testing on non-biotin-based platforms, or reanalyze the sample on the same platform after biotin depletion procedures. Alternatively, laboratory may request new blood samples after the patient has abstained from biotin for a longer period, if it is not medically contraindicated.

Conclusion

We reported a case of very high 25-OHD results due to biotin interference which clinically mimicked vitamin D intoxication. We also demonstrated normalization of test results using a biotin-depletion protocol. Majority of the reported biotin interferences cases involved thyroid function tests; other endocrine tests, especially 25-OHD, were less frequently reported. Spurious test results of 25-OHD may potentially lead to misdiagnosis and inappropriate management. Laboratory personnel and clinicians should have increased awareness in preventing and detecting such interference especially when results do not appear to be in accord with the clinical picture.

References

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