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A RARE CAUSE OF DISCORDANT TSH: MACRO-TSH

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Abstract

Background. When the laboratory results are not compatible with the clinical features of the patient, the presence of assay interference should be considered.

Patient and Methods. Here, we report a case of macro-thyroid stimulating hormone (macro-TSH) in a 31 year-old woman who had hypothyroidism due to thyroidectomy as well as discordant TSH levels with the clinical findings. Her TSH level was spuriously high with low levels of free T3 and T4 on levothyroxine treatment and she had only mild fatigue. To screen for the presence of interference, we performed TSH measurements in different platforms and serial dilution of the sample. Her rheumatoid factor was found to be negative.

Results. The testing for heterophile blocking and non-specific anti TSH antibodies suggested lack of interference. We did further test for the clinical suspicion of interference and TSH decreased to 29.8 μIU/mL from 210.5 μIU/mL (recovery: 14.1%) after polyethylene glycol (PEG) precipitation indicating the existence of macro-TSH. After two months of increased dosage of levothyroxine, her TSH level was still very high (192.0 μIU/mL), but free T3 and free T4 increased to normal levels. PEG precipitation test was reperformed and TSH was decreased to 46.0 μIU/mL from 192.0 μIU/mL (recovery: 24.0 %). Her levothyroxine replacement dosage was not increased since free T3 and T4 levels were normal.

Conclusions. If there is a suspicion for the discordant TSH level, the presence of macro-TSH by PEG precipitation should be investigated even though first step investigations for interference were found to be negative.

Key words: macro-TSH, interference, PEG.

INTRODUCTION

Diagnosis and treatment of thyroid diseases are based on the patient's clinical findings and laboratory results. TSH measurement as the first screening test is used for the evaluation of thyroid function (1). Although measurement techniques for TSH have advanced, interference for TSH measurement is still a problem in the laboratories. When the laboratory results are not compatible with the clinical features of the patient, the presence of assay interference should be considered (2). In a multicenter study, false positive hormone results have been reported as nearly 6 % (3). The presence of anti-animal and heterophile antibodies, rheumatoid factor, paraprotein and macro-thyroid stimulating hormone (macro-TSH) have been suggested as the causes of assay interference (4 - 10). In addition, there are method-related interferences defined on different assays despite developing technology (11,12).

Macro-TSH is an immunoglobulin complex including TSH and anti-TSH antibody (13). It has been suggested as a rare cause of laboratory interference presented with spuriously high TSH levels. Macro-TSH prevalence was established as 0.6 %.

PATIENT

A 31 year-old female was admitted to our hospital for abnormal thyroid function test results while using levothyroxine treatment despite dose adjustment. She had a history of total thyroidectomy for multinodular goiter two years ago. The histopathology was found to be benign. After the operation levothyroxine replacement treatment was started. While she was taking the drug regularly, euthyroid state could not be achieved. Despite mild clinical hypothyroid symptoms, spuriously high TSH levels with low free T3 and free T4 were found (Table 1). TSH was normalised quickly after increasing the levothyroxine dosage. But, this euthyroid state could not be maintained despite taking the same dosage. She was complaining for only mild fatigue. Therefore, her symptoms and physical examination findings were not concordant with the markedly elevated TSH concentration. She had no gastrointestinal symptoms suggesting malabsorption. She had no other medical problems and was not taking any other drugs. She was

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taking the replacement therapy regularly. Her TSH level was 650.0 µIU/mL (reference interval: 0.35-4.94 µIU/mL) with low levels of free T3 and free T4 on 150 µg/d levothyroxine (Table 1). The anti-thyroglobulin and anti-thyroid peroxidase antibodies were negative. Informed consent of the patient was taken.

METHODS

Serial Dilution

We performed serial dilutions (1/2,1/4,1/8) of the serum sample using the manufacturer's diluents.

Repeating TSH in Alternative Platforms

The measurements were repeated on the same patient sample using chemiluminescence (CLIA) and electrochemiluminescence (ECLIA) immunoassays on four different analytical platforms (Abbott Architect, Siemens Advia Centaur XP, Beckman Dxi, Roche Cobas).

Testing for Heterophile Antibodies

Sample was analyzed with nonspecific antibody blocking tubes and heterophile-antibody blocking tubes (Scantibodies, Santee, CA).

Presipitation with Polyethylene glycole

A solution of 25% PEG 6000 (VWR, Lutterworth, UK) was prepared in water. A serum aliquot of 0.2mL was added to 0.2mL of the 25% PEG 6000 solution (TSHPEG) and to 0.2mL of deionized water ($TSHH_2O$) as a control. The samples were vortex mixed, incubated for 15 min at room temperature and then centrifuged for 15 min at 3500 rotations per minute. The TSH concentration of the supernatant was assayed using the Abbott Architect. The percentage recovery of TSH was calculated.

RESULTS

To screen a laboratory interference that may cause discordant TSH levels, we performed a serial dilution of the blood sample. TSH measurement of the 1/2, 1/4 and 1/8 dilution of the blood sample resulted in linear recovery (Table 2). Additionally, TSH measurement was repeated with four alternative platforms (Abbott Architect, Siemens Advia Centaur XP, Beckman Dxi, Roche Cobas); three of them were using CLIA method while the latter was using ECLIA method. All the results were high and there were little differences in TSH measurements between alternative platforms that could not be attributed to analytical bias

Table 1. Thyroid function test results and levothyroxine replacement dose of the patient after thyroidectomy

Date	TSH (µIU/mL)	fT4 (ng/dL)	fT3 (pg/mL)	LT4 dosage
February 2007	478 (0.34-5.6)	<0.25 (0.58-1.64)	1.88 (2.5-3.9)	(-)
April 2007	0.25 (0.2-4.3)	1.44 (0.9-1.7)	3.57 (2.5-4.5)	100 mcg/d
July 2009	>75 (0.4-4.0)	1.24 (0.9-1.7)		150 mcg/d
January 2010	3.22 (0.27-5.0)	1.34 (0.93-1.7)	2.54 (2.0-4.4)	150 mcg/d
November 2011	5.69 (0.4-4.0)	2.04 (0.93-1.7)	3.77 (2.0-4.4)	150 mcg/d
April 2012	4.45 (0.4-4.0)	1.09 (0.93-1.7)	2.95 (2.0-4.4)	125 mcg/d
September 2013	650 (0.35-4.94)	0.54 (0.7-1.48)	2.23 (1.7-3.71)	150 mcg/d

Table 2. Serial dilution of the sample

	1/1	1/2 dilution	1/4 dilution	1/8 dilution
TSH (µIU/ml) (0.35-4.94)	210.5	200.0	191.8	190.7

Table 3. TSH results from different platforms

	Abbott Architect (CLIA)	Siemens Advia Centaur XP (CLIA)	Beckman Dxi (CLIA)	Roche Cobas (ECLIA)
TSH (µIU/ml)	210.5 (0.35-4.94)	>200.0 (0.34-5.60)	186.2 (0.34-5.60)	203.4 (0.27-4.20)

Table 4. TSH measurements taken before and after PEG precipitation

	Initiation (Under Levothyroxine 150 mcg/d)		After 2 months (Under Levothyroxine 175 mcg/d)	
	TSH(µIU/ml)	Recovery (%)	TSH(µIU/ml)	Recovery (%)
Pre-PEG	210.5		192.0	
		14.2		24.0
Post-PEG	29.8		46.0	

PEG; polyethylene glycol

between the assays (Table 3). Although repeating the test in different platforms and serial dilution were not associated with laboratory interference, we did further tests as laboratory interference was still suspected. Her rheumatoid factor was negative. Incubation of the sample with heterophile blocking tube and non-specific antibody tube returned 95 % and 97 % recovery, suggesting lack of interference. We could not perform gel filtration chromatography test for detecting macro-TSH. However, TSH decreased to 29.8 μIU/mL from 210.5 μIU/mL (recovery: 14.1 %) after polyethylene glycol (PEG) precipitation (Table 4).

Clinical Follow-up

After these investigations, her levothyroxine dosage was increased to 175 μg/d. We checked the thyroid function tests again after two months. Her TSH level was still very high (192.0 μIU/ml), but free T3 and free T4 increased to normal levels and her mild fatigue was improved. Her levothyroxine replacement dosage was not increased according to normal T3 and T4 levels. PEG precipitation test was performed and TSH was decreased to 46.0 μIU/mL from 192.0 μIU/mL (recovery: 24%) (Table 4).

DISCUSSION

Our presented case was a female patient who had hypothyroidism due to thyroidectomy as well as discordant TSH levels with the clinical findings. Assay interference was suspected despite similar results of TSH in different platforms and linear serial dilution of the sample. Finally, macro-TSH was found by PEG precipitation. With this case, we wanted to point out the existence of macro-TSH even in patients with overt hypothyroidism.

A previous study detected laboratory interference for TSH and gonadotropin levels in 28 of 5310 patients. Eighty two percent of these interferences caused a modification in the clinical approach of the patients (2).The study of Marks *et al.* reported that 2 of 10 donors with illnesses known to be associated with rheumatoid factor were found to have falsely high TSH values (3). Loh *et al.* also reported a case with macro-TSH and suggested a screening algorithm for the interference of TSH in which serial dilution of the sample is firstly performed, after that, the TSH measurement is repeated on different platforms (10). Detection of linear recovery in serial dilution and similar results taken from different laboratory platforms can support lack of interference (10). If there is still a suspicion of possible interference, testing of blood sample with heterophile blocking tube and rheumatoid factor measurement are also recommended in the algorithm. If the results are negative, the gel filtration chromatography test which is a gold standard test is performed to detect macro-TSH. In the present case, discordant TSH level with the clinical features of the patient caused a suspicion of interference despite negative screening tests including serial dilution and repeat of the samples on different platforms. Therefore, other interference tests which are positivity of rheumatoid factor, heterophile and non-specific antibodies were investigated and they all were found to be negative.

Although PEG precipitation is particularly used to detect the interference of prolactin in clinics, it is also used in cases suspected to have interference of TSH. In the study by Mills *et al.*, (9) low recovery rate after PEG precipitation which is considered as < 25 % was determined in 15 (3 %) of 495 TSH measurements. Macrocomplex of immunoglobulin-TSH was identified in 3 of 15 samples by gel filtration chromatography. In consistent with our case, Sakai *et al.* (8) detected 4 % of recovery and Loh *et al.* (10) found 3.2 % of recovery after PEG precipitation in cases with macro-TSH. In our case, we performed the PEG precipitation and observed recovery rates as 14.1 % at hypothyroid phase and 24.0 % after increasing levothyroxine dose. At this step, gel filtration chromatography test is the suggested test for the diagnosis of macro-TSH. Test could not be performed since no laboratory performing this test exists in our country because of expense and hard work of this assay as in many centers in the world. However, gel filtration chromatography may rarely not differentiate the causes of interference such as macrohormone, heterophile antibodies (9).

Most cases previously described in the literature had abnormal thyroid function tests due to interference although they were euthyroid (2,10,14). The treatments which were started in order to abnormal results have been stopped after detecting the presence of interference in these patients (2). But, our case had a history of total thyroidectomy and true hypothyroidism. Therefore, in contrast to previous studies, we had the chance to follow the levels of macro-TSH at different stages of hypothyroidism as an original feature of the present case. Such that, after increasing thyroid replacement dose, recovery value of TSH was observed to be increased compared to the first recovery. As a hypothesis, this increase seen in recovery of TSH might be partly explained by an improvement in renal clearance of TSH-Ig complex as a macromolecule after achieving euthyroidism. On the other hand, the

variability of the recovery values of TSH after PEG might be expected since PEG precipitation is not a fully reliable method for macromolecules like TSH.

There are not enough data for the PEG precipitation of macro-TSH in the literature. Only very few case reports were published (8,10). Most of the previous case reports showed normalisation of TSH after PEG precipitation. But, our report presented the first case of macro-TSH who required levothyroxine therapy due to previous history of thyroidectomy. We think that the patient might have elevated TSH consisting both of macro-TSH and also true TSH due to hypothyroidism at initiation. Two months after increasing the levothyroxine dose, we observed that TSH level was still elevated after PEG precipitation. Potential reasons for this persistent elevation are firstly, the existing hypothyroidism in tissue level despite normal serum free thyroid hormones, secondly, the existing macromolecules that could not be precipitated with PEG. This issue needs to be supported by further studies.

The presence of TSH interference has led to some difficulties in our dose adjustment. Because of discordant TSH levels, TSH could not be the target for the dose adjustment. Additionally, different recovery rates in repeated PEG precipitations during follow-up showed that repeated PEG precipitation may not be a reliable method during the treatment of hypothyroid patients while it is an important method for the assessment of interference. Therefore, our follow-up treatment was planned according to the free thyroid hormone levels.

In conclusion, if there is a suspicion for the discordant TSH level with the clinical features of the patient, the clinician should warn the laboratory to investigate possible assay interference one of them being macro-hormones. In this respect, the presence of macro-TSH by PEG precipitation, which is a simple and cheap method, should be explored even though first step investigations for interference are found to be negative.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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