CLINICAL VIGNETTE

Hyperthyroxinemia with Non-Suppressed TSH: Case Report of Thyroid Immunoassay Interference

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Case Presentation

A 35-year-old man with a past medical history of anxiety and depression presented to his primary care provider for evaluation of worsening anxiety and insomnia. Thyroid function tests showed normal TSH and elevated Free T4. The patient was referred to endocrinology for suspicion of hyperthyroidism.

In addition to increased anxiety and insomnia, the patient also noted palpitations. However, he had no weight loss, sweating, diarrhea, hair loss, proptosis, and dry eyes and had no personal or family history of thyroid disease. He also denied taking supplements containing thyroid hormone or high dose biotin, although he reported taking protein powder with biotin. He had no exposure to lithium, amiodarone and IV contrast and was free of neck pain or recent viral illness.

Physical exam includes normal blood pressure, pulse and temperature, with elevated BMI at 25.5kg/m^2. Otherwise, his exam was unremarkable, including a normal thyroid exam without enlargement, palpable nodules or tenderness to palpation.

The Initial differential diagnosis for hyperthyroxienmia included lab error, assay interference, Graves' disease, toxic nodule, painless thyroiditis, and TSH secreting pituitary adenoma. However, the patient's history and physical exam findings were not congruent with hyperthyroidism, therefore, repeat labs were drawn 2 weeks after stopping the protein powder containing biotin.

Initial thyroid function tests showed normal TSH of 1.6 mica/mL (0.3- 4.7 mcIU/mL) and elevated Free T4 of 2.7 ng/dL (0.8-1.7 ng/dL). Repeat tests 2 weeks after stopping protein powder containing biotin showed similar results with normal TSH and elevated Free T4 with the addition of elevated Total T4 and thyroid peroxidase (TPO) antibodies but normal Total T3. Discrepancy between TSH and T4, along with the patient's lack of physical signs of thyrotoxicosis further suggested the possibility of lab interference. We worked with the clinical laboratory to repeat the thyroid function tests using heterophile antibody blocking tubes. Results using heterophile antibody blocking tubes showed a decrease in the levels of free T4, total T4 and TPO antibodies compared to what was previously reported and therefore suggesting lab interference was likely.

To confirm this, we also checked free T4 and free T3 using equilibrium dialysis, which resulted in normal hormone levels. All labs are summarized in Table 1.

Patient was reassured that initial abnormal thyroid function tests were due to laboratory interference, most likely due to heterophile antibody interference with the immunoassays, rather than from actual thyroid pathology.

Discussion

HPT Axis Physiology and Thyroid Hormone

Thyroid hormone production is tightly regulated by TRH release from the hypothalamus and subsequent TSH release from the pituitary gland. The most common thyroid diseases primarily involve the thyroid gland (primary hypothyroidism and hyperthyroidism) and result in TSH changes that are concordant with T4/T3 changes. There are however rare disorders, such as TSH secreting pituitary adenoma or resistance to thyroid hormone, that can result in discordance in TSH and T4/T3. These should be considered after exclusion of more common causes of discordance in TSH and T4/T3, which include non-thyroidal illness, medication, and immunoassay interference.

Prevalence and Types of Lab Interference

Immunoassays are the most frequently used method of testing thyroid function as they are automated, efficient and inexpensive. However, immunoassays are vulnerable to different types of interferences. Assay interference in thyroid function testing has been estimated to be approximately 1% of all thyroid tests performed.[1]

The presence of heterophile antibodies in a sample is one of the causes of interference in immunoassays. Heterophile antibodies form due to exposure to external antigens, including animal antigens. Incidence of interference due to heterophilic antibodies may be 0.05-6% depending on the analyte considered.[1] Interference due to heterophilic antibodies may lead to falsely low or high levels of one or more thyroid function tests, including TSH, FT4, FT3 assay.[2] Manufacturers have modified

their current assays to reduce interferences with addition of nonimmune sera or purified immunoglobulins as well as variable blocking agents, however, if a patient's serum contains a high level of interfering antibodies, they can still cause interference.[2] This interference may be overcome by using different antibody/antigen assay, dilution assay or use of heterophile blocking tube test.[1]

Macro-TSH, a large circulating form of TSH composed of monomeric TSH complexed with autoimmune anti-TSH antibodies, can lead to falsely elevated TSH with normal FT4 and FT3. The presence of Macro-TSH can lead to misdiagnosis of subclinical hypothyroidism and unnecessary initiation or increase of thyroid hormone replacement. The presence of Macro-TSH can be evaluated by adding polyethylene glycol to serum which precipitates out gamma globulin fractions. If the percentage of PEG-precipitable TSH exceeds 90% in serum, macro TSH should be suspected and confirmed by gel chromatography.[3]

Biotin has been reported to cause interference in many immunoassay platforms including thyroid function tests. Biotin or vitamin B7 is widely used in dietary supplements for alopecia and brittle nail, as well as part of B complex vitamins and other multivitamins. When ingested at high levels, biotin can cause interference depending on the type of assay used. Biotin is used in immunoassays due to its ability to bind streptavidin, making it useful as a general bridge system. In competitive assays, like those usually used to measure free T4, biotin competes with biotinylated T4 for binding to streptavidin which leads to falsely high results. In sandwich assays, like those usually used for measurement of TSH, biotin competes with the biotinylated sandwich complex for binding with streptavidin causing a low signal and falsely low results.[4] For these reasons, patients should be questioned about the use of biotin as part of the routine history when evaluating for abnormal thyroid function tests.

Thyroid hormone auto-antibodies (antibody towards T4/T3) can be present in about 1.8% in healthy population and up to 20% in autoimmune thyroid disease, and can lead to falsely elevated T4/T3 leading to misdiagnosis of hyperthyroidism. Only about 2% of patients with these antibodies demonstrate lab interference. Lab interference is less likely to occur if FT4 is measured with equilibrium assay or two step immunoassay. Radio-immunoprecipitation with PEG can identify thyroid hormone auto-antibody presence.[2]

Methods to Test or Reduce Interference

Prior to pursuing more complicated testing, repeat lab testing should be the first step in case of lab error. Doubling serial dilution is a simple and fast way to assess for interfering antibodies, as their presence can distort the expected linear results of serial dilution. This method can identify up to 60% of cases of interference from endogenous antibodies.[5]

Heterophile blocking tubes can also be used to detect and overcome interference. These tubes contain a blocking reagent composed of specific binders that bind to interfering heterophile antibodies so they are no longer available to cause immunoassay interference.[6] In our case, using heterophile antibody blocking tubes was very helpful to determine that interference was present, since they showed a decrease in the previously elevated level of free T4.

Equilibrium dialysis or ultrafiltration can also be used to avoid interference. These tests directly measure thyroid hormone by physically separating free hormones from protein bound hormones as opposed to the immunoassay which estimates the level of free hormone.[7] These tests were used in our case to confirm that the level of free T4 and free T3 were normal and no thyroid pathology was present.

While no single test may be sufficient to determine the cause or reduce interference, combined use of comparison methods, dilution tests and blocking agents are reported to identify antibody interference in about 90% of suspected samples.[6] PEG precipitation, affinity extraction and size exclusions can be used to deplete interfering antibodies, and may be used if suspicion of laboratory interference remains high as they require more sophisticated testing.

It is estimated that approximately 50% of all reported cases of thyroid function test interference were initially mis-diagnosed and resulted in negative clinical consequences including inappropriate medication prescriptions including both thyroid hormone and anti-thyroid drugs and unnecessary imaging.[1] It is important for clinicians to consider possible laboratory interference when there is discrepancy between clinical and laboratory data to avoid erroneous diagnosis and treatment.

Table 1. Laboratory testing

Test	Reference range	Initial blood sample	Second blood sample- 2 weeks later	Re-run of initial blood sample	Re-run of initial sample with Heterophile blocking tube	Third blood sample
TSH	0.3 - 4.7 mcIU/mL	1.6	1.74	1.53	1.96	
Free T4	0.8 - 1.7 ng/dL	2.7	3.74	3.42	1.72	
Total T4	4.9 - 11.4 mcg/dL		12.1	11.2	9.71	
Total T3	85 - 185 ng/dL		180	158.3	152.4	
TPO ab	<=20 IU/mL	81			34.15	
Free T4 by Equilibrium Dialysis/HPLC-Tandem Mass spec	1.1-2.4 ng/dL					1.8
Free T3 by Equilibrium Dialysis/LC-MS/MS	1.81-4.06 pg/dL					2.68

Roche Cobas assays were used for TSH, Free T4, total T3, total T4 and TPO tests. A sandwich immunoassay was used for TSH. A competitive immunoassay was used for Free T4 tests analogues. Competitive immunoassays are used for total T3 and total T4 tests. Competitive immunoassay is used for TPO antibodies.
TSH- thyroid stimulating hormone
TPO ab- thyroid peroxidase antibodies
HPLC- High Performance Liquid Chromatography
LC-MS/MS- Liquid Chromatography with tandem mass spectrometry

REFERENCES

1. **Favresse J, Burlacu MC, Maiter D, Gruson D.** Interferences With Thyroid Function Immunoassays: Clinical Implications and Detection Algorithm. *Endocr Rev*. 2018 Oct 1;39(5):830-850. doi: 10.1210/er.2018-00119. PMID: 29982406.
2. **Després N, Grant AM**. Antibody interference in thyroid assays: a potential for clinical misinformation. *Clin Chem*. 1998 Mar;44(3):440-54. PMID: 9510847.
3. **Hattori N, Ishihara T, Yamagami K, Shimatsu A**. Macro TSH in patients with subclinical hypothyroidism. *Clin Endocrinol (Oxf).* 2015 Dec;83(6):923-30. doi: 10.1111/cen.12643. Epub 2014 Dec 19. PMID: 25388002.
4. **Elston MS, Sehgal S, Du Toit S, Yarndley T, Conaglen JV**. Factitious Graves' Disease Due to Biotin Immunoassay Interference-A Case and Review of the Literature. *J Clin Endocrinol Metab*. 2016 Sep;101(9):3251-5. doi: 10.1210/jc.2016-1971. Epub 2016 Jun 30. PMID: 27362288.
5. **Ismail AA**. On detecting interference from endogenous antibodies in immunoassays by doubling dilutions test. *Clin Chem Lab Med*. 2007;45(7):851-4. doi: 10.1515/CCLM.2007.152. PMID: 17617026.
6. **Ismail AA**. Interference from endogenous antibodies in automated immunoassays: what laboratorians need to know. *J Clin Pathol*. 2009 Aug;62(8):673-8. doi: 10.1136/jcp.2008.055848. PMID: 19638536.
7. **Demers LM, Spencer CA**. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. *Clin Endocrinol (Oxf).* 2003 Feb;58(2):138-40. doi: 10.1046/j.1365-2265.2003.01681.x. PMID: 12580927.