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CASE REPORT
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Macro-Thyroid-Stimulating Hormone Is a Rare Cause of Elevated Thyroid-Stimulating Hormone Levels: Two Cases and Review of the Literature

ABSTRACT

In patients with elevated thyroid-stimulating hormone levels but clinically euthyroid, non-thyroid causes of thyroid-stimulating hormone elevation should be considered. A careful approach is required when making a treatment decision for these patients because there are many different conditions, including macro-thyroid-stimulating hormone, that can increase thyroid-stimulating hormone levels. We present 2 patients, a 38-year-old man and an 87-year-old woman. Both of them applied to the endocrinology outpatient clinic because of their high thyroid-stimulating hormone levels. Both patients were using levothyroxine treatment at the time of admission. While thyroid-stimulating hormone levels were high in both patients under levothyroxine treatment, free thyroxine and free tri-iodothyronine levels were within the normal range. Both were clinically euthyroid. When levothyroxine doses were increased in both patients due to high thyroid-stimulating hormone levels, symptoms of thyrotoxicosis developed and free thyroxine levels increased, but thyroid-stimulating hormone levels were still high. No pathological physical examination findings were detected. No discrepancy was found in the detailed query in terms of treatment compliance. There was no drug use or acute stress situations. Thyroid hormone resistance and heterophile antibody interference were excluded. The thyroid-stimulating hormone level was measured again with polyethylene glycol precipitation method in terms of immunoglobulin and thyroid-stimulating hormone complex macromolecule (macro-thyroid-stimulating hormone). Polyethylene glycol -precipitated thyroid-stimulating hormone levels were found to be 0.16 and 0.128 μIU/mL (0.27-4.2), respectively. Macro-thyroid-stimulating hormone is a macromolecule formed as a result of the complex binding of thyroid-stimulating hormone with immunoglobulins. Macro-thyroid-stimulating hormone is considered biologically inactive. These patients are clinically euthyroid. False-elevated thyroid-stimulating hormone may be detected in patients harboring macro-thyroid-stimulating hormone. Misdiagnosing these patients may result in unnecessary treatment.

Keywords: Immunoassays, interferences, macro-TSH, thyroid function tests

Introduction

Elevated thyroid-stimulating hormone (TSH) levels are a common clinical entity. There may be thyroidal and non-thyroidal diseases that increase TSH levels. Clinical and subclinical hypothyroidism is one of the most common thyroid-related causes. Subclinical hypothyroidism is found in 4%-20% of the adult population.[1] Elevated TSH levels is a condition that should be approached carefully when making a treatment decision. Temporary thyroid dysfunctions that may increase TSH levels (such as painless thyroiditis), poor thyroxine compliance, and some non-thyroidal diseases, such as non-thyroidal illness healing phase, medications and assay interference need to be excluded.[2] Despite the methodological developments over the years, the immunological methods used in the detection of thyroid functions are exposed to many interferences. They include macromolecules like macro-TSH as well as heterogeneous antibodies and anti-mouse antibodies. The complicated interaction of TSH with immunoglobulins (Igs) results in the formation of macromolecules called macro-TSH. Macro-hormones, such as macro-TSH, are thought to be physiologically inactive. Thyroid-stimulating hormone is still detectable by the current TSH assays despite being bound into such complexes, which causes elevated values. False-elevated TSH may be detected in patients harboring macro-TSH. This can lead to clinical mistreatment. Macro-TSH is a relatively uncommon condition, with a reported prevalence of 0.6%-1.6%.[3] We present 2 cases of macro-TSH, who received levothyroxine therapy due to elevated TSH levels despite being in the euthyroid clinic. Verbal informed consent was obtained from the patients who agreed to take part in the study.

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Case Presentation

The first case is a 38-year-old male patient with no known comorbidity. He was referred from a local hospital with persistently elevated TSH levels. When the TSH level was found to be high during routine laboratory tests performed at the age of 24, 50 mcg/day levothyroxine treatment was started. At this time, plasma levels of free thyroxine (fT4) and free tri-iodothyronine (fT3) were in the normal range. Anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG) antibodies were found to be negative. There was no pathological finding in physical examination and he was clinically euthyroid. Thyroid ultrasonography was normal regarding size and texture. During his follow-ups, he did not use the drug regularly and no thyroid function test was performed again. Ten years later, the levothyroxine dose was increased to 100 mcg/day because the TSH value was still high and the patient started to use the drug regularly from this date on. While using 100 mcg/day levothyroxine treatment regularly and appropriately for a month, the measured TSH level was 13.69 µIU/mL (0.35-4.94) and the fT4 level was 1.31 ng/dL (0.7-1.48) (16.8 pmol/L). The dose of levothyroxine was increased to 125 mcg/day. After 6 weeks on this levothyroxine treatment, the TSH level was 7.17 µIU/mL (0.27-4.2), the fT4 level was 23.50 pmol/L (11-22), the fT3 level was 4.41 pmol/L (3.1-6.8), calcium level was 6.0 mg/dL (8.6-10.2), phosphorous level was 6.20 mg/dL (2.5-4.5), 25-OH vitamin D level was 11.23 µg/L, albumin level was 49.9 g/L (35-52), and parathormone level was 7 pg/mL (15-65). The patient had no symptoms or pathological physical examination findings related to hypocalcemia. Primary hypoparathyroidism was detected incidentally. However, after the levothyroxine dose was increased, the fT4 level increased above normal limits and the patient developed mild palpitations and sweating. Serum TSH, fT4, and fT3 levels were measured with chemiluminescent immunoassay (Roche Cobas 8000 Modular e801). No medication including biotin or accompanying acute stress was detected. The anterior pituitary hormone panel was evaluated. No pathology was detected.

It was also investigated for the etiology of hypoparathyroidism. No pathology was detected. A diagnosis of idiopathic hypoparathyroidism was made. The patient was started on active vitamin D and calcium replacement at appropriate doses.

The TSH measurement was confirmed in another laboratory. However, electro-chemiluminescence immunoassay was used as the measurement method in both laboratories. The TSH level was measured using a heterophile antibody blocking tube to exclude heterophile antibody interference and similar results were obtained. Heterophilic antibodies were not considered. Plasma TSH in both parents was within the reference range. Due to the development of thyrotoxicosis symptoms under levothyroxine treatment, thyroid hormone resistance was not considered in the foreground.

Thereupon, the TSH level was measured again with polyethylene glycol (PEG) precipitation method in terms of Ig and TSH complex macromolecule (macro-TSH). The result was 0.16 µIU/mL (0.27-4.2). This result demonstrated that the presence of macro-TSH was what caused the plasma TSH to be so elevated. Levothyroxine replacement therapy was discontinued. Although the TSH level was still high in the thyroid function tests performed during the follow-ups, fT3 and fT4 levels were within the normal range. The patient was clinically euthyroid.

The second case is an 87-year-old female patient with known diagnoses of atrial fibrillation, primary hypertension, and primary hypothyroidism. The patient was diagnosed with primary hypothyroidism 27 years ago, but the thyroid function tests could not be found for that time. But during the last 27 years of follow-up, her TSH and fT4 levels were found to be within the normal range under levothyroxine replacement therapy. Although the drug dose or drug compliance did not change, she was referred to the endocrinology outpatient clinic because the TSH level was found to be high. In the thyroid function tests performed at the time of admission, the TSH level was 23.4 µIU/mL (0.27-4.2), the fT4 level was 16.10 pmol/L (11-22), and the fT3 level was 4.57 pmol/L (3.1-6.8). Anti-TPO and anti-TG antibodies were negative. There was no pathological finding in her physical examination. She was clinically euthyroid. The drugs she used regularly were levothyroxine 100 mcg/day, rilmenidine, edoxaban, candesartan, hydrochlorothiazide, metoprolol, and benidipine. In the ultrasonography, the thyroid volume was decreased and the parenchyma was slightly heterogeneous. No nodule was detected. Thereupon, the levothyroxine dose taken by the patient was increased from 100 mcg/day to 125 mcg/day. In the thyroid function tests performed 3 months later, the TSH level was 30.1 µIU/mL (0.27-4.2), the fT4 level was 24.50 pmol/L (11-22), and the fT3 level was 4.80 pmol/L (3.1-6.8). Meanwhile, the patient had a newly developed palpitation complaint. Serum TSH, fT4, and fT3 levels were measured with chemiluminescent immunoassay (Roche Cobas 8000 Modular e801). No medication including biotin or accompanying acute stress was detected. Thereupon, the patient was questioned in detail in terms of drug compliance and usage. It was found that she used levothyroxine treatment regularly. Thyroid hormone receptor beta gene mutation was examined since there were individuals with thyroid dysfunction in the family history. No mutation was detected. Thyroid hormone resistance and heterophile antibody interference were ruled out. Thereupon, the TSH level measuring again with the PEG precipitation method in terms of Ig and TSH complex macromolecule (macro-TSH). After precipitation with PEG, the TSH level was 0.128 µIU/mL (0.27-4.2). The levothyroxine dose was reduced to 100 mcg/day. The TSH level measured after 3 months was 21.2 µIU/mL (0.27-4.2), the fT4 level was 19.6 pmol/L (11-22), and the fT3 level was 2.88 pmol/L (3.1-6.8). The TSH level precipitated by PEG was 0.20 µIU/mL (0.27-4.2). The levothyroxine level was reduced to 75 mcg/day. Control was scheduled after 8 weeks.

MAIN POINTS

- In clinically euthyroid patients with biochemically determined subclinical hypothyroidism, careful evaluation is required when making a treatment decision.
- Assay interference and macro-thyroid-stimulating hormone (TSH) should be considered when clinically incompatible TSH values are detected.
- Macro-TSH is considered biologically inactive. False-elevated TSH may be detected in patients harboring macro-TSH.
- Misdiagnosing these patients may result in unnecessary treatment.

Discussion

Here we report 2 cases with elevated TSH levels. Thyroid function evaluation with immunoassay may contain errors.[2] Both endogenous and exogenous chemicals can disrupt immunoassays. Several substances, including megadoses of biotin, anti-streptavidin antibodies,

human anti-mouse/animal antibodies, and heterophile antibodies, have been associated with the disorder.[4] Biotin may cause significant interference with thyroid function tests when used in high doses and should be discontinued before the reevaluation of thyroid functions. Consideration must be given to heterophile antibodies in addition to biotin interference. Heterophile antibodies are a subset of natural antibodies and autoantibodies that can react with a variety of diverse and poorly defined antigens and may cause abnormal thyroid function tests. Using serial dilution with manufacturer-recommended diluents is another method for screening for interference. The existence of the interference is indicated by nonlinear serial dilution.[5] In the detailed questioning of our patients, there was no history of biotin use that could lead to interference. In addition, TSH measurement was repeated in a different laboratory and a heterophile blocking tube was used to exclude heterophile antibody interference.

Macromolecules, like macro-TSH, are one of the causes of immunoassay interference. Macro-TSH with a molecular weight of >150 kDa is quite large compared to the original TSH molecule with a molecular weight of 28 kDa, which is secreted from the pituitary.[6] It is composed of TSH and anti-TSH autoantibodies—mostly IgG.[3] Due to its large molecular size, its renal clearance is thought to be later than free TSH.[7] This enables the accumulation of macro-TSH in the circulation, which raises serum TSH levels. The characteristic features of patients with macro-TSH are high TSH levels, normal fT4 and fT3 levels, and clinical euthyroidism. The measured plasma TSH levels are usually markedly elevated (>100). However, as in our cases, mildly elevated TSH levels have also been reported in the literature.[3] Patients with macro-TSH may biochemically mimic subclinical hypothyroidism.[5] The patient continues to be clinically euthyroid due to the poor biological activity of macro-TSH.[8] Macro TSH, like macro PRL, likely has limited bioactivity since TSH and its autoantibodies may compete for binding to TSH receptors.[9] Most of these patients are mistakenly started on levothyroxine therapy. Our patients had also used levothyroxine therapy for a while. The TSH levels can be suppressed with levothyroxine therapy.[3] This does not rule out the presence of macro-TSH. In our male patient, when the levothyroxine treatment dose was increased from 100 mcg/day to 125 mcg/day, TSH levels measured 1 month later decreased from 13.69 µIU/mL to 7.97 µIU/mL (0.27-4.2). On the other hand, in our female patient, the TSH level was 23.4 µIU/mL (0.27-4.2) at the time of diagnosis, while the TSH level was measured as 30.1 µIU/mL (0.27-4.2) after levothyroxine treatment.

No routine TSH immunoassay can detect macro-TSH. Special measurement techniques are required for this. The gold standard method is gel filtration chromatography (GFC).[5] This method can separate different TSH fractions according to their molecular weights. Another method for macro-TSH detection is the dilution method. The patient's serum can be diluted with manufacturer-recommended diluents. Nonlinearity in diluted samples with higher recovery may indicate the existence of macro-TSH.[10] Nonetheless, it should be noted that the diluting process is neither specific nor sensitive. Therefore, we did not prefer this method in our patients. Polyethylene glycol precipitation is a popular and simple technique for screening for macroprolactin that has also been used to screen for macro-TSH.[11] Hattori et al[3] suggest that in cases with TSH levels >10 µIU/mL and >90% precipitation with the PEG method, the clinician should strongly consider macro-TSH. In our cases, precipitation with PEG was used because the GFC method could not be reached. Polyethylene glycol-precipitable TSH exceeds 90% in both of our patients.

Although the underlying cause of macro-TSH formation is not known exactly, anti-human TSH antibodies have been shown in most cases.[12] Hattori et al[12] found that 4 of 16 patients with macro-TSH had Hashimoto's disease, while other patients had accompanying autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis. While the patient presented in the first case had accompanying idiopathic hypoparathyroidism, in the patient presented in the second case, although thyroid autoantibodies were negative, sonographically, the thyroid gland shrunk and its heterogeneity increased.

The etiology underlying macro-TSH is still unknown. There is no general recommendation that predicts who should be considered for macro-TSH. Mills et al[13] suggested that macro-TSH should be suspected in case of TSH above 10 mIU/L, while Hattori et al[14] found macro-TSH in 0.17% of women of childbearing age and suggested that macro-TSH should be excluded before starting levothyroxine therapy for patients in this group. In addition, in the study conducted on neonates, the frequency of macro-TSH was found to be 0.43%, and macro-TSH was detected in the mothers of all these babies. When high TSH and normal fT4 levels are detected in neonates, it is recommended to check the presence of macro-TSH in the mother.[15] However, under current circumstances, this approach does not seem to be cost-effective for this rare situation. Regular patient follow-up could be considered in order to learn more about this issue.[16] In the 4-year follow-up of 16 patients diagnosed with macro-TSH, macro-TSH disappeared in 2 patients and TSH levels returned to normal.[12]

Conclusion

Measurement interference and macro-TSH should definitely be considered in patients with high TSH levels but who develop thyrotoxicosis symptoms under levothyroxine treatment. As in our cases, macro-TSH can be seen at slightly elevated TSH levels.

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