Macro-TSH: A Diagnostic Challenge

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What Is Known about This Topic?

- Macro-TSH is a rare condition, which is caused by binding of TSH to other plasma proteins, most often immunoglobulins. This results in falsely elevated TSH measurement.
- The biochemical profile mimics subclinical hypothyroidism and may lead to inappropriate LT4 treatment.
- No immunoassay can reveal the presence of macro-TSH. Gel filtration chromatography is the state-of-the-art method for detection of macro-TSH.

What Does This Case Report Add?

- This report highlights the importance of screening for macro-TSH, in addition to other analytical problems, in patients with a markedly and isolated elevated plasma TSH, particularly if other signs of thyroid dysfunction are absent.

Keywords

Macro-TSH · Analytic errors · Gel filtration chromatography

Abstract

Introduction: Analytical problems should be considered in case of a discrepancy between the results of biochemical tests and the clinical findings. Macro-hormones often artefactually elevate biochemical tests. **Case Presentation:** A young male was referred with persistently elevated TSH (148 mIU/L) measured by a sandwich electrochemiluminescence immunoassay, ECLIA (Cobas; Roche, Basel, Switzerland).

The patient's complaints were unspecific, and he appeared clinically euthyroid. The plasma levels of free T4 and free T3 were within the normal range, thyroid autoantibodies were negative, and thyroid ultrasonography was normal. During a short trial of thyroid hormone substitution, the level of TSH decreased to near-normal levels, but hyperthyroid symptoms emerged. TSH analysed by a different immunoassay (Architect; Abbott, Chicago, IL, USA) yielded similar results. In addition, serial dilutions were performed showing linearity, without detection of heterophilic antibody interference. Gel filtration chromatography confirmed the presence of macro-TSH. **Conclusion:** The patient har-

boured macro-TSH, which is a rare condition. The complex binding of TSH to other plasma proteins, most often immunoglobulins, results in elevated plasma TSH. However, the biologically active fraction of TSH is normal, reflected by clinical and biochemical euthyroidism.

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Introduction

Despite methodological improvements, immunoassays used for evaluation of the thyroid function are still exposed to different types of interferences. Macro-thyrotropin (macro-TSH) is caused by a large amount of monomeric TSH complexed with anti-TSH antibodies – mostly immunoglobulin G [1, 2]. Macro-hormones, including macro-TSH, are considered biologically inactive, and only a small fraction of the total amount of TSH in plasma remains free [3].

Although being bound into such complexes, TSH is still detected by the current assays used for TSH measurement, causing elevated results. Thus, none of the commercially available immunoassays employed for TSH testing can discriminate macro-TSH from the bioactive freely circulating TSH. In patients harbouring macro-TSH, the falsely elevated TSH may lead to clinical mismanagement. Macro-TSH is a rare condition, with a reported prevalence ranging from 0.6 to 1.6% [4–6]. Here, we present a case with isolated TSH elevation, illustrating the challenges of diagnosing macro-TSH.

Case Presentation

A 29-year-old male was referred from a local hospital with persistently elevated TSH (148 mIU/L) measured by a sandwich electrochemiluminescence immunoassay (ECLIA) on Cobas 8000 (Roche, Basel, Switzerland). The patient's complaints were unspecific of nature, like tiredness, headache, dizziness, and discomfort in the chest. Plasma levels of free T4 and free T3 were within the normal range, and thyroid autoantibodies were negative. The physical examination was without abnormal findings, and he appeared clinically euthyroid. Thyroid ultrasonography was normal regarding size, texture, and echogenicity. In addition, MRI of the pituitary gland and an ACTH-stimulation test were normal. The symptoms and the increased TSH had prompted a trial of levothyroxine (LT4) replacement therapy on the suspicion of thyroid failure. Up to 150 µg LT4 per day was given. Hereby, plasma TSH decreased to near-normal levels within a few weeks, but hyperthyroid symptoms emerged in parallel with the thyroid hormones rising to supranormal levels (Fig. 1). Therefore, the treatment was withdrawn.

Supplementary Laboratory Tests

Measurement of TSH by use of a different immunoassay (Architect; Abbott, Chicago, IL, USA) yielded a similar high TSH level (>100 mIU/L) as did the assay from Roche. Serial dilution of the patient's plasma was analysed using both immunoassays to rule out possible interference from human anti-mouse antibodies targeting TSH assay antibodies. For both assays, linear and almost identical dilution patterns were found (Fig. 2a), making interference from heterophilic antibody highly unlikely. Further analyses included search for a mutation in the TSH-receptor-gene and the TSH-beta-gene, also with negative results [7]. Plasma TSH in both parents was within the reference range.

Thyroid failure was considered unlikely, based on the current results, the very high TSH level contrasting the normal levels of free T3 and free T4, as well as the appearance of the patient. The possibility of a complex formation (i.e., macro-TSH) of TSH and an unknown component in the plasma was considered. Therefore, gel filtration chromatography (GFC) was performed on plasma samples from the patient, a control person with slightly elevated TSH, and a pool of healthy controls, respectively (Fig. 2b). A total of 100 µL plasma was fractionated on a Superdex S-75 column (PBS, pH 7.4; 1 mM EDTA) via ÄKTA FPLC. Fractions of 500 µL were collected, and TSH was analysed by Cobas 8000 e801 (Roche, Basel, Switzerland). The TSH peak of the control person eluted at 24 kDa, corresponding to free TSH (theoretically 28 kDa), while the patient's TSH peak eluted at 154 kDa, compatible with a TSH-IgG complex (theoretically 178 kDa). This finding confirmed that the highly elevated plasma TSH was caused by the presence of macro-TSH. Thus, the patient had no thyroid disorder and his symptoms were non-thyroid related.

Discussion

Characteristics of patients with macro-TSH include elevated TSH, normal thyroid hormone levels, and no clinical symptoms of thyroid dysfunction. Plasma TSH is often markedly increased, like in our patient, but cases with only slightly elevated TSH have been published [5]. Biochemically, this mimics subclinical hypothyroidism and may lead to inappropriate LT4 therapy [2]. However, a decrease in plasma TSH in response to LT4 therapy cannot exclude the presence of macro-TSH [5, 8]. As demonstrated in our case, plasma TSH decreased significantly within a few weeks during LT4 replacement therapy, reflecting a suppression of TSH secretion of the pituitary gland. This observation seems to indicate that TSH is bound rather weakly to the immunoglobulins and is in a state of equilibrium with the free TSH in the circulation. If the complex binding was irreversible, the decline in TSH would probably have been more prolonged, considering the long half-life of immunoglobulins. To a large extent, macro-TSH mimics the situation seen with the thyroid hormones, where only a minor fraction is freely circulating due to protein binding.

Fig. 1. The response to LT4 replacement therapy in the patient with macro-TSH. Initially, plasma TSH was high but decreased during LT4 replacement therapy, in parallel with a rise in plasma free T4. Reference interval of TSH: 0.3–4.0 mIU/L; free T4: 12–21 pmol/L. LT4 was initiated at week = 0 and withdrawn at week = 28. LT4, levothyroxine.

No routine TSH immunoassay can disclose the presence of macro-TSH. For this purpose, the gold standard method is GFC, which can separate the various TSH fractions according to the molecular weight. There is no clear answer when to screen for macro-TSH. This condition may be more common than hitherto reported. Mills et al. [6] suggested that macro-TSH should be suspected in case of TSH above 10 mIU/L, while Hattori et al. [4] recommended screening for macro-TSH, especially in women of childbearing age in whom LT4 therapy for subclinical hypothyroidism is considered. Since macro-TSH is present in less than 1% in most populations, such an ap-

proach is controversial and will significantly increase the costs of thyroid function tests.

For screening of macroprolactinemia, polyethylene glycol (PEG)-mediated precipitation has been widely adopted by clinical laboratories [9]. This method is based on either the post-PEG recovery level of monomeric prolactin or the use of post-PEG monomeric prolactin reference intervals. Dilution studies and measurement of TSH after addition of PEG might be helpful in identifying assay interference, without being definitive regarding the cause of such interference. Considering lower costs and higher accessibility than with GFC, such an approach may be a

Fig. 2. a Serial dilution analyses were made with two different assays (Cobas and Architect), both showing similar and normal linear TSH dilution response. The upper limit of measurement in both assays is 100 mIU/L, undiluted measurements are thus not shown. **b** Analysis of TSH monomer and macro species in plasma. Plasma (0.1 mL) from the patient (TSH case), a control person with slightly elevated TSH, and a pool of healthy controls, respectively, was subjected to GFC. TSH immunoreactivity was analysed in the resulting fractions using the Cobas method. The expected (interpolated) molecular weights (kDa) of free TSH and TSH bound to IgG are indicated by arrows. GFC, gel filtration chromatography; IgG, immunoglobulin G.

valid alternative for detection of macro-TSH, although results must be confirmed by performing GFC [2].

The aetiology behind the development of macro-TSH is unknown. Likewise, little is known whether macro-TSH may persist lifelong or if it is transient of nature. Apart from jeopardizing the diagnostic process, the presence of macro-TSH is probably of no harm to the patient.

Nevertheless, it may be considered to follow up the patients regularly, in order to gain more knowledge about this condition [1, 10].

In conclusion, macro-TSH, in addition to other analytic interferences, should always be considered in patients with a markedly and isolated elevated plasma TSH, particularly if other signs of thyroid dysfunction are absent.

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Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The patient gave consent to publication of the case.

Disclosure Statement

The authors have no conflicts of interest to declare.

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All authors participated in drafting the article or revising it critically for important intellectual content and gave their final approval of the version to be submitted.

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