



# Falsely Elevated Thyroid Stimulating Hormone in Two Cases Requiring Special Follow-up

## Özel Takip Gerektiren İki Olguda Yalancı Tiroid Stimulan Hormon Yüksekliği

● Serpil YANIK ÇOLAK<sup>1</sup>, ● Eray ÖZGÜN<sup>2</sup>, ● Burak ANDAÇ<sup>1</sup>, ● Mine OKUR<sup>1</sup>, ● Buket YILMAZ BÜLBÜL<sup>1</sup>, ● Mehmet ÇELİK<sup>1</sup>

<sup>1</sup>Trakya University Faculty of Medicine, Department of Internal Disease, Division of Endocrinology and Metabolic Diseases, Edirne, Turkey

<sup>2</sup>Trakya University Faculty of Medicine, Department of Biochemistry, Edirne, Turkey

### ABSTRACT

"Inappropriate thyroid stimulating hormone (TSH)" refers to an elevation in TSH levels that does not match the clinical findings and free T3 and free T4 levels. Several conditions can cause this, such as pituitary tumors that produce TSH, resistance to thyroid hormones, macro-TSH, and antibody interference. Macro-TSH is a condition that causes TSH to be measured high in the blood for a long time by forming a complex with immunoglobulins, mostly IgG. However, patients are clinically euthyroid because macro-TSH is not a bioactive complex. It is essential to exclude the diagnosis of falsely elevated TSH to protect patients from unnecessary or high-dose levothyroxine therapy. In our first case, we presented a patient in whom subclinical hypothyroidism was detected during in vitro fertilization treatment, and levothyroxine was started. The other case was an operated papillary thyroid cancer patient. In both cases, although the dose of levothyroxine was increased, insufficient TSH response to increased FT4/FT3 levels suggested inappropriate TSH elevation. The polyethylene glycol (PEG) precipitation method was used to detect the assay variability. TSH recovery after PEG was 0.96% and 21%, respectively, supporting the diagnosis of macro-TSH. In both cases, detecting Macro-TSH was crucial in preventing thyrotoxicosis caused by excessive levothyroxine dosage. In addition, delay in treatment for infertility was prevented in the first case.

**Keywords:** Macro-TSH, subclinical hypothyroidism, polyethylene glycol precipitation method, interference, heterophilic antibody

### ÖZ

"Uyumsuz tiroid stimüle edici hormon (TSH)" terimi, klinik bulgularla ve serbest T3/T4 düzeyleriyle uyumlu olmayan TSH yüksekliğini ifade eder. TSH üreten hipofiz tümörleri, tiroid hormon direnci, makro-TSH ve antikor interferansı gibi çeşitli durumlar buna neden olabilir. Macro-TSH, başta IgG olmak üzere immünoglobulinler ile TSH'in kompleks oluşturarak kanda uzun süre yüksek olarak ölçülmesi durumudur. Ancak hastalar klinik olarak tiroiddir; çünkü makro-TSH biyoaktif bir kompleks değildir. Hastaları gereksiz veya yüksek doz levotiroksin tedavisinden korumak için hatalı TSH yüksekliğini dışlamak önemlidir. İlk olgumuzda yardımcı üreme teknikleri tedavisi sırasında subklinik hipotiroidi saptanan ve levotiroksin başlanan bir olguyu sunduk. Diğer olgumuz, opere papiller tiroid kanseri hastasıydı. Her iki olguda da, levotiroksin dozu artırılmış olmasına rağmen, artan serbest T3/T4 seviyelerine yetersiz TSH yanıtı, uygunsuz TSH yüksekliğini akla getirmiştir. Laboratuvar interferansını değerlendirmek için polietilen glikol (PEG) ile çöktürme metodu kullanıldı. PEG sonrası TSH recovery oranları sırasıyla %0,96 ve %21 olup, makro-TSH tanısını desteklemekteydi. Her iki olgumuzda da, yüksek levotiroksin dozunun neden olabileceği tirotoksikozun önlenmesinde makro-TSH'nin saptanması önem arz etmekteydi. Ayrıca, ilk olguda infertiliteye yönelik tedavi gecikmenin önüne geçilmiş oldu.

**Anahtar Kelimeler:** Makro-TSH, subklinik hipotiroidizm, polietilen glikol çöktürme yöntemi, interferans, heterofil antikor

### INTRODUCTION

The term inappropriate thyroid stimulating hormone (TSH) describes a TSH elevation inconsistent with clinical findings and free T3 (FT3) and free T4 (FT4) levels<sup>1-3</sup>. TSH-producing pituitary

adenomas (TSHoma), thyroid hormone receptor resistance (RTH), macro-TSH, and interference by endogenous antibodies may cause this situation<sup>4</sup>. The first condition to be evaluated in the differential diagnosis is the presence of clinical findings.

**Address for Correspondence:** Serpil YANIK ÇOLAK MD, Trakya University Faculty of Medicine, Department of Internal Disease, Division of Endocrinology and Metabolic Diseases, Edirne, Turkey **Phone:** +90 531 690 57 69 **E-mail:** serpilynk@hotmail.com **ORCID ID:** orcid.org/0000-0003-3602-7697

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In the presence of thyrotoxicosis symptoms and signs, TSHoma and RTH should be considered first. However, depending on the mutation subtype in RTH, it may also present with hypothyroidism. Sometimes the symptoms of both conditions can be seen together in patients with RTH. In addition, in the differential diagnosis of TSHoma and RTH, pituitary magnetic resonance imaging (MRI), TR- $\beta$  mutation analysis, TSH alpha subunit level, thyrotropin releasing hormone (TRH) stimulation test, and t3 suppression test are performed as further tests<sup>5,6</sup>.

In the absence of hypothyroid symptoms, the situations causing falsely elevated TSH levels, such as macro-TSH and interference by endogenous antibodies, should be considered as rare causes. The concept of interference by endogenous antibodies is used whenever one suspects a patient's sample contains antibodies that cause false results by binding to the assay antibodies<sup>7</sup>. There are three types of endogenous antibodies that cause interferences in immunoassays: autoantibodies, heterophilic antibody, and antianimal antibody<sup>8</sup>. Macro-TSH is caused by a large amount of monomeric TSH complexed with anti-TSH antibodies—mostly immunoglobulin G (IgG)<sup>9,10</sup>. Macro-TSH is at least 150 kDa that likely accumulates in the circulation, resulting in measurements indicating falsely increased TSH levels<sup>9,11,12</sup>. This molecule is not bioactive but immunoreactive. It is crucial to identify laboratory interferences to ensure the accurate diagnosis of thyroid diseases, proper treatment, and prevention of the adverse effects of levothyroxine overtreatment.

In this article, we discuss two cases where precise TSH level measurement is crucial for treatment planning and monitoring. The first case involves a female patient who is undergoing in vitro fertilization (IVF) treatment, while the second case is a patient with papillary thyroid cancer, who requires TSH suppression therapy after surgery.

## CASE REPORTS

### Case 1

A 32-year-old female patient was referred to our clinic after detecting TSH: 25  $\mu$ U/mL (reference interval: 0.27–4.20), fT4: 1.3 ng/dL (reference interval: 0.93–1.7) fT3: 2.6 pg/mL (reference interval: 2.0–4.4) in the tests performed during IVF treatment. There were no signs or symptoms of thyroid dysfunction and no personal or family history of thyroid or autoimmune disorders. In the patient's control examination, TSH: 14.8  $\mu$ U/mL, fT4: 1.41 ng/dL and fT3: 2.8 pg/mL were detected. Anti-thyroglobulin and anti-thyroid peroxidase were found to be negative. The thyroid ultrasound pattern was normal. The patient had a pregnancy plan. Levothyroxine was started at a dose of 1.25 mcg/kg. Six weeks later, TSH: 11.1  $\mu$ U/mL, fT4: 1.56 ng/dL and fT3: 3.21 pg/mL were detected. The levothyroxine dose was adjusted to 1.6 mcg/kg in the

patient. In the follow-up examination one month later, TSH: 10.2  $\mu$ U/mL, fT4: 1.68 ng/dL and fT3: 3.70 pg/mL were detected. Although the levothyroxine dose was increased, sufficient TSH response to the increase in fT3 and fT4 levels could not be obtained. The patient had drug compliance. There was no drug use to interact with levothyroxine. There were no laboratory or clinical findings suggestive of malabsorption. Rheumatoid factor was negative. In the differential diagnosis of the patient who was clinically euthyroid since diagnosis, polyethylene glycol (PEG) precipitation test was applied to exclude laboratory interference and to screen for monomeric TSH. The post-PEG TSH recovery was 0.96% (Table 1).

### Case 2

A 31-year-old male patient was referred to our clinic for follow-up after a total thyroidectomy operation. The pathology result was reported as encapsulated follicular variant papillary thyroid carcinoma (pT1bNx Mx). The postoperative laboratory evaluation results were as follows: TSH: 30.8  $\mu$ U/mL, fT4: 0.05 ng/dL and fT3: 1.32 pg/mL. Levothyroxine treatment was started at a dose of 1.6 mcg/kg/day. At the follow-up evaluation after six weeks, TSH: 27.5  $\mu$ U/mL, fT4: 1.32 ng/dL and fT3: 2.1 pg/mL were detected. Therefore, the levothyroxine dose increased to 1.9 mcg/kg/day. One month later, TSH: 15.0  $\mu$ U/mL fT4: 1.34 ng/dL, fT3: 2.2 pg/mL were detected. Macro-TSH was considered in the differential diagnosis of the patient since the suppression in the TSH level did not show sufficient correlation with the increase in the fT4/T3 levels. The patient was previously evaluated for other possible causes. The patient had drug compliance. There was no clinical or laboratory finding to suggest malabsorption. Rheumatoid factor was negative. PEG precipitation test was performed on the patient (Table 1). The post-PEG TSH recovery rate was 21%.

The recovery rates following PEG indicated that both patients had macro-TSH. Since the levels of monomeric TSH were normal after PEG precipitation, conditions such as TSHoma and RTH were ruled out as potential diagnoses. As a result, there was no need to conduct further tests such as pituitary MRI, TR- $\beta$  mutation analysis, TSH alpha subunit level assessment, TRH stimulation test, and t3 suppression tests. Also, endogenous antibodies might have caused falsely elevated TSH results as an assay interference in our patients, and PEG precipitation

**Table 1. PEG precipitation**

	TSH concentration ( $\mu$ U/mL) Before PEG	TSH concentration ( $\mu$ U/mL) After PEG	Recovery rate (%)
Case 1	12.5	0.12	0.96
Case 2	15.24	3.16	21

PEG: Polyethylene glycol, TSH: Thyroid stimulating hormone

might have precipitated these antibodies as well. In any case, monomeric TSH detected after PEG determines our clinical approach.

In the first case, our patient's monomeric TSH level was at the target value (TSH <2.5) before undergoing IVF, so we continued their current levothyroxine treatment dose. For the second case, we adjusted the levothyroxine suppression dose to maintain fT4 at the upper limit of 1/3, and no recurrences were observed during the patient's follow-up.

### PEG Precipitation

The patients' serum samples were mixed with equal volumes of a 25% solution of PEG 6000 (Sigma, dissolved in distilled water) and equal volumes of distilled water (as a control). The mixtures were centrifuged at 10000 g for 5 minutes and the supernatants were collected for TSH assay. TSH levels were measured by Roche E801 analyzer using its original kits with electrochemiluminescence immunoassay (ECLIA) method and results were given by multiplying by two (dilution factor). The recovery (%) rates were calculated by using following formula: Recovery %=(the TSH value measured after the addition of PEG/the TSH value measured after the addition of distilled water)×100. A recovery rate that was lower than 40% suggests the high molecular weight proteins, such as immunoglobulins<sup>4</sup>. In the case reports in the literature, the TSH recovery after PEG precipitation was less than 25%<sup>10</sup>. In only two cases, the recoveries were about 50%<sup>13,14</sup>. According to Sakai et al.<sup>4</sup>, a recovery rate lower than 40% suggests high molecular weight proteins, such as immunoglobulin. We based this study's method and cut-off values to evaluate our cases.

### Other Laboratory Assays

Serum TSH, fT4, fT3, anti-thyroid peroxidase assays were also measured by Roche E801 analyzer using its original kits with ECLIA method. Anti-thyroglobulin was measured through Siemens Immulite 2000 Xpi by using its original kit with chemiluminescent immunometric method. Rheumatoid factor levels were measured by Roche C702 chemistry analyzer using its original kits.

### DISCUSSION

Measurements of serum TSH levels are the first step test in the thyroid function evaluation algorithm. If there is an increase in TSH levels, the next step should be to check the fT4/fT3 levels. Subclinical hypothyroidism is characterized by normal levels of serum fT4/fT3 and high levels of serum TSH. Moreover, there are various situations where a high serum TSH concentration does not accurately fit the subclinical hypothyroidism definition. These include TSHoma, RTH and occasional mutations of the TSH receptor, variability in assays, the phase of recuperation from nonthyroidal illness<sup>5</sup>.

Elevated TSH in patients with TSHoma or RTH is generally associated with high serum fT4 and/or fT3 concentrations. Diagnosing this case is relatively straightforward, but sometimes fT4/T3 levels can be normal. Thyrotoxicosis clinic is a common symptom of TSHoma. In addition, symptoms and signs of hyperthyroidism or hypothyroidism may be observed depending on the subtype of the mutation in RTH. To confirm our diagnoses, laboratory tests were conducted to prevent assay variability that might lead to inappropriate TSH elevation. In both cases, normal monomeric TSH levels were found after precipitation with PEG, which ruled out the diagnoses of TSHoma and RTH. As a result, further tests such as pituitary imaging, genetic mutation analysis, TSH alpha subunit level, TRH stimulation test, and t3 suppression test were not required.

Interference by endogenous antibodies (autoantibodies, heterophilic antibody, and antianimal / human anti-mouse antibodies), rheumatoid factor and macro-TSH should be kept in mind at differential diagnosis<sup>15</sup>. TSH is a small bioactive hormone of 28 kDa easily filtered by the kidney, macro-TSH is a large molecule of at least 150 kDa that likely accumulates in the circulation, resulting in measurements indicating falsely increased TSH levels<sup>9,11,12</sup>. The binding of IgG to TSH has been reported as the leading cause of macro-TSH<sup>12,16</sup>. Macro-TSH is not bioactive but immunoreactive. The etiology of macro-TSH is unknown.

The PEG precipitation method to screen for macroprolactinemia has also been transposed to macro-TSH detection<sup>9</sup>. Multiple PEG precipitation procedures are available, with percent recovery typically performed<sup>16</sup>. Information about monomeric TSH levels can be obtained by precipitating macro-TSH and interfering endogenous antibodies with PEG. If TSH recovery is low, macro-TSH and interferences by endogenous antibodies should be kept in mind.

The prevalence of macro-TSH has yet to be well known but is considered a rare condition. Hattori et al.<sup>9</sup> evaluated 681 patients with elevated TSH concentrations; macro-TSH was detected in 11 patients from 681 serum samples (1.61%). Ismail et al.<sup>17</sup>, in their study, found that 6 of 5310 patients had high TSH levels due to interference by endogenous antibodies. In some of these 6 cases, falsely elevated TSH might occur due to macro-TSH.

One of our cases is the first macro-TSH case diagnosed during IVF treatment in the literature. For women with laboratory values for subclinical hypothyroidism and scheduled for IVF, achieving a TSH concentration of <2.5 mU/L with levothyroxine replacement is recommended.<sup>18</sup> However, in the presence of macro-TSH, patients may receive levothyroxine treatment unnecessarily. Therefore, especially in clinical and laboratory incompatibility cases, the diagnosis of macro-TSH

will be important in terms of not delaying IVF and protecting the mother and fetus from exogenous thyrotoxicosis caused by the overtreatment of levothyroxine during pregnancy. Hattori et al.<sup>19</sup> proposed that Macro-TSH should be excluded before giving levothyroxine replacement therapy in patients with subclinical hypothyroidism to avoid unnecessary treatment. However, it is challenging to confirm every case routinely. It is also not cost-effective. Therefore, it seems reasonable to keep it in mind in the differential diagnosis and to perform a further examination for macro-TSH in selected cases. In our case, the clues that initiated further investigation were the consistently high TSH levels despite appropriate levothyroxine treatment and the fT4 level close to the upper limit of the reference range. Moreover, malabsorption, drug incompatibility, and interactions with other drugs were all ruled out, and there were no clinical signs of hypothyroidism since diagnosis. Although there is no guideline for treatment monitoring in the presence of Macro-TSH during IVF treatment in the literature, since TSH levels of individuals planning pregnancy cannot be trusted, it may be a reasonable approach to set fT4 levels at 1/3 upper limit as the treatment goal before IVF.

No data are available on thyroidectomized patients with macro-TSH except for two case reports with thyroid cancer and nodular goiter. Macro-TSH was present in one female patient with a history of low-risk papillary thyroid carcinoma<sup>20</sup>. Our second case is the second macro-TSH case reported in the literature, diagnosed due to insufficient TSH suppression after a papillary thyroid cancer operation.

In cases of operated papillary thyroid cancer followed up with TSH suppression, macro-TSH should be considered if a higher than expected dose of levothyroxine is needed to achieve euthyroidism. There are no definitive data on levothyroxine dose adjustment in these patients. Levothyroxine dose adjusted for body weight is the main factor in TSH suppression therapy. In such cases, levothyroxine replacement dose can be determined so that fT4 is kept at the upper limit of 1/3. Thus, patients are protected from exogenous hyperthyroxinemia. In the adults, while macro-TSH disappears and serum TSH level returns to normal in some patients, macro-TSH may persist for up to 4 years in some patients<sup>10</sup>. Considering that the renal clearance of macro-TSH is slower than monomeric TSH, long-term follow-up of patients is required. More studies are needed to assess the balance between TSH suppression and avoiding unnecessary exogenous hyperthyroxinemia.

## CONCLUSION

In conclusion, falsely elevated TSH should be kept in mind in clinically euthyroid<sup>18</sup> patients who are planning pregnancy and having subclinical hypothyroidism, and the patients with operated thyroid cancer who require a higher-than-expected dose of levothyroxine to achieve targeted TSH suppression.

## Ethics

**Informed Consent:** Consent form was filled out by all participants.

**Peer-review:** Externally peer-reviewed.

## Authorship Contributions

Surgical and Medical Practices: S.Y.Ç., B.A., M.O., M.Ç., Concept: S.Y.Ç., B.Y.B., M.Ç., Design: S.Y.Ç., E.Ö., B.Y.B., M.Ç., Data Collection or Processing: S.Y.Ç., E.Ö., B.A., M.O., Analysis or Interpretation: S.Y.Ç., B.A., M.O., B.Y.B., M.Ç., Literature Search: S.Y.Ç., E.Ö., B.A., M.O., Writing: S.Y.Ç.

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