Falsely Elevated Thyroid-Stimulating Hormone (TSH) Level Due to Macro-TSH

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Abstract. We encountered a 60-year-old woman with remarkably elevated thyroid-stimulating hormone (TSH) level as measured by electrochemiluminescent immunooassay (ECLIA), but with no specific symptoms, and with normal levels of free T3 and free T4. We performed the following investigations: polyethylene glycol (PEG) precipitation test, human antimouse IgG antibody (HAMA) interference test, and 3 additional TSH measurements by chemiluminescent immunooassay (CLIA). We then performed 2 gel filtration chromatography (GFC) procedures; one was at pH 7.2, and the other was at pH 3.0. Although the recovery of TSH shown by the PEG precipitation test was 4% which was extremely low, no HAMA interference was observed. Moreover, 3 CLIA instruments also showed various high values. The first GFC showed that the main peak of TSH immunoreactivity by ECLIA was located at a slightly larger molecular weight position than that of IgG. By the second GFC, the sample from the peak fraction of the first GFC showed that the TSH peak disappeared completely at the previous retention time but newly appeared at the same retention time as the TSH monomer. Protein G-Agarose gel removed the majority of the TSH complex. In conclusion, the majority of TSH in her serum was macro-TSH; TSH and anti-TSH IgG autoantibody complex. We should keep the possibility of macro-TSH in mind in cases with unexpectedly high TSH values, especially in autoimmune thyroidal disorders.

Key words: macro-TSH, anti-TSH autoantibody, IgG, interference, ECLIA

THYROID-STIMULATING hormone (thyrotropin; TSH) is a key parameter to evaluate thyroidal condition [1, 2]. Recently, the treatment of pregnant women with subclinical hypothyroidism has been discussed and the relation between cardiovascular disease and subclinical hypothyroidism has become a focus of attention [3, 4]. Thus, the reliability of the TSH value is important for subclinical hypothyroidism.

Methods for the measurement of TSH have developed from immunoradiometric assay (IRMA) to recent chemiluminescence enzyme immunoassay (CLEIA), chemiluminescence immunoassay (CLIA) and electrochemiluminescence immunoassay (ECLIA). These latter immunoassays provide greater precision, accuracy and a wider range of measurement [5].

A high level of TSH generally indicates hypothyroidism. However, we should consider the cases of TSH-producing tumor, resistance to thyroid hormone (RTH: Refetoff syndrome), and false elevation of TSH due to heterophile antibodies specific to the reagents [6]. Autoimmunity against TSH is rare and has not been evaluated in sufficient detail, although several reviews have been published on heterophile antibodies.

We examined a case with extremely high TSH levels. We decided to perform the polyethylene glycol (PEG) precipitation test. Furthermore, the TSH value was shown to be markedly low by PEG precipitation test. We eventually found macro-TSH, a unique anti-TSH autoimmune complex which may have caused the unexpectedly high elevation of TSH.
Case description

In December 2000, a 54-year-old woman lost consciousness watching television at home. In our emergency room she was resuscitated from idiopathic ventricular fibrillation by cardiologists.

In October 2002, laboratory data showed that high TSH (17.0μU/ml), normal freeT3 (3.30pg/ml) and freeT4 (1.22ng/dl) by IRMA (reference range by IRMA in our hospital: TSH 0.3–4.0μU/ml, freeT3 2.47–4.34pg/ml, freeT4 0.95–1.80ng/dl). Similar data were seen in April, July and October 2003. We began to use ECLIA for TSH, freeT3 and freeT4 measurement instead of IRMA in March 2005 (reference range by ECLIA: TSH 0.5–5.0μU/ml, free T3 2.3–4.3 pg/ml, freeT4 0.9–1.7 ng/dl). In June 2005 at the first measurement by ECLIA, she showed even higher TSH (96.0μU/ml), normal freeT3 (2.69 pg/ml) and freeT4 (1.19 ng/dl) without symptoms. TSH further increased to 274 μU/ml in June 2006, and 210 μU/ml in March 2007 with the same level of freeT3 and freeT4. The cardiologists had previously prescribed amiodarone at 200 mg/day in May 2002. TSH level did not decrease although they reduced the amount of amiodarone to 100 mg/day in November 2003 and 50 mg/day in July 2006.

She was introduced to us and we considered Hashimoto’s thyroiditis because of her elevated anti-thyroglobulin antibody (12.3U/mL) and ultrasound showed diffuse heterogeneous hypoechoic pattern. At first we suspected that there might have been an association of human anti-mouse IgG antibody (HAMA) interference.

We collected blood specimens after obtaining her consent and proceeded to the subsequent examination.

Materials and Methods

Materials

Patient’s blood specimens were collected regularly and centrifuged. The supernatant of the sample was stored at -80°C. We used the Elecsys 2010 and Modular Analytics E170 (Roche Diagnostics K.K. Tokyo, Japan) [5] for the measurement of TSH, free T3 and free T4 based on the ECLIA. A blood specimen collected in February 2007 underwent PEG precipitation test and HAMA interference test. A specimen collected in May 2007 was used for additional examinations such as gel filtration chromatography.

PEG precipitation test

We performed PEG precipitation test according to a previous report which used this test to identify macroprolactinemia [7]. We dissolved 2.5 g of PEG 6000 (Wako Junyaku Kogyo K.K., Tokyo, Japan) in 10 mL of distilled water. Equal volumes of a 25% solution of PEG and patient’s serum were mixed and centrifuged at 3000 x g for 5 min. Supernatant was collected and TSH level was measured by Elecsys. The recovery (%) was calculated by the following formula: recovery (%) = 2 x TSH (after absorption) / TSH (before absorption) x 100. A recovery rate which was lower than 40% suggests the influence of high molecular weight proteins, such as immunoglobulin, in the specimen.

HAMA interference test

Elecsys TSH is a sandwich assay using 2 different anti-mouse TSH monoclonal antibodies. It is rarely interfered with by HAMA because it is made with human and mouse chimeric antibodies [8]. Therefore, a positive control against HAMA is not available. For this interference test, a special TSH reagent was utilized in which one of the antibodies, the biotinylated mouse anti-human TSH monoclonal antibody, was changed to the biotinylated mouse anti-human hCG monoclonal antibody (Bt-hCG Ab). In assays with this reagent, the Bt-hCG Ab never binds to TSH. Therefore a high value suggests the presence of high concentrations of HAMA. High TSH levels would suggest a falsely elevated TSH level due to HAMA effect.

Measurements by other CLIA methods

Three additional TSH measurements based on chemiluminescent immunoassay (CLIA) were performed to compare TSH levels, using ADVIA Centaur (Siemens Medical Solutions Diagnostics, Tokyo, Japan), Lumipulse f (Fuji Rebio Inc., Tokyo, Japan), and Architect i2000 (Abbott Japan, Tokyo, Japan) systems.
FALSELY ELEVATED TSH LEVEL DUE TO MACRO-TSH

Table 1. Polyethylene glycol (PEG) precipitation test.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Elecsys TSH (μIU/mL) Before PEG treatment</th>
<th>After PEG treatment</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>210.6</td>
<td>3.81</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2. Human anti-mouse immunoglobulin G (IgG) antibody (HAMA) interference test.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Elecsys TSH reagent **</th>
<th>Non-sense assay reagent ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient, x4 diluted</td>
<td>48.34</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>control serum 1*</td>
<td>2.30</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>control serum 2*</td>
<td>2.08</td>
<td>&lt; 0.1</td>
</tr>
</tbody>
</table>

* HAMA negative control serum
** Sandwich assay reagent with two anti-TSH mouse monoclonal antibodies
*** Sandwich assay reagent with anti-TSH mouse monoclonal antibody and anti-hCG-β mouse monoclonal antibody

**Gel filtration chromatography (GFC)**

GFC was performed with coupled Shodex Protein KW-804 columns (Showa Denko, Tokyo, Japan). We performed 2 gel filtration procedures, one at pH 7.2, and the other at pH 3.0. In the first experiment, we eluted 200 μL of injected samples with 50 mM Tris-HCl buffer (pH 7.2) containing 150 mM NaCl. In the second experiment, we used 200 mM Glycine buffer (pH 3.0). The flow rate was controlled at 0.75 mL per min, and fractions were collected every 30 sec.

**Protein G addition test**

Protein G Agarose (Roche Diagnostics) was utilized for the absorption of serum IgG. After addition of an equal volume of Protein G Agarose, the patient serum was incubated at 4 °C for 3 h, centrifuged at 12000 g for 5 min, and then the supernatant was measured by Elecsys TSH.

**Results**

**PEG precipitation test, HAMA interference test, and measurements by other CLIA methods**

PEG precipitation test showed extremely low TSH recovery (4%, Table 1), indicating the influence of large molecules such as immunoglobulin. There was no significant elevation in the HAMA interference test (Table 2), and high TSH concentrations above each reference range were shown by all three of the other kinds of CLIA TSH measurements (Table 3). These findings suggested that the high TSH level in the ECLIA assay was not due to any heterophile antibodies specific to the assay reagent.

**Table 3. TSH measurement by different methods.**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Manufacturer</th>
<th>Reference range</th>
<th>TSH(μIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elecsys</td>
<td>Roche</td>
<td>0.5 ~ 5.0</td>
<td>152.0</td>
</tr>
<tr>
<td>Centaur</td>
<td>Siemens</td>
<td>0.4 ~ 4.0</td>
<td>20.47</td>
</tr>
<tr>
<td>Lumipulse</td>
<td>Fuji Rebio</td>
<td>0.610 ~ 4.684</td>
<td>112.436</td>
</tr>
<tr>
<td>Architect</td>
<td>Abbott</td>
<td>0.35 ~ 4.94</td>
<td>9.809</td>
</tr>
</tbody>
</table>

TSH levels of the patient serum were measured by 3 additional different methods.

**Table 4. Protein G addition test.**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>TSH Patient (μIU/mL)</th>
<th>Control *</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before absorption</td>
<td>151.95</td>
<td>2.04</td>
<td>31</td>
</tr>
<tr>
<td>After absorption</td>
<td>23.4</td>
<td>1.21</td>
<td>119</td>
</tr>
<tr>
<td>IgG Patient (mg/dL)</td>
<td>1150</td>
<td>1186</td>
<td>1</td>
</tr>
<tr>
<td>Control *</td>
<td>8</td>
<td>14</td>
<td>2</td>
</tr>
</tbody>
</table>

* Pooled serum

**Results**

**PEG precipitation test, HAMA interference test, and measurements by other CLIA methods**

PEG precipitation test showed extremely low TSH recovery (4%, Table 1), indicating the influence of large molecules such as immunoglobulin. There was no significant elevation in the HAMA interference test (Table 2), and high TSH concentrations above each reference range were shown by all three of the other kinds of CLIA TSH measurements (Table 3). These findings suggested that the high TSH level in the ECLIA assay was not due to any heterophile antibodies specific to the assay reagent.

**GFC**

Serum GFC showed that the main peak of TSH immunoreactivity by ECLIA assay was located at a slightly larger molecular weight position than that of regular IgG. When the gel filtration analysis was first performed, the main peak of TSH was recognized in the fraction at 27.5 min, which corresponded to a slightly larger side than the IgG peak (Fig. 1A.). The main peak retention time of the pseudo TSH was the same as that of IgG if the high value of TSH was a pseudo TSH due to non-specific IgG type heterophile antibodies.

This result clearly ruled out the possibility of IgG/IgM interference like HAMA because the main peak fraction of TSH for this specimen was obviously different from that of IgG (retention time 29.0 min) and IgM (21.5 min). In order to identify the presence of the immune complex comprised of TSH and IgG type anti-TSH autoantibody, we then made a sample from the TSH peak fraction (27.5 min) in GFC.

We later investigated the subsequent GFC with an...
acid elution buffer (pH 3.0) in order to dissociate the antigen-antibody complex.

When the TSH main peak fraction in the first GFC was applied to the second GFC column, the TSH peak disappeared at the previous retention time (27.5 min) but newly appeared at the same retention time as that of TSH monomer (TSH control, 32.0 min, Fig. 1B.).

In contrast, the peak fraction of IgG as a control remained at 29.0 min in the dissolving time under acidic conditions. The change (from 27.5 min to 32.0 min) in the dissolving time of the TSH peak represents the molecular weight change of the detected material because IgG showed at the same fraction (29.0 min) under acidic conditions. The TSH peak of the control reagent was also detected at 32.0 min. This supported the view that the TSH peak appearing at the above-mentioned 32.0 min is a monomeric TSH origin.

Protein G addition test

TSH recovery showed a remarkable decrease in the Protein G addition test (Table 4). This result suggested that the majority of the TSH complex was removed by Protein G Agarose gel which mainly absorbs IgG. Therefore, most of the TSH in blood of this case exists in the form of macro-TSH which is an immune complex of the anti-TSH autoantibody and TSH.

Discussion

We set out to determine why the TSH level was markedly higher than expected from the free T3 and free T4 levels in this patient. We were eventually able to clearly identify the presence of macro-TSH through examination of GFC and Protein G addition test. The results of GFC strongly suggested that most of the TSH was dissociated from a certain complex under acidic condition, and was detected as a monomer. Our subsequent GFC method is extremely useful to distinguish macro-TSH from other highly elevated TSHs, which is due to true TSH monomers or non-specific reactions, and can be used under a wide range of pH conditions.

Moreover, it was presumed that most of anti-TSH autoantibodies which bound TSH in the sample were IgG because a remarkable decrease was shown in TSH recovery after addition of Protein G. It is possible that, after the addition of Protein G, almost all of the IgG was adsorbed and removed, including the anti-TSH autoantibody, because IgG recovery was only 1%. However, the TSH recovery remained at 31% after Protein G was added, suggesting that some of the anti-TSH autoantibody in this specimen was the immunoglobulin which was neither adsorbed nor removed by Protein G.

The specimen of this patient showed various high TSH values in response to other TSH measurement re-
agents used in CLIA (Table 3). Therefore, we suggested that it was unavoidable that various TSH measurement reagents based on the sandwich method might be influenced by the anti-TSH autoantibody. The differently elevated TSH levels by CLIA were possibly due to the various epitopes and affinities of the sandwich assay reagents. There are many reports investigating macroprolactin based on gel filtration analysis [9, 10]. Those reports suggested an association of immune complex of the autoantibody and the hormone, i.e. the formation of macrobodies.

However, there are almost no reports on the formation of macro-TSH except in cases of neonates and their mothers both of whom had the anti-TSH autoantibody [11-13]. In the present case we were able to clearly demonstrate that the majority of TSH in the patient serum was present in the form of macro-TSH consisting of the immune complex bound to the anti-TSH autoantibody.

Halsall et al. used GFC assay to study neonates with high TSH levels to demonstrate the interfering substance. They compared the fraction between the patient’s serum and serum after incubation with serum from a control with increased TSH [14]. However, our method appears more certain since we added an acid elution buffer to GFC, which can clearly identify the monomeric TSH and separate it from immunocomplex. To the best of our knowledge, there is no other report describing this method in detail.

In general, the macroform lacks hormonal activity. It is assumed that no treatments were needed on the macroform-caused high values of TSH. There is a possibility of falsely elevated TSH due to anti-TSH autoantibody by ECLIA and CLIA. We were able to diagnose it in this case because the TSH value was extremely high. However, it would have been difficult if there had been only a small amount of immunocomplex. The limitations of this study included the fact that there was only one case of macro-TSH and the evaluation method is expensive and unusual.

Ismail et al. reported that 6 cases out of 5310 patients with high TSH value by CLIA were considered to immunological interference by endogenous antibodies. These 6 cases were independent from HAMA interference although another 22 cases out of 5310 patients showed HAMA interference [15]. It is likely that falsely elevated TSH due to macro-TSH would occur in some of these 6 cases. Therefore, there may be more patients with macro-TSH than expected. A great elevation of TSH value in our case was observed by the change of measurement from IRMA to ECLIA. These unexpected values can be detected by using new methods of analysis.

In conclusion, to avoid misdiagnosis of subclinical hypothyroidism, we should consider the possibility of macro-TSH or non-specific reaction to the reagent, in cases with TSH values higher than expected based on free T3 and free T4 levels.

**Acknowledgement**

The authors are indebted to Prof. J. Patrick Barron of the International Medical Communication Center of Tokyo Medical University for reviewing this manuscript.

**References**


