Macro Thyrotropin-IgG Complex Causes Factitious Increases in Thyroid-Stimulating Hormone Screening Tests in a Neonate and Mother

To the Editor:

We read with interest the case reported by Newman et al. (1) of factitious neonatal hyperthyrotoxemia, and we noted that the authors were unable to identify the maternally transmitted interfering substance. We report a similar case in which we demonstrated that the interference was caused by the presence of a macro thyrotropin-IgG complex (macroTSH).

Routine neonatal screening performed 7 days after an uncomplicated delivery in a full-term infant revealed a blood spot thyroid-stimulating hormone (TSH) of 213 mIU/L (Perkin-Elmer DELFIA assay, reference range <10 mIU/L). The neonate was clinically euthyroid, and repeat serum thyroid function tests at 10 days (Roche Elecsys) confirmed the increased serum TSH (826 mIU/L, reference interval 0.27–4.20 mIU/L) but showed a free thyroxine (FT₄) concentration within the reference interval, at 17.2 pmol/L (reference interval 12.0–22.0 pmol/L). The mother was also clinically euthyroid, and her thyroid function tests also showed increased TSH (308 mIU/L, reference interval 0.27–4.2 mIU/L) and an FT₄ of 13.5 pmol/L, within the reference interval of 12.0–22.0 pmol/L, both measured with the Roche Elecsys.

The discrepant thyroid function test results and the absence of clinical symptoms prompted investigations for interference in the TSH assay. In the mother’s serum TSH, results varied with different assays (reference intervals are in parentheses); Bayer Centaur, 4.0 mIU/L (0.35–5.5 mIU/L); DPC Immulite, 16 mIU/L (0.4–4.0 mIU/L); Roche Elecsys, 308 mIU/L (0.27–4.2 mIU/L), and Delfia 146 mIU/L (0.4–4.0 mIU/L). With the Immulite and Delfia assays, TSH values did not dilute linearly (other systems were not tested). A 5-fold dilution of maternal serum in TSH-free diluent (provided by the manufacturers) increased estimated serum TSH concentration to 58.1 and 225 mIU/L, respectively. With the Elecsys assay, adsorption with protein G-Sepharose (Sigma) removed 99.6% (routine 1%) of the TSH immunoreactivity, indicating interference involving an IgG antibody. Gel filtration chromatography of serum from the mother and infant, performed with a 40-cm Sephacryl S-300 column (Pharmacia), showed that the majority of the TSH immunoreactivity in the Elecsys assay eluted in a peak with molecular mass consistent with a TSH-IgG complex (Fig. 1). This peak was not present on chromatography of serum after adsorption with protein G-Sepharose.

Incubation of maternal serum with serum from a patient with hypothyroidism and increased TSH, followed by chromatography, showed an increase in the concentration of macroTSH (Fig. 1), confirming the presence of excess anti-TSH antibodies. Serum TSH in the neonate remained increased (>100 mIU/L) at 3 months of age, but an accurate result after dilution was not obtained. It is possible that the macroTSH in the neonate resulted from maternal transmission of the anti-TSH antibody, but the mother refused further investigations on herself and the infant, and we were not able to determine whether the macroTSH persisted.

In conclusion, we report a case in which macroTSH caused a false-positive screening test result for congen-
ital hypothyroidism. In their case report, Newman et al. (1) emphasized that increased serum TSH results detected in screening programs for neonatal hypothyroidism should be confirmed with repeat TSH and FT₄ measurements and investigation of discrepant results. It has also been recommended that maternal thyroid function tests be carried out in these cases (2). MacroTSH has been described in a mother and her neonates by Tamaki et al. (3), and we report our case to raise awareness of this particular form of assay interference and to illustrate the value of gel filtration chromatography in identifying interference from macro forms of peptide hormones (4–6) and in distinguishing this form of interference from that caused by heterophile antibodies.

References


The authors of the article cited above respond:

To the Editor:

Falsely increased thyrotropin (TSH) results caused by heterophile antibodies have been well documented in the literature. Although demonstration of macro forms of hormones (especially macroprolactin) is well known, there have been very few reports in the literature of factitious TSH increases caused by macro TSH in euthyroid patients.

Halsall and colleagues, in their letter above, are to be commended for their elegant use of Sephacryl S-300 chromatography to analyze macro TSH in the serum of a mother and her neonate.

Unfortunately, it is unclear whether the maternally transmitted interfering substance that we detected in our study (1) is similar to the macro TSH reported by Halsall et al. In the neonate investigated by Halsall et al., the increase in neonate TSH values was an order of magnitude greater than in our patient (Roche Elecsys result of 826 mIU/L, compared with 60.9 mIU/L, measured on the Dade Dimension in our study). Like Halsall et al., we believe that we excluded the presence of heterophile antibodies, but there remains a possibility that we were also dealing with a macro TSH. Protein A-sepharose did not decrease the high maternal serum TSH concentrations in our study, in marked contrast to the protein G-sepharose in the study of Halsall et al. Two possible explanations are the presence of an IgG3-TSH binding antibody not bound by protein A-sepharose but bound by protein G-sepharose; or dissociation of an IgG-TSH complex under the conditions of the protein A-sepharose separation, perhaps a result of the ionic strength, as demonstrated by Tamaki et al. (2).

We reiterate the importance of following up abnormal TSH results for which clearly increased TSH is inconsistent with the clinical state of the patient and/or the normal free thyroxine (FT₄). This precaution is particularly important in newborn screening, but will apply generally to any situation. In the case of neonates, confirmation of the abnormality in maternal serum (by measuring both TSH and FT₄) will support the conclusion that the interference is caused by a maternally derived Ig. Alternative assays may confirm the artificial nature of the TSH. It may be necessary, however, to provide more than 1 alternative assay, because we found that 4 of 7 TSH assays were affected in our study (1), and Halsall et al. found that 3 of 4 assays were affected. Other tests to elucidate whether the interference is a result of a heterophile antibody or macro TSH should include measurements of dilutions of sample with manufacturers diluent, sample pretreatment with protein A-sepharose, and addition of heterophile blocking agents such as Scantibodies.

We would support the approach of Halsall et al. in definitively demonstrating the presence of a macro TSH by the use of gel filtration. In conclusion, the presence of macro TSH has been poorly recognized but may be more common than is currently appreciated.

References