Partial prediction of postpartum Graves’ thyrotoxicosis by sensitive bioassay for thyroid-stimulating antibody measured in early pregnancy

Akane Ide1), Nobuyuki Amino1), Eijun Nishihara1), Takumi Kudo1), Mitsuru Ito1), Yukiko Kimura2), Nobuya Tatsumi2), Mineo Yamazaki2) and Akira Miyauchi3)

1) Department of Internal Medicine, Kuma Hospital, Kobe, Japan
2) Department of Obstetrics and Gynecology, Palmore Hospital, Kobe, Japan
3) Department of Surgery, Kuma Hospital, Kobe, Japan

Abstract. Graves’ disease often occurs after delivery. However, it has been difficult to predict who will develop Graves’ hyperthyroidism. We attempted to predict postpartum onset of Graves’ disease by measuring anti-TSH receptor antibodies (TRAb) and thyroid-stimulating antibodies (TSAb) in early pregnancy. TRAb was measured by a third generation assay and TSAb was measured by a newly developed sensitive bioassay. In 690 early pregnant women, 2 showed borderline TRAb positive reactions. However, none of them developed Graves’ disease after delivery. Thirty-eight of 690 pregnant women were positive for anti-thyroid peroxidase antibodies (TPOAb) and 4 were positive for TSAb. Two of these 4 women developed postpartum Graves’ hyperthyroidism. These findings indicate that the third generation TRAb assay was not useful, but that the sensitive TSAb bioassay was moderately useful for predicting the postpartum onset of Graves’ hyperthyroidism.

Key words: Graves’ disease, Postpartum onset, Prediction, Thyroid-stimulating antibody (TSAb), Anti-TSH receptor antibody (TRAb)

Materials and Methods

In this study, we used frozen serum samples that were obtained in a previous study that clarified the relationship between maternal thyroid function and fetal and child development [8].

Subjects

We examined 690 consecutive pregnant women that had visited the Palmore Hospital for their first checkup during early pregnancy between July 2005 and January 2006. All subjects were at 7–15 wk gestation. Pregnant women who had present or past history of Graves’ disease were excluded. Serum concentrations of TSH, free T4 (FT4), free T3 (FT3), and TPOAb were mea-
sured at the initial obstetrical visit. Thirty-eight subjects positive for TPOAb were followed for 1-6 months postpartum in order to clarify the presence of postpartum thyroid dysfunction. When thyroid dysfunction was revealed after delivery, patients were referred from Palmore Hospital to Kuma Hospital.

Graves’ disease and painless thyroiditis were diagnosed according to the diagnostic guidelines of the Japan Thyroid Association [9], defined by clinical findings and the determination of serum FT4, FT3, TSH, and TRAb. Final diagnosis was confirmed by following up the patients.

Methods

TSH, FT4, and FT3 concentrations were measured by electrochemiluminescent immunoassays (ECLusys TSH, ECLusys FT4, and ECLusys FT3, respectively; Roche Diagnostics K.K., Tokyo, Japan) [8]. TPOAbs were measured using a sensitive RIA system (TPOAb Cosmic 2; Cosmic Co., Tokyo, Japan). The normal range for TPOAb is less than 0.3 U/mL [8].

For the prediction of postpartum onset of Graves’ disease, all 690 frozen serum samples from early pregnancy were measured for TRAb using a third generation kit (electrochemiluminescence immunoassays; ECLusys 2010; Roche Diagnostics Japan Co., Tokyo, Japan). Cut off was 1.9 IU/L [10]. Thirty-eight TPOAb-positive samples were assayed for TSAb activity by sensitive bioassay [6]. In this assay kit, effect of serum TSH is negligible because of using anti-TSH antibody in buffer, and serum hCG up to 125,000 mU/mL did not influence TSAb activity [6]. In order to increase the sensitivity, the original method [6] was modified as follows: Serum (50 μL) was mixed with 200 μL of ice-cold 15% PEG6000 by vortexing. The serum was centrifuged for 20 min at 3,000 rpm. The pellet was dissolved in 75 μL of buffer1. The frozen cells stored at -80°C (3 × 10^6 cells/mL) were thawed in a water bath at 37°C for 2 min, transferred to 10 mL of buffer 1, and then centrifuged for 5 min at 1,000 rpm. The cell pellets were suspended in 12 mL of buffer 2, and 25 μL of luminescence substrate Viviren™ (Promega, Madison, WI, USA) by gentle pipetting. A neutralizing antibody against hTSH was included in buffer 2. The diluted sample (1:1.5) was added to a 96-well white plate (25 μL duplicate), followed by 100 μL of cell suspension, and then incubated for 4 h at 25°C. Light luminescence of each well was recorded for 7 seconds by adding 100 μL of detection buffer containing 100 mM of calcium chloride using an injector equipped with a luminometer (Tecan IF200, Männedorf, Switzerland). Activities of aequorin TSAb were calibrated using the international standard, NIBSC 08/204, and expressed as mIU/L. This procedure increased sensitivity 5 fold more than the original method [6]. The cut off value of TSAb in postpartum women was fixed at 12 mIU/dL.

Informed consent was obtained from each subject. Protocols were approved by the ethics committees of the Palmore and Kuma Hospitals.

Results

Positive TRAb was found in 2 of 690 pregnant women. However, actual values were borderline at 2.5 and 2.1 IU/L. None of these subjects developed postpartum Graves’ disease.

Thirty-eight of 690 pregnant women showed positive TPOAb (23.99 ± 35.43 U/mL: range 0.5-100), and 4 of them showed positive TSAb (Fig. 1). These 4 subjects were different from the above 2 women with borderline positive TRAb. Two of 4 women with positive TSAb developed postpartum Graves’ hyperthyroidism. One of them developed painless thyroiditis (Fig. 1).

Discussion

We found postpartum onset of Graves’ hyperthyroidism in 3 (7.9%) of 38 pregnant women with positive TPOAb, that is, subclinical autoimmune thyroiditis.

This prevalence was very similar to the 7.0% found in our previous study [5]. Two of 690 pregnant women showed borderline TRAb positive reactions, but it is possible that these may have been induced by nonspecific reactions, as other findings from our group have shown that around 1% of healthy subjects showed borderline TRAb positive reactions (unpublished data). However, this third generation TRAb assay [7] is more sensitive than previous TBII assays, and showed that these TRAb reactions were not related to postpartum Graves’ hyperthyroidism.

This newly introduced TSAb assay is very sensitive and specific. Indeed, 98.9% of 199 untreated patients with Graves’ disease showed positive reactions. In contrast, all 185 normal control subjects were negative [6]. In this study, we introduced a modified assay procedure to increase the sensitivity up to 5 fold more than the original assay system (see method). Four subjects showed positive TSAb, and 2 of them developed postpartum
Prediction of postpartum Graves’ disease

confirm the importance of TSAb measurement in early pregnancy. In conclusion, the third generation TRAb assay was not useful, but a sensitive TSAb bioassay was moderately useful for predicting the postpartum onset of Graves’ hyperthyroidism.

Acknowledgement

We would like to thank Dr. Naohiro Araki for his help to assay TSAb.

Disclosure Statement

The authors state that they have no conflict of interest.

References

1. Kashiwai T, Hidaka Y, Takano T, Tatsumi KI, Izumi Y, et al. (2003) Practical treatment with minimum maintenance dose of anti-thyroid drugs for prediction of remission in Graves’ disease. Endocr J 50: 45-49.

2. Jansson R, Dahlberg PA, Winsa B, Meirik O, Safwenberg J, et al. (1987) The postpartum period constitutes an important risk for the development of clinical Graves’ disease in young women. Acta Endocrinol (Copenh) 116: 321-325.

3. Tada H, Hidaka Y, Tsuruta E, Kashiwai T, Tamaki H, et al. (1994) Prevalence of postpartum onset of disease within patients with Graves’ disease of childbearing age. Endocr J 41: 325-327.

4. Benhaim Rochester D, Davies TF (2005) Increased risk of Graves’ disease after pregnancy. Thyroid 15: 1287-1290.

5. Hidaka Y, Tamaki H, Iwatani Y, Tada H, Mitsuda N, et al. (1994) Prediction of post-partum Graves’ thyrotoxicosis by measurement of thyroid stimulating antibody in early pregnancy. Clin Endocrinol (Oxf) 41: 15-20.
6. Araki N, Iida M, Amino N, Morita S, Ide A, et al. (2015) Rapid bioassay for detection of thyroid-stimulating antibodies using cyclic adenosine monophosphate-gated calcium channel and aequorin. *Eur Thyroid J* 4: 14-19.

7. Yoshimura Noh J, Miyazaki N, Ito K, Takeda K, Hiramatsu S, et al. (2008) Evaluation of a new rapid and fully automated electrochemiluminescence immunoassay for thyrotropin receptor autoantibodies. *Thyroid* 18: 1157-1164.

8. Orito Y, Oku H, Kubota S, Amino N, Shimogaki K, et al. (2009) Thyroid function in early pregnancy in Japanese healthy women: relation to urinary iodine excretion, emesis, and fetal and child development. *J Clin Endocrinol Metab* 94: 1683-1688.

9. Japan Thyroid Association 2010 Guidelines for the diagnosis of thyroid disease. available at: www.japanthyroid.jp/doctor/guideline/english.html (accessed April 28, 2016).

10. Ide A, Amino N, Kang S, Yoshioka W, Kudo T, et al. (2014) Differentiation of postpartum Graves' thyrotoxicosis from postpartum destructive thyrotoxicosis using antithyrotropin receptor antibodies and thyroid blood flow. *Thyroid* 24: 1027-1031.