TSH-receptor Antibodies Determined by the First, Second and Third Generation Assays and Thyroid-stimulating Antibody in Pregnant Patients with Graves’ Disease

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Abstract. The measurement of TSH receptor antibody (TRAb) has been recommended to predict the risk of neonatal hyperthyroidism (NH) in pregnant women with Graves’ disease (GD). For the first generation TRAb (TRAb1) assay with commercial kit (Brahms, Berlin, Germany; or Cosmic co., Tokyo, Japan) an arbitrary limit of 40 U/l or 50% was suggested to indicate risk when measured late in pregnancy. In order to substitute TRAb1 with the second generation TRAb using porcine TSH receptor (pTRAb2) and human recombinant TSH receptor (hTRAb2) and the third generation TRAb (TRAb3) assay for this purpose, we measured TRAb in these four methods late in pregnancy in a total of 62 pregnant women with Graves’ disease. The data showed that no cases with TRAb1 >50% has been missed if the TRAb1 assay was replaced by the pTRAb2, hTRAb2 or TRAb3 assay using their equivalent cut-off value of 70%, 10 IU/l, and 75%, respectively, but that an additional group of women would have been included in the risk group, especially in the TRAb3 assay. Next, the effect of maternal TRAb on thyroid function of offspring was studied in the 47 pregnant women with GD (43 with TRAb1 <50% and 4 with TRAb1 >50% during late pregnancy). In 2 women who gave birth to hyperthyroid children at days 6 and 14 of life, the maternal sera had strongly positive levels of TRAb1 (73.5% and 84.1%), pTRAb2 (84.9% and 91.5%), hTRAb2 (40.68 IU/L and 89.70 IU/L) and TRAb3 (92.1% and 93.5%) late in pregnancy, with one case displaying high positive (1114.3%) thyroid stimulating antibody (TSAb) level and the other case had moderate positive (433%) TSAb level. Of the remaining 45 women, 43 had TRAb1 <50% and the other 2 had TRAb >50% including 1 with low TSAb positive and 1 with positive thyroid stimulating blocking antibody (TSBAb) and negative TSAb; all of them gave birth to euthyroid children. Finally, a serial study regarding TRAb in 23 women with Graves’ disease during pregnancy showed that TRAb1, pTRAb2, hTRAb2, TRAb3 value and TSAb level decreased significantly as pregnancy progressed. In conclusion, the present study supported TRAb as a useful marker to predict the risk of NH.

Key words: Neonatal hyperthyroidism, TRAb, Pregnancy

PREGNANCY has profound effects on the thyroid which can be demonstrated both during gestation and postpartum. Even though the interrelations are not fully understood, recent studies have shown that various immunosuppressive factors may partly explain the protection of the fetal allograft during pregnancy [1, 2]. Maternal autoimmune thyroid disorders may affect fetal and neonatal thyroid function through placental transfer of anti TSH-receptor antibodies (TRAb). These antibodies, mainly of the IgG class, usually elicit thyroid stimulating activity but in rare cases may inhibit thyroid stimulation. Thyroid stimulating antibodies (TSAb) are usually encountered in Graves’ disease (GD). Children born to women with high titers of TRAb are therefore at risk of developing passive thyroid autoimmune hyperthyroidism. It has thus been recommended to determine TRAb late in pregnancy in women with GD to predict the risk of neonatal hyperthyroidism (NH). An arbitrary limit of 50% [3–5] or 40 U/L [6–8] using 1st generation TRA (TRAb1) assay was suggested to indicate risk when measured late in

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pregnancy. In order to substitute TRAb1 assay with the 2nd generation TRAb assay using porcine TSH receptor (pTRAb2) and human recombinant TSH receptor (hTRAb2) and the 3rd generation TRAb (TRAb3) assay, we measured TRAb in these four methods late in pregnancy. Also we studied serially the changes in the levels of anti-TSH receptor antibody (TRAb) during pregnancy.

Subjects and methods

Sixty-two patients were studied for comparison of TRAb determined by TRAb1, pTRAb2, hTRAb2 and TRAb3. Of the 62 women with GD, 44 had active GD (PTU in 40 and MMI in 4) throughout pregnancy. MMI or PTU was stopped in 7 cases during pregnancy because of remission. Two had a past history of GD for which they had undergone subtotal thyroidectomy. An other 2 who had a past history of antithyroid therapy for GD, were receiving thyroid hormone replacement therapy. The remaining 7 cases had inactive GD throughout pregnancy. Also we observed 47 pregnant women with GD and their offspring. Blood samples were obtained from the women during the third trimester of pregnancy. Of these, 34 patients were euthyroid and 3 were just above of normal range according to free T4 estimated with chemiluminescent immunoassay (Siemens Medical Solutions Diagnostics K.K.). The remaining 10 cases were subclinically hyperthyroid. Of the 47 pregnant women with GD, 32 had active GD (PTU in 29, MMI in 1 and KI in 1) and 13 had inactive GD that were clinically and biochemically euthyroid without medication at the time of study. Another one of 47 women was receiving l-thyroxine due to hypothyroidism after subtotal thyroidectomy for GD. The remaining one had a past history of GD and was euthyroid at the time of blood sampling, with l-thyroxine replacement therapy for hypothyroidism which developed upon the appearance of TSBAb.

Maternal diagnosis of GD was made on the basis of elevated FT3 and FT4 levels, suppressed TSH levels, positive TRAb values, and exaggerated thyroid uptake of 99mTc scintigraphy before pregnancy.

The assay methods for determining serum TRAb1, pTRAb2, hTRAb2, TRAb3 and thyroid-stimulating antibody (TSAb) have been previously reported [9–11]. The values determined by each TRAb and TSAb were expressed as percentages, except IU/L of hTRAb2. TSAb activity was assayed by measuring inhibition of TSH-induced cAMP production. Values above 45% indicated the presence of TSBAb activity. Serial serum samples from each patient were all stored at –20°C. All patients were informed of the purpose of this study and gave their consent.

Statistical significance was analyzed using Wilcoxon signed-rank test.

Results

The determination of TRAb levels in pregnant women with Graves’ disease has been recommended to predict the risk of NH. An arbitrary limit of 50% using TRAb1 assay was suggested to indicate risk when measured late in pregnancy [3–5]. The effect of substituting TRAb1 with pTRAb2, hTRAb2 and TRAb3 for this purpose is shown in Figure 1. An overall significant correlation was obtained between TRAb1 and pTRAb2 (n = 182; r = 0.893, p<0.0001) (Fig. 1a), between TRAb1 and hTRAb2 (n = 135, r = 0.829, p<0.0001) (Fig. 1b), and between TRAb1 and TRAb3 (n = 164, r = 0.856, p<0.0001) (Fig. 1c). No cases with TRAb1 >50% would have been missed if TRAb1 assay was replaced by the pTRAb2, hTRAb2 and TRAb3 using the cutoff values of 70%, 10 IU/L and 75%, respectively, but 12.5% (3 of 24 cases) in pTRAb2 assay, 7.7% (1 of 13 cases) in hTRAb2 assay and 39.2% (11 of 28 cases) in TRAb3 assay would have been included in the risk group, as shown in Table 1.

Next, this study observed 47 pregnant women with GD and their offspring, as shown in Figure 2. Blood samples were obtained from the women late in pregnancy. Of 47 women with Graves’ disease, 43 had TRAb1 <50% and 4 had TRAb1 >50%. Two women gave birth to clinically and biochemically hyperthyroid children at days 6 and 14 of life. Their sera had strongly positive levels of TRAb1 (73.5% and 84.1%), pTRAb2 (84.9% and 91.5%), hTRAb2 (40.68 IU/L and 89.70%), and TRAb3 (92.1% and 93.5%) late in pregnancy, respectively. TSAb in one case was remarkably elevated
at 1114.3%, although another case showed moderate TSAb positivity at 433%. Both cases were kept at the subclinical hyperthyroidism by PTU, 100 mg three times daily and by MMI, 10 mg once daily. The remaining 45 women (43 has TRAb1 <50% and 2 had TRAb1 >50%) gave birth to clinically and biochemically euthyroid children. The maximum values of pTRAb2, hTRAb2, TRAb3 and TSAb values were 58.8%, 5.47 IU/L, 70.1% and 604.6% in the pregnant 43 women with GD who had TRAb1 <50%. Of them, the one who gave birth to a euthyroid infant, was receiving l-T4 replacement therapy because of the presence of a strongly positive TSBAb activity of 98.7% (normal, <45%) late pregnancy. She had a previous history of MMI treatment for Graves’ disease and had TRAb1, 50.7%, pTRAb2, 74.8%, hTRAb2, 16.24 IU/L, TRAb3, 84.5% and TSAb, 159%.

Finally, the effect of pregnancy on the levels of TRAb1, pTRAb2, hTRAb2, TRAb3 and TSAb was studied serially in 23 patients with Graves’ disease during pregnancy, as shown in Table 2. A significant difference was found for the levels of TRAb1, pTRAb2, hTRAb2, TRAb3 and TSAb between early and late pregnancy. In addition, there were significant differences in pTRAb2, hTRAb2, TRAb3 and TSAb levels between early and middle pregnancy and in TRAb1, hTRAb2, TRAb3 and TSAb between middle and late pregnancy, although this study found no significant difference in TRAb1 levels between early and middle pregnancy and in pTRAb2 levels between middle and late pregnancy.
Discussion

An arbitrary limit of 40 U/L or 50% using TRAb1 assay was suggested to indicate the risk of NH when measured in late in pregnancy. Clavel et al. [6] reported that all seven women who gave birth to hyperthyroid infants had TRAb1 values of more than 40 U/L and the only false positive result was in a woman with a TRAb1 value of 67 U/L and whose baby was euthyroid. The remaining 27 women who gave birth to euthyroid infants showed TRAb1 values of below 40 U/L. It is also pointed out by Laurberg et al. [7] and Wallace et al. [8] that with TRAb1 method of TRAK (Brahms, Berlin, Germany) the levels above approximately 40 U/L are considered high enough to indicate risk of neonatal hyperthyroidism. Interestingly, Momotani reported that the levels above 50% of TRAb1 (Cosmic corporation, Tokyo, Japan) are useful for the prediction of NH and the data obtained from the literature by Matsuura et al. [4] and Hale et al. [5] strongly supported the view that positive TRAb1 >50% conveyed high likelihood of NH. The effect of sub-
STITUTING TRAb1 WITH hTRAb2 FOR THIS PURPOSE SHOWED THAT A TRAb1 OF 40 U/L WAS EQUIVALENT TO THE hTRAb2 OF 10 IU/L [12], WHICH WAS ALSO DEMONSTRATED TO BE COMPARABLE TO 50% OF TRAb1 IN THE PRESENT STUDY. IT IS THUS SUGGESTED THAT A TRAb1 VALUE OF 40 U/L IS THE EQUIVALENT CUT-OFF VALUE OF TRAb1 50%. IN ADDITION, THIS STUDY CLARIFIED THAT NO CASES WITH TRAb1 50% WOULD HAVE BEEN MISSED IF THE TRAb1 ASSAY WERE REPLACED BY pTRAb2 AND TRAb3 USING THE EQUIVALENT CUT-OFF VALUE OF 70% AND 75%, RESPECTIVELY, ALTHOUGH ON ADDITIONAL GROUP OF WOMEN WOULD BE INCLUDED IN THE RISK GROUP, ESPECIALLY IN TRAb3 ASSAY. ALSO THIS STUDY SHOWED THAT ALL 43 WOMEN WHO HAD TRAb1 VALUES OF <50% GAVE BIRTH TO EUTHYROID INFANTS AND THAT THE UPPER LIMIT OF TRAb1, pTRAb2, hTRAb2, TRAb3 AND TSAb IN THESE WOMEN WERE 32.3%, 58.8%, 5.47 IU/L, 70.1% AND 604.6%, RESPECTIVELY. IN CONTRAST, 2 OF 4 WOMEN WITH TRAb1 >50% WHO GAVE BIRTH TO HYPERTHYROID INFANTS, HAD STRONGLY POSITIVE LEVELS OF TRAb1, pTRAb2, hTRAb2 AND TRAb3 LATE IN PREGNANCY, WHILE HIGH-POSITIVE (1114.3%) AND MODERATE-POSITIVE (433%) TSAb RESULTS WERE OBSERVED IN THE 2 PREGNANT WOMEN.

The two women who had TRAb1 values >50% including one low-positive (261.5%) TSAb and another one positive for TSBA and negative TSAb gave birth to euthyroid infants. Finally, this study investigated sequentially the early, middle and late pregnancy with the purpose of analyzing changes in TRAb1, pTRAb2, hTRAb2, TRAb3 and TSAb in pregnant women with GD.

Compared to the data early in pregnancy, the findings in late pregnancy indicated TRAb1, pTRAb2, hTRAb2, TRAb3 and TSAb were significantly decreased. As has been reported, immunosuppressive factors originating from fetus, the placenta, or the mother (decreased circulating T helper cells), could play a role in the median decrease of TRAb levels between early and late pregnancy [1,2]. Gonzalez-Jimenez et al. [13] also reported that the levels of TRAb1 decreased during pregnancy in both active and remission GD patients and Tamaki et al. [14] indicated TRAb1 levels often fell during pregnancy in active GD.

References
