Effect of thyroid peroxidase antibodies on thyroid-stimulating hormone reference limits in a primarily Latina population

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Summary: The aim of the paper is to determine the prevalence of thyroid peroxidase antibodies (TPOAb) and assess its effect on the thyroid-stimulating hormone (TSH) reference range during pregnancy in a primarily Latina population. Serum samples were collected from healthy pregnant women and non-pregnant controls. TSH reference ranges were calculated when TPOAb-positive patients were either included or excluded. A total of 134 pregnant women and 107 non-pregnant controls were recruited. Positive TPOAb titres were found in 23 (17.2%) of the 134 pregnant women, and in 14 (13.1%) of the 107 non-pregnant controls. When the TPOAb-positive women were included in the TSH analysis, the upper reference limit using two different methods was consistently higher: 0–2.2 fold in the non-pregnant women, 2.01–2.78 fold in the first trimester, 3.18–4.7 fold in the second and 1.05–1.42 fold in the third. The lower TSH reference limit was not affected by the inclusion of TPOAb-positive subjects. In conclusion, inclusion of TPOAb-positive patients results in higher upper reference limits during pregnancy.

Keywords: thyroid, pregnancy, reference, Latina

INTRODUCTION

It is now well established that thyroid-stimulating hormone (TSH) levels are lower in pregnancy as a result of beta-human chorionic gonadotropin (β -hCG) stimulation of the thyroid.^{1,2} For the clinician taking care of the pregnant woman, the ability to properly interpret TSH test results requires that the manufacturer-provided reference ranges take these physiological changes into account.

Experts recognize the necessity for trimester and method-specific TSH reference ranges in pregnancy.^{3–6} It has been emphasized that specific criteria be used in patient selection when establishing TSH reference ranges.⁷ These criteria include the absence of personal or family history of thyroid dysfunction, no visible or palpable goitre, being free of medications, drawing of samples in the fasting state and in the morning hours, iodine sufficient state, use of modern TSH immunoassays and being thyroid peroxidase antibody (TPOAb) negative. It is the last of these criteria that is the focus of our research.

TPOAbs are associated with future thyroid dysfunction, specifically autoimmune thyroiditis (Hashimoto's thyroiditis). Despite the high prevalence (i.e. 11–18%) of TPOAbs in the general population, pregnant patients who are TPOAb positive should be distinguished from those patients without TPOAbs.^{8–10} This is

primarily because of data that associate TPOAb positivity with adverse pregnancy outcomes such as spontaneous abortion, overt hypothyroidism and postpartum thyroiditis.^{11,12}

The objective of this study was to evaluate the effect of including or excluding TPOAb-positive women when determining trimester-specific TSH reference ranges in a primarily Latina population.

METHODS

Pregnant women who were obtaining prenatal care at the Los Angeles County and University of Southern California Women's and Children's Hospital were recruited in the first trimester of pregnancy. Participants had sequential samples of venous blood drawn in the first trimester (<14 weeks), second trimester (14–27 6/7 weeks) and in the third trimester (≥ 28 weeks). A spot urine was obtained to measure iodine at each visit. All participants were over the age of 18 years and had singleton, viable pregnancies confirmed by ultrasound performed in the first trimester. Exclusion criteria included known thyroid disease, autoimmune disease, multiple gestation, diabetes, hyperemesis gravidarum or pregnancy within the last 12 months.

Pre-menopausal non-pregnant subjects aged 18–45 years were recruited from the gynaecology clinic and matched for ethnicity. Exclusion criteria included known thyroid disease, autoimmune disease, use of hormone-containing contraceptives or pregnancy within the last 12 months. Both blood and urine were collected once. These subjects were different from the pregnant subjects.

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Table 1 Urinary iodine and TPO antibody positivity

	N	Gestational age (weeks)	Urinary iodine ($\mu\text{g/dL}$)	TPO antibody positive n (%)
Non-pregnant	107	0	19.6 ± 29.4	14 (13.1)
First trimester	134	9.2 ± 2.3	16.1 ± 10.5	23 (17.3)
Second trimester	52	24.7 ± 3.1	24.1 ± 58.7	10 (19.2)
Third trimester	83	32.4 ± 4.6	19.8 ± 15.7	20 (24.4)

TPO = thyroid peroxidase

TSH was measured using the Elecsys (Roche, IN, USA) platform (method A). When sufficient specimen was available, TSH values were also obtained using the Tosoh A1A-600 analyser (Ramsey, MN, USA) (method B). TPOAb were measured using the Nichols Advantage platform (San Juan Capistrano, CA, USA) as well as by the Kronus (Boise, ID, USA) radioimmunoassay method. The TSH reference ranges established by the respective laboratories for methods A and B were 0.3–3.0 mIU/L.

Iodine was measured as previously described and iodine sufficiency for the study population was defined as a median urinary concentration greater than $5 \mu\text{g/dL}$.¹³

Comparison of demographic data between pregnant and non-pregnant subjects was performed using independent *t*-test and χ^2 . Comparison of thyroid function tests was performed using non-parametric tests and χ^2 . $P < 0.05$ was considered significant. Statistical analysis was performed using SPSS v.13.0 (Chicago, IL, USA).

The Institutional Review Board of the Los Angeles County + University of Southern California Medical Center approved the study.

RESULTS

Non-pregnant women were older (31.8 ± 6.0 versus 27.4 ± 6.4 years, $P = 0.001$) and more parous (1.9 ± 1.6 versus 1.3 ± 1.3 , $P = 0.02$). There was no difference in gravidity and the percentage with Hispanic ethnicity was also similar: 98.2% of the pregnant and 94.0% of the non-pregnant women. The mean

gestational age in the first trimester was 9.2 weeks, in the second 24.7 weeks and in the third 32.4 weeks. TPOAbs were detected in 23/134 (17.2%) of pregnant and in 14/107 (13.1%) of non-pregnant women. Our population was iodine sufficient in both the non-pregnant and pregnant state. The prevalence of TPO antibody positivity was not significantly different among the entire cohort ($P = 0.25$; Table 1).

Table 2 shows the non-pregnant and pregnant subjects' TSH median and 95% reference ranges for methods A and B when TPO Abs are included and excluded in the analysis. When subjects with TPO antibodies were included in the analysis, the TSH medians did not statistically differ in each trimester; however, when TPOAb-positive subjects were included, the upper 95% reference range was higher across all time periods for method A and all time periods during pregnancy for method B. For method A, when including TPOAb-positive subjects, the upper 95% reference range increased 2.2-fold in the non-pregnant state, 2.01-fold in the first trimester, 3.2-fold in the second trimester and 1.05-fold in the third trimester. For method B, when including TPOAb-positive subjects, the upper 95% reference range increased 0-fold in the non-pregnant state, 2.78-fold in the first trimester, 4.7-fold in the second trimester and 1.42-fold in the third trimester. The lower 95% range did not greatly differ with the inclusion of TPOAb-positive patients; of note, in the first trimester, the lower TSH range remained the same for both methods.

DISCUSSION

The diagnosis of thyroid dysfunction in pregnancy depends on having a TSH test that accounts for the physiological changes that occur with each trimester. The inclusion of TPOAb-positive subjects in the calculation of pregnancy-specific TSH reference ranges will significantly elevate the upper reference limit. Our findings confirm prior studies that show a widening of the TSH reference range and show an elevation in the mean TSH value when TPOAb-positive subjects are included in the calculations.^{5,14}

Table 2 TSH and the effect of included TPO antibody patients on the median and 95% reference range (mIU/L)

	TPO antibodies excluded			TPO antibodies included		<i>P</i>
	Median	2.5–97.5 reference range		Median	2.5–97.5 reference range	
Non-pregnant						
Method A <i>n</i> = 93	1.60	0.63–3.90	Method A <i>n</i> = 107	1.70	0.65–8.48	0.51
Method B <i>n</i> = 26	2.10	0.79–3.96	Method B <i>n</i> = 30	2.09	0.79–3.96	0.87
First trimester						
Method A <i>n</i> = 111	1.20	0.03–2.60	Method A <i>n</i> = 134	1.30	0.03–5.24	0.42
Method B <i>n</i> = 77	1.32	0.02–3.11	Method B <i>n</i> = 99	1.38	0.02–8.66	0.41
Second trimester						
Method A <i>n</i> = 42	1.60	0.53–4.16	Method A <i>n</i> = 52	1.65	0.60–13.21	0.75
Method B <i>n</i> = 34	1.80	0.72–3.70	Method B <i>n</i> = 44	1.88	0.76–17.35	0.88
Third trimester						
Method A <i>n</i> = 63	1.60	0.20–4.80	Method A <i>n</i> = 83	1.70	0.33–5.08	0.74
Method B <i>n</i> = 44	1.91	0.14–3.84	Method B <i>n</i> = 59	1.94	0.19–5.47	0.83

TPO = thyroid peroxidase; TSH = thyroid-stimulating hormone

The reason to exclude patients with TPOAb when establishing thyroid test reference ranges is because of the association between TPOAb and autoimmune thyroiditis. Moreover, the clinical manifestations of autoimmune thyroiditis (e.g. overt hypothyroidism, subclinical hypothyroidism) have been associated with adverse pregnancy outcomes such as poor fetal neurodevelopmental outcome, fetal death, preterm delivery and placental abruption.^{15–17} However, even without overt clinical or biochemical manifestations of thyroid dysfunction, patients who are euthyroid but have TPOAb positivity are at increased risk for adverse pregnancy outcomes (e.g. abortion, preterm birth, postpartum thyroiditis).⁹ Interestingly, Negro *et al.*¹⁸ showed that thyroxine supplementation reduced the risk for preterm birth and spontaneous abortion in euthyroid TPOAb-positive pregnant subjects.

It should be emphasized that the TSH reference ranges are assay-dependent and population-dependent, and the clinician should be aware of the specific assay being used by the laboratory. The Endocrine Society has recommended lowering the upper TSH limit to 2.5 mIU/L in the first trimester of pregnancy.¹⁹ In our study, when TPOAb-positive women were excluded, the upper TSH reference range in the first trimester was 2.6 mIU/L for method A and 3.11 mIU/L for method B. Our data partially support this recommendation, but more importantly, it shows that different assays can result in different ranges. That said, we acknowledge that the sample size for method B is small and recruitment of more patients is needed before any conclusions can be made on the final reference range for method B. Ethnicity may also affect the TSH 95% reference range. The TSH range in our mostly Hispanic, non-pregnant women was consistent with the 95% reference range data provided by the National Health and Nutrition Examination Survey (NHANES) trial, where the median and 95% reference range for non-pregnant Mexican American women between the age of 12 and 49 was 1.37 (0.38–4.69). However, in NHANES's pregnant Mexican American population, the TSH median and 95% reference range was 1.34 (range 0.04–10.85) and was noticeably different from our trimester-specific TSH ranges whether or not TPOAb status was accounted for.²⁰

In conclusion, we have shown that the inclusion of TPOAb-positive patients has a significant influence on TSH values and can significantly increase the upper 95% reference range. When establishing pregnancy-specific reference ranges, TPOAb-positive subjects should be excluded from the analysis.

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