Assessment of thyroid function during pregnancy: first-trimester (weeks 9–13) reference intervals derived from Western Australian women

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ABSTRACT

Objective: To establish first-trimester-specific reference intervals for thyroid function tests in pregnant Australian women.

Design, setting and participants: Serum samples were collected from 2159 pregnant women (9–13 weeks' gestation) attending a private pathology practice for first-trimester screening during October and November 2006. Levels of serum thyrotropin (TSH), free thyroxine (fT_4), free triiodothyronine (fT_3), thyroid peroxidase antibodies (TPOAb), and thyroglobulin antibodies (TgAb) were measured by chemiluminescent immunoassay (Abbott ARCHITECT analyser).

Main outcome measures: Reference intervals based on 2.5th and 97.5th percentiles for TSH, fT_4 and fT_3, after exclusion of 338 women with positive TPOAb or TgAb tests; comparison with reference intervals for non-pregnant women (TSH, 0.40–4.0 mU/L; fT_4, 9–19 pmol/L; fT_3, 3.0–5.5 pmol/L).

Results: Derived reference intervals for thyroid function tests during the first trimester of pregnancy were: TSH, 0.02–2.15 mU/L; fT_4, 10.4–17.8 pmol/L; and fT_3, 3.3–5.7 pmol/L. If the non-pregnant TSH reference range was applied to the study participants, 344 women (16.0%) whose serum TSH concentration was within the first-trimester-specific reference range would be misclassified as having subclinical hyperthyroidism, and 98 women (4.5%) with a TSH concentration above the first-trimester-specific upper reference limit would not be identified.

Conclusions: The reference interval for TSH during the first trimester of pregnancy differs substantially from that for non-pregnant women, and applying the general laboratory reference range to pregnant women results in misclassification of thyroid status for 20.5% of women. Australian pathology laboratories should adopt pregnancy-specific reference intervals for thyroid function tests.

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The importance of normal maternal thyroid function in pregnancy and fetal development is well established.[1-4] During the first trimester, the fetus is reliant on transplacental passage of maternal thyroxine, as the fetal thyroid is not fully functional until about 16 weeks' gestation, whereas thyroid hormone receptors in fetal tissues are present and functional much earlier.[1-4] Neuropsychomotor development is impaired and mean IQ scores are reduced in children born to women who had thyroid deficiency during pregnancy.[5-7] Pregnancy complications such as spontaneous miscarriage and gestational hypertension are associated with maternal hypothyroidism. Rates of placental abruption and premature delivery are also increased with both maternal hypothyroidism and subclinical hypothyroidism.[6,8,9]

Laboratory reference intervals for thyroid function tests are based on the central 95% of results from non-pregnant subjects without thyroid disease. Their validity in pregnant women is questionable because of the physiological changes in thyroid function that occur during pregnancy. In particular, human chorionic gonadotropin (hCG) stimulates thyroid hormone secretion, resulting in reduced serum thyrotropin (TSH) concentrations because of a negative feedback effect. This effect is likely to be particularly relevant during the first trimester of pregnancy, when hCG secretion is maximal.

Several studies have attempted to derive pregnancy-specific reference ranges for thyroid function tests, with inconsistent results[10-14] — perhaps reflecting differences in iodine status between studies and, in some studies, the inclusion of women with thyroid autoimmunity.[10,11] No relevant studies have been conducted with Australian women. It has been suggested that, until trimester-specific and method-specific reference ranges are established, an upper limit for TSH in pregnant women of 2.5 mU/L (compared with 4.0–4.5 mU/L in non-pregnant women) should be adopted.[15]

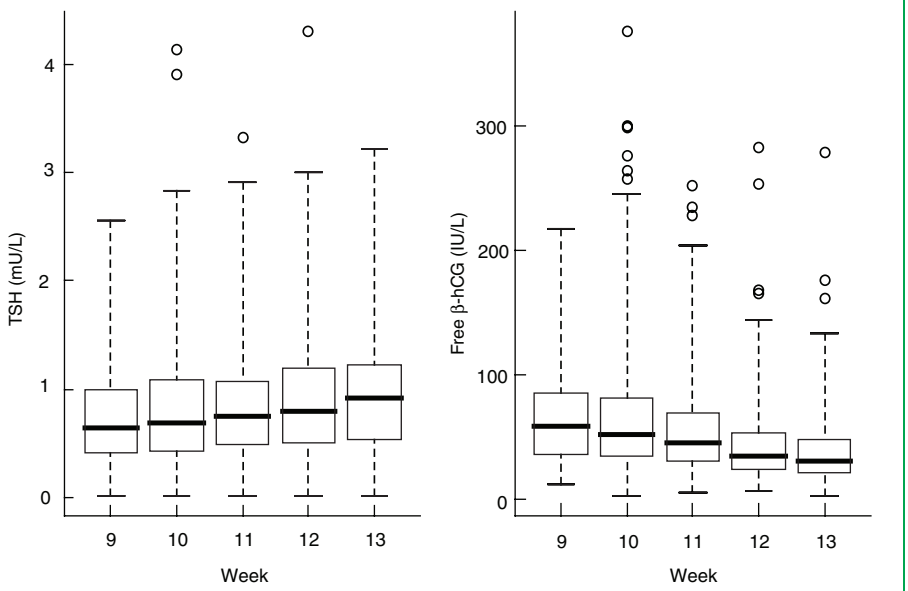
We aimed to establish first-trimester-specific reference intervals for TSH, free thyroxine (fT_4) and free triiodothyronine (fT_3) in women in Western Australia. The iodine status of pregnant Western Australian women has not been specifically studied, but a recent study of school children in WA suggests it is an iodine-sufficient region.[16]

METHODS

Study subjects were 2159 women who consecutively attended Western Diagnostic Pathology laboratories for first-trimester screening during October and November 2006. Based on number of births and the number of first-trimester screens performed at local pathology practices (Peter O'Leary, Director, Office of Population Health Genomics, WA Health, personal communication), we estimate that 73% of pregnant women in WA undergo first-trimester screening and, of these, 84% are performed at Western Diagnostic Pathology. Gestational age was determined by ultrasound for 41% of women and by date of last menstrual period for the remainder.

Patient serum samples were de-identified and stored at – 20° C. Analysis was performed at PathWest Laboratory Medicine WA in January and February 2007. Levels of serum TSH, fT_4, fT_3, thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb) were measured by automated two-step chemiluminescent immunoassay on an ARCHITECT analyser (Abbott Diagnostics, Sydney, NSW). For TSH, the limit of detection was 0.01 mU/L, and inter-assay coefficients of variation were 6.4%, 3.9% and 2.9% at TSH concentrations of 0.07 mU/L, 0.39 mU/L and 5.3 mU/L, respectively. The intra-assay imprecision of TSH at 2.1 mU/L was 1.5%. The inter-assay coefficients of variation for fT_4, fT_3, TPOAb and TgAb were all less than 6%. Free β-hCG

1 Serum thyrotropin (TSH) and free β-human chorionic gonadotropin (hCG) concentrations in 1817 antibody-negative women, by week of gestation



was measured at Western Diagnostic Pathology (as part of first-trimester screening) on a Kryptor analyser (Brahms, Berlin, Germany) by time-resolved cryptate emission analysis. The detection limit for free β-hCG was 0.16 IU/L, and inter-assay coefficients of variation during testing were 5.0%, 3.7% and 5.6% at 7.96 IU/L, 20.2 IU/L and 80.1 IU/L, respectively.

The local laboratory reference intervals for TSH, fT_4 and fT_3 for non-pregnant women (established from 100 healthy adult blood donors) are: TSH, 0.4–4.0 mU/L; fT_4, 9–19 pmol/L; and fT_3, 3.0–5.5 pmol/L. Reference intervals for TPOAb (< 5.61 mU/L) and TgAb (< 4.11 kU/L) were taken from the manufacturer's kit insert.

For each analyte, medians and the 2.5th and 97.5th percentiles were determined by the Harrell–Davis quantile estimator method[17] for each week within the 9–13-week gestation range, and overall for the first trimester. For the purposes of determining reference intervals, women who were positive for TPOAb and/or TgAb were excluded. We also calculated 95% confidence intervals for the lower and upper limits of the first-trimester reference ranges, using the Harrell–Davis jackknife variance estimator.[17] Shifts in the distribution of age, gestation and TSH, fT_4 and fT_3 between the antibody-positive and antibody-negative groups were assessed non-parametrically using Wilcoxon tests.

In the antibody-negative cohort, differences in median TSH and free β-hCG in gestation weeks 9–13 were tested using quantile regression analysis. The relationship between free β-hCG and TSH was examined using multiple linear regression, with adjustment for week of gestation, maternal age and the remaining thyroid variates. In this analysis, TSH was square root transformed to ensure statistical adequacy of the model.

The study was approved by the Human Research Ethics Committee of Sir Charles Gairdner Hospital.

RESULTS

Serum samples were collected from 2159 pregnant women between 9 and 13 completed weeks' gestation. Three subjects with insufficient sample volume for analysis were excluded, as was one subject with overt hypothyroidism (TSH, 32 mU/L; fT_4, 8 pmol/L; negative TPOAb and TgAb).

Of the remaining 2155 women, 338 (15.7%) were positive for TPOAb and/or TgAb. In the 1817 antibody-negative women, the median serum TSH concentration increased significantly across the 9–13-week gestation range ($P = 0.020$ from quantile regression analysis), whereas median serum free β-hCG concentrations decreased significantly over the same period ($P < 0.001$) (Box 1). There was a strong inverse relationship between TSH and free β-hCG ($P = 0.002$ from multiple regression analysis), which remained significant after adjustment for other statistically significant covariates (maternal age, week of gestation, fT_4 and pregnancy-associated plasma protein A).

There was statistical evidence of a shift effect for TSH, fT_4 and fT_3 between the antibody-negative and antibody-positive groups of women ($P < 0.001$ for each comparison) (Box 2). There was no evidence of a difference in distribution of maternal age between antibody-negative and antibody-positive groups ($P = 0.672$). There was statistical evidence of a small shift in gestational age ($P = 0.013$); however, the magnitude of the mean difference was 1 day.

The reference group for deriving reference intervals comprised the 1817 antibody-negative women (maternal age range, 14.3–45.8 years; mean, 30.9 years) (Box 2). Reference ranges by gestation week for TSH, fT_4 and fT_3 derived from non-parametric analysis (with 95% confidence intervals for the upper and lower limits of the reference ranges) are shown in Box 3. For the whole reference group, the first-trimester-specific reference intervals were: TSH, 0.02–2.15 mU/L; fT_4, 10.4–17.8 pmol/L; and fT_3, 3.3–5.7 pmol/L. The TSH reference range is substantially lower than the established non-pregnant TSH reference range of 0.4–4.0 mU/L.

If the general laboratory TSH reference intervals were applied to our study participants, misclassification of maternal thyroid function would occur. Three hundred and forty-four women (16.0%) with serum TSH concentrations that are normal for early pregnancy would be misclassified as having subclinical hyperthyroidism (reduced TSH, normal fT_4). Of these women, 25 (7.3%) were positive for TPOAb or TgAb. Conversely, 98 study subjects (4.5%) with a TSH above the first-trimester-specific upper reference limit would not have been identified, and, of these women, 56 (57.1%) were positive for either TPOAb or TgAb.

DISCUSSION

In this study, we have derived reference intervals for TSH, fT_4 and fT_3 on a common automated immunoassay platform for pregnant women in weeks 9–13 of their first trimester. For fT_4 and fT_3, the differences between the trimester-specific and conventional reference ranges are relatively minor, but for TSH, our results differ substantially from the conventional reference range. This is clinically important, because TSH is the most sensitive marker of thyroid dysfunction and is recommended as the initial screening test.

2 Demographic and thyroid function details for pregnant women at 9–13 weeks' gestation*

	All women	Antibody-positive women	Antibody-negative women (R)
Number	2155	338	1817
Age (years)	30.9 (5.3)	30.7 (5.4)	30.9 (5.2)
Gestation (weeks)	11.2 (1.0)	11.1 (1.0)	11.3 (1.0)
TSH* (mU/L)	0.78 (0.03, 2.78)	1.13† (0.06, 6.11)	0.74† (0.02, 2.15)
fT_4 (pmol/L)	13.5 (2.1)	13.0† (2.4)	13.5† (2.0)
fT_3 (pmol/L)	4.30 (0.67)	4.24† (0.85)	4.35† (0.64)

R = reference group. TSH = serum thyrotropin. fT_4 = free thyroxine. fT_3 = free triiodothyronine.
* Values are mean (SD) except for TSH, which are median (2.5th, 97.5th percentiles). † $P < 0.001$ from a Wilcoxon test of shift effect between the antibody-positive and antibody-negative groups. ◆

3 First-trimester-specific reference intervals for TSH, fT_4 and fT_3, by gestation week and for the whole group

Gestation week	No. of women	TSH (mU/L)	fT_4 (pmol/L)	fT_3 (pmol/L)
9^0–9^6	101	0.05–2.20	11.0–17.4	3.3–5.5
10^0–10^6	679	0.02–2.13	10.6–17.6	3.3–5.6
11^0–11^6	515	0.02–2.16	10.4–18.6	3.3–5.6
12^0–12^6	408	0.06–2.00	10.3–17.9	3.3–5.9
13^0–13^6	114	0.06–2.54	9.7–16.7	3.2–6.0
All	1817	0.02–2.15*	10.4–17.8*	3.3–5.7*
95% CI for LL†		(0.01–0.04)	(9.6–11.2)	(3.3–3.3)
95% CI for UL†		(2.02–2.28)	(17.2–18.4)	(5.6–5.8)

TSH = serum thyrotropin. fT_4 = free thyroxine. fT_3 = free triiodothyronine.
* Corresponding reference intervals for non-pregnant subjects are: TSH, 0.4–4.0 mU/L; fT_4, 9–19 pmol/L; fT_3, 3.0–5.5 pmol/L. † 95% CIs for the lower limit (LL) and upper limit (UL) of each reference range for the whole group. ◆

Our derived TSH reference interval of 0.02–2.15 mU/L is narrower than that reported by a study in the United States (0.01–4.05 mU/L).[11] However, most of the women in that study were Hispanic, and there is evidence for differences between ethnic groups in thyroid function tests.[18] Another US study also found a higher TSH upper limit of 3.61 mU/L, despite excluding TPOAb-positive women.[12] In both of these studies,[11,12] TSH was analysed on a different immunoassay platform using the DPC Immulite analyser (Diagnostic Products Corporation, Los Angeles, Calif, USA). In a recent US study of 585 thyroid disease-free and TPOAb-negative women of less than 14 weeks' gestation, the derived TSH reference interval was 0.04–3.6 mU/L using the Bayer Advia Centaur analyser (Bayer Diagnostics, Tarrytown, NY, USA).[13] In a study from Switzerland, researchers using the same automated immunoassay analyser as in our study (Abbott ARCHITECT) obtained a first-trimester-specific reference range for TSH in TPOAb- and TgAb-negative women of 0.09–2.83 mU/L.[14] The higher upper limit in these studies[13,14] may be due to the inclusion of women at earlier stages of gestation than in our study (less than 9 weeks), when the effects of hCG on the thyroid are not maximal. The diversity of reported first-trimester-specific reference intervals for thyroid function tests illustrates the importance of recruiting a local population and acknowledging methodological variations.

The strengths of our study include its large sample size, the inclusion of consecutively screened women from both metropolitan and regional areas, and the exclusion of women with thyroid autoimmunity in determining thyroid function test reference intervals. The prevalence of thyroid antibodies in our sample was similar to previous reports,[6,12,14] suggesting that our sample was representative of pregnant women in general in this respect. None of the previous studies[10-12] have evaluated iodine status in pregnant women. It has been documented that schoolchildren in WA are iodine-sufficient, based on the National Iodine Nutrition Study.[16] Our assumption in this study that pregnant women in WA are also iodine-sufficient needs to be confirmed.

Our study has some weaknesses. It was limited to women between 9 and 13 weeks' gestation, and it cannot be assumed that the derived reference intervals apply to earlier gestation in the first trimester, or to the second and third trimesters. We did not have access to information on maternal medical history or medication use, and it is possible that some antibody-negative women being treated with thyroid hormone replacement or other relevant medication were included in the reference group.

Our study shows that applying the conventional TSH reference interval to pregnant women results in misclassification of thyroid status in over 20% of women. Some 16% of women would have been erroneously classified as having subclinical or mild hyperthyroidism, which might lead to inappropriate investigation or treatment, and maternal anxiety. It has been shown that pregnant women with subclinical hyperthyroidism based on TSH at or below the 2.5th percentile are not at increased risk of adverse pregnancy outcomes.[19]

In a further 4.5% of the women, TSH was elevated according to the first-trimester-specific reference range; these women would not be identified with the conventional reference interval. More than half of these women (57%) tested positive for thyroid antibodies, suggesting they do have thyroid disease rather than being healthy outliers whose TSH concentrations happen to fall outside the reference range. The optimal management of these women is not known. In a recent randomised clinical trial, thyroxine replacement in pregnant women with thyroid antibodies and serum TSH within the conventional reference range (0.27–4.2 mU/L) was associated with a reduced rate of miscarriage and other pregnancy complications.[20] On that basis, and until further data are available, it may be reasonable to offer thyroxine replacement therapy to women whose serum TSH concentration is above the upper limit of the first-trimester-specific reference interval, if thyroid antibodies are present.

Our study is timely, as there is growing debate about the merits of screening for thyroid dysfunction during pregnancy. Currently, there is controversy over whether universal screening or targeting of high-risk individuals is more appropriate.[6,21] Whatever the outcome of that debate, it is likely that Australian pathology laboratories will receive increasing numbers of samples from pregnant women, and it is now appropriate for laboratories to move towards establishing trimester-specific (and method-specific) reference intervals for TSH, fT_4, and fT_3.

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COMPETING INTERESTS

None identified.

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