Free T4 immunoassays are flawed during pregnancy

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Background and Objective

Although thyrotropin (thyroid-stimulating hormone; TSH) is generally considered the primary test for evaluating thyroid status during pregnancy, in some situations it is imperative for clinicians taking care of pregnant patients to have access to an accurate and reliable way to estimate free thyroxine (FT4) concentrations. This is especially true in light of reports suggesting that isolated hypothyroxinemia may be associated with impaired fetal psychomotor development and a decreased intelligence quotient.

Pregnancy poses unique challenges to FT4 methodologies. Current FT4 immunoassays are tests used to estimate FT4 concentrations; they do not measure FT4 directly and are known to be sensitive to alterations in binding proteins. It is thus not surprising that pregnancy-induced increases in thyroxine-binding globulin (TBG), as well as decreases in albumin, can influence FT4 immunoassays in a method-specific manner. Whereas the law of mass action dictates that some lowering of FT4 should be expected in pregnancy because of the high TBG state, the unexpected high prevalence of low third-trimester FT4 immunoassay values vs the nonpregnant lower limit questions its reliability. Measurement of FT4 by the current reference methods (equilibrium dialysis, gas chromatography/mass spectrometry) reveals less than a 10% decrease in third-trimester mean vs nonpregnant controls. Thus, FT4 immunoassays may not reliably identify hypothyroxinemic pregnant patients.

We evaluated the performance of 2 commonly used FT4 immunoassays in a select population of clinically euthyroid subjects in the nonpregnant state and through each trimester of pregnancy. Total T4 (TT4) and the free T4 index (FT4I) have been used for clinical management of thyroid disease in pregnancy for 3 decades with consistent results. In recent years, FT4 immunoassays have largely replaced these 2 tests; nevertheless, method-specific and trimester-specific data for these direct immunoassays are limited. These limitations have caused considerable confusion about the interpretation of thyroid tests during pregnancy. The primary purpose of this study was to compare the diagnostic performance of 2 different FT4 immunoassays to traditional approaches for estimating free thyroxine (total T4 and FT4I) relative to the physiologic TSH changes that occur throughout pregnancy.

★ EDITORS' CHOICE ★

OVERVIEW

The use of nonpregnant reference ranges for free thyroxine measured by immunoassay will result in an overdiagnosis of hypothyroxinemia during pregnancy.

Materials and Methods

We recruited women obtaining prenatal care in the first trimester of pregnancy at the Los Angeles County and University of Southern California Women's and Children's Hospital. Sequential samples of venous blood were drawn in the first trimester (< 14 weeks), second trimester (14-27 6/7 weeks), and third trimester (≥ 28 weeks). A spot urine was obtained to measure iodine at each visit. All participants were over age 18 years and had viable singleton pregnancies confirmed by ultrasound performed in the first trimester. Premenopausal nonpregnant subjects aged 18-45 years were recruited from the gynecology clinic and matched with subjects for ethnicity.

Values measured included TSH, TT4, a thyroid hormone-binding ratio (THBR) estimate of TBG, and an FT4 estimate made by immunoassay. Free hormone index corrections for TBG effects (FT4I) were calculated by dividing total hormone values by the THBR result. Thyroid peroxidase (TPO) antibodies were measured. TPO antibody-positive subjects were excluded from the analysis. Batches of specimens were separated and the sera stored at 4°C for ≤ 3 days before analysis.

Reference ranges were TSH (methods A and B), 0.3-3.0 mIU/L: TT4, 4.5-12.5 μg/dL; THBR, 0.72-1.24; FT4I, 4.5-12.5 μg/dL; and FT4 immunoassay (method A, 0.93-1.7 ng/dL; method B, 0.75-1.54 ng/dL). Iodine sufficiency for the study population was defined as a median urinary concentration > 5 μg/dL.

Results

From January 2004 through April 2007, 134 pregnant subjects and 107 nonpregnant subjects were enrolled. After TPO antibody-positive subjects had been excluded, 111 pregnant patients and 107 nonpregnant controls remained for analysis. Of the 111 pregnant subjects recruited in the first trimester, follow-up samples were obtained in 47 in the second trimester and 63 in the third trimester. Both nonpregnant and pregnant

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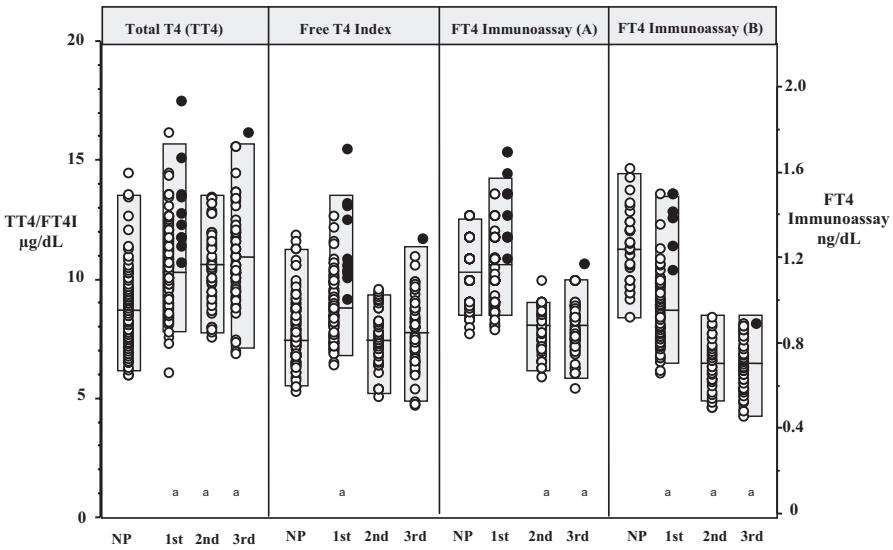
subjects were iodine sufficient among all trimesters.

TSH values were lower in the first trimester compared with nonpregnant controls and returned to nonpregnant values in the second and third trimesters using both methods. Ten patients had first-trimester TSH values below 0.3 mIU/L using method A; these patients also had low values measured by method B. Only 3 patients (2.7%) had values above 2.5 mIU/L (2.6, 2.6, and 3.1 mIU/L) using method A. Method B values available for 2 of these patients were 3.3 and 3.1 mIU/L.

The Figure shows the different approaches for estimating free T4 status (total T4, FT4I, and FT4 immunoassays). As expected, the estrogen-mediated rise in TBG resulted in higher TT4 values relative to nonpregnant subjects in all 3 trimesters ($P < .001$). The TBG effect was present very early in gestation because first-trimester median TT4 values were not statistically different from second-trimester values. The FT4I appeared to correct for the TBG effects in that first-trimester values were higher than in nonpregnant subjects due to the peak human chorionic gonadotropin (hCG) stimulatory effect, returning to nonpregnant levels in the second and third trimesters. The inverse relationship between free thyroxine and TSH appeared appropriate for all methods.

In contrast, neither of the 2 FT4 immunoassays demonstrated the expected first-trimester increase in FT4 relative to the nonpregnant controls. The median FT4 measured by method B was significantly lower than in nonpregnant subjects in the first trimester (1.30 [0.90-1.60] vs 0.97 [0.68-1.50] ng/dL; $P < .001$). In the second and third trimesters, a high percentage of women had FT4 immunoassay results below the manufacturer's lower limit (Table). By method A, 7.5% of nonpregnant subjects and 5.4% of first-trimester subjects had FT4 values below the manufacturer's lower limit, whereas FT4 was low in 66.0% during the second trimester and in 57.1% during the third trimester. For method B, 0% of nonpregnant and 6.6% of first-trimester subjects were below the manufacturer's lower limit, while 63.9% and 67.5% were below this threshold in the second and third trimesters, respectively. In contrast, neither the FT4 index nor TT4 fell below the manufacturer's lower limit in any of the groups.

FIGURE
TT4, FT4I, and FT4 immunoassay A+B results



● Low TSH < 0.3 mIU/L
[a] $P < .05$ vs NP

Individual and median values for nonpregnant (NP) vs 1st, 2nd, and 3rd trimesters for total thyroxine (TT4) vs 3 free thyroxine estimate tests: free T4 index (FT4I) and 2 different immunoassays (A = Roche Elecsys; B = Tosoh A1A). [a]Significance relative to the nonpregnant group. Patients with TSH below 0.3 mIU/L are indicated by *solid symbols*. Sample sizes are as listed in the Table.

FT4, free thyroxine; *T4,* thyroxine; *TSH,* thyroid-stimulating hormone.

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Comment

We have demonstrated that in an iodine-sufficient, TPO antibody-negative population, the measurement of free thyroxine by 2 different immunoassays did not reflect the expected physiologic hCG-mediated rise in the first trimester. In addition, the expected return to nonpregnant concentrations in the second and third trimesters was not seen. Instead, a continued decline in FT4 was identified, resulting in 57-68% of women falling into a range that would be classified as hypothyroxinemic by the manufacturer's recommended ranges. In contrast, the FT4I performed as expected, with a physiologic increase in the first trimester and normalization to nonpregnant levels in the second and third trimesters. This pattern of change during pregnancy corresponds to that described using the gold standard methods of equilibrium dialysis and tandem mass spectrometry. Because these latter methods are expensive and technically demanding, they are not suitable for widespread clinical use, but to the extent that they validate the trends seen with the FT4I, they support its use.

An impediment to the use of the FT4I in current practice is that it requires 2 distinct tests, adding to expense. Alternatively, TT4 may serve as an appropriate approximation of FT4. The TT4, whether measured directly or indirectly, has shown remarkably consistent ranges throughout pregnancy over many years: approximately 143-158% of nonpregnant values. Adjusting the TT4 in pregnancy by a factor of 1.5 compared with nonpregnant reference ranges yields a workable estimate of FT4. In practice, the nonpregnant reference range of 4.5-12.5 µg/dL would become 6.75-18.75 µg/dL. Applying this adjustment to our study population, we found

TABLE
Subjects with thyroid values below the manufacturer's lower reference limit

Variable	FT4 method A[a]	FT4 method B[b]	FT4 index[c]	TT4[d]	TT4 × 1.5[e]
Nonpregnant	7/93 (7.5)	0/30 (0)	0/93 (0)	0/93 (0)	—
1st trimester	6/111 (5.4)	5/76 (6.6)	0/111 (0)	0/111 (0)	1/111 (0.9%)
2nd trimester	31/47 (66.0)	23/36 (63.9)	0/47 (0)	0/47 (0)	0/47 (0)
3rd trimester	36/63 (57.1)	27/40 (67.5)	0/63 (0)	0/63 (0)	0/63 (0)

Results are expressed as n/N (%).

[a] Free thyroxine (FT4) (0.93 ng/dL); [b] FT4 (0.75 ng/dL); [c] FT4 index (4.5 μg/dL); [d] Total thyroxine (TT4) below (4.5 μg/dL); [e] TT4 multiplied by ratio of 1.5 to adjust for pregnancy (6.75 μg/dL).

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no statistical difference in the percentage of subjects below the reference range for the FT4 index and the pregnancy-adjusted TT4. Since TT4 is an inexpensive assay well suited to automation, the simple adjustment described above would provide clinicians with an inexpensive and readily available method for estimating free thyroxine.

In conclusion, FT4 immunoassays in pregnant women yield results that do not correspond to well-established pregnancy-related changes in the thyroid axis or to estimates of FT4 by equilibrium dialysis and tandem mass spectrometry. The results diverge so significantly during the second and third trimesters that the vast majority of women would be diagnosed incorrectly as hypothyroxinemic by laboratory criteria alone. Unless normal and abnormal values are determined for each immunoassay for the pregnant state, immunoassay results may underestimate FT4. Alternatively, the FT4 index or TT4 adjusted for pregnancy may be used reliably for estimating FT4 status in pregnancy.

CLINICAL IMPLICATIONS

- Clinicians evaluating thyroid function during pregnancy should be aware that free thyroxine (FT4) immunoassays in pregnant women yield results that do not correspond to well-established pregnancy-related changes in the thyroid axis or to estimates of FT4 by equilibrium dialysis and tandem mass spectrometry.
- FT4 immunoassay results diverge so significantly during the second and third trimesters that the vast majority of women would be diagnosed incorrectly as hypothyroxinemic by laboratory criteria alone.
- Alternatively, the FT4 index or total T4 adjusted for pregnancy may reliably be used to estimate FT4 status in pregnancy. ■