CLINICAL LABORATORY STRATEGIES
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Analyzing Free T4 in Pregnancy

Immunoassays May Not Measure Levels Accurately

By Gina Rollins

Pregnancy brings on well-described hormone changes that alter thyroid function. TSH is the preferred test for evaluating thyroid status in pregnancy, but in some instances free T4 also is of diagnostic importance. FT4 immunoassays have largely replaced the older free thyroxine index tests but are known to be sensitive to changes in binding proteins, and some studies have suggested that they are prone to inaccurate readings, especially in the third trimester. New research examines the performance of two FT4 immunoassays throughout pregnancy. This issue of Strategies *explores those findings.*

Human chorionic gonadotropin (hCG) and estrogen alter thyroid function in pregnancy, causing increases in thyroxine binding globulin (TBG), as well as increased thyroid synthesis and secretion of T3 and T4. The blood volume expansion that occurs in pregnancy also causes hemodilution, which affects levels of certain analytes, such as albumin, that bind to thyroxine. The thyroidal stimulation caused by high hCG levels causes a slight lowering of TSH in the first trimester, which usually returns to normal during the second and third trimesters. Otherwise, FT4 typically remains within the nonpregnant reference range throughout gestation, when measured directly by reference techniques, whereas total thyroid hormone levels—TT4 andTT3— typically are higher.

While overt and subclinical hypothyroxinemia are relatively rare in pregnancy, they have important consequences for pregnancy complications and neuropsychological development of the child. But reference methods for directly measuring FT4, equilibrium dialysis /tandem mass spectrometry, are technically cumbersome and impractical for many clinical labs to use. FT4 immunoassays provide only an estimate of free thyroxine and are prone to report inappropriately low values when binding proteins are grossly abnormal such as during pregnancy or nonthyroidal illness, according to co-author Carole Spencer, PhD, FACB, professor of medicine at the Keck School of Medicine at the University of Southern California in Los Angeles. (DOI:10.1016/J.ACOG.2008.10.042. Accessed January 2009.) Problems in interpreting the tests in pregnant women led the authors to perform this study.

“In practice, we had reports of a lot of FT4 tests coming back with low results when compared with the manufacturers’ reference ranges, which made us want to assess how they performed in pregnancy. We wanted to find out whether the tests were reliable and what the implications would be for clinical practice,” explained lead author Richard Lee, MD, assistant professor of obstetrics and gynecology at the Keck School of Medicine.

The researchers collected venous blood samples from 111 pregnant women and 107 nonpregnant control subjects, all of whom were TPOAb negative, and did not have thyroid or autoimmune disease, multiple gestation, diabetes, hyperemesis gravidarum, or had not been pregnant within the last 12 months. Samples from all 111 pregnant women were drawn in the first trimester, and sequentially from 47 and 63 in the second and third trimesters, respectively. The immunoassay platforms used to conduct the study included Roche’s Elecsys and the Tosoh A1A-600 analyzer.

The researchers found that three tests gave appropriate values for pregnancy. Specifically, TT4 values were higher in pregnant subjects than non-pregnant controls in all three trimesters, TSH values lower in the first trimester compared with non-pregnant controls and then normalized, and FT4I values slightly elevated in the first trimester in relation to non-pregnant subjects and returned to normal values in the second and third trimesters. In contrast, neither FT4 immunoassay reported the expected first trimester rise in FT4 in comparison to non-pregnant controls, nor the expected normalization in the second and third trimesters. In fact, 66% of pregnant subjects in the second trimester and 57.1% in the third trimester had FT4 values below Roche's lower limit for the Elecsys, while 63.9% of pregnant subjects in the second trimester and 67.5% in the third trimester had levels below the Tosoh A1A-600 Analyzer's lower limit. In contrast, none of these subjects had FT4I or TT4 values below the manufacturer's lower limit.

The results puzzled the researchers. "The values just got lower and lower. We don't know why, but it doesn't reflect what happens physiologically with pregnant women. Their values should stay within the reference range," noted Spencer. The researchers speculate that the immunoassays may not work well during pregnancy due to their sensitivities to alterations in binding proteins, which can cause alterations in a "method specific manner." Laurence Demers, PhD, FACB, distinguished professor of pathology and medicine at Pennsylvania State University in Hershey, Pa. believes there is an explanation. "The FT4I used in this study is accounting for TBG and not albumin, so it's missing out on one of the more important binders of thyroid hormone —albumin—which has been shown to decline as pregnancy progresses," he cautioned. Spencer agreed that albumin levels become low in pregnancy, but stated that although TBG is the dominant thyroxine binder, free T4 methods are supposed to measure FT4 independent of binding proteins such that changes in albumin should not affect free T4 concentrations. Furthermore, FT4 measured by direct reference methods is consistent with the FT4I values but not the low FT4 immunoassay values.

Demers also pointed out that the researchers did not assess other available immunoassays and did not compare the two in this study against the gold standard. Spencer explained that the Elecsys and Tosoh A1A-600 Analyzer are commonly used immunoassays and that another study, cited in this paper, evaluated the performance of a range of immunoassays in the third trimester and found they were all prone to low values.

The researchers acknowledge limitations of their study, in that 94% of pregnant subjects and 98.2% of nonpregnant participants were Hispanic, and that they were unable to recruit more than 120 subjects per trimester as recommended by the NACB in its guidelines on laboratory support for the diagnosis and monitoring of thyroid disease. Still, with further confirmation in other studies, the results may help establish a reference range for FT4 immunoassays used in serum from pregnant women. "You can't develop a reference range that's based on an artifact," said Spencer.

Until further studies are completed and a robust, reliable reference range is developed, "clinicians should be very cautious in interpreting FT4 results by immunoassay," said Lee. When it is necessary to assess the FT4 status of a pregnant patient, this study showed that FT4I measurement was more reliable than two FT4 immunoassays. A CDC-American Thyroid Association conference held in 2004 acknowledged problems with current FT4 immunoassays and recommended the use of TT4 measurements with the nonpregnant reference range adjusted by a factor of 1.5 to compensate for the elevated TBG. Laboratory directors should monitor developments as evidence regarding the FT4 immunoassays evolves.

Guidelines from several professional organizations disagree about the need for thyroid screening in pregnant women. In addition to NACB, further information is available from the American Association for Clinical Endocrinology (www.aace.org), the American College of Obstetricians and Gynecologists (www.acog.org), and The Endocrine Society (www.endosociety.org)