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Thyroid Function Testing in Pregnancy and Thyroid Disease: *Trimester-specific Reference Intervals*

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Abstract

During pregnancy the thyroid is hyperstimulated, resulting in changes in thyroid hormone concentrations. Accurate assessment of thyroid function during pregnancy is critical, for both the initiation of thyroid hormone therapy, and for the adjustment of thyroid hormone dose in those already receiving thyroid hormone. Trimester-specific intervals are especially important during pregnancy when thyroid insufficiency may be associated with adverse obstetric outcome and fetal neurodevelopmental deficits. Gestational age-specific reference intervals are now available for thyroid function tests. Knowing the expected normal changes in hormone concentrations throughout pregnancy allows individualized supplementation when necessary.

Keywords

gestation; thyroxine T4; triiodothyronine T3; thyroid-stimulating hormone; TSH; free T4; FT4; reference ranges; LC/MS/MS

Thyroid disease is the second most common endocrine disease to affect women of reproductive age. Thyroid disorders can have adverse reproductive and pregnancy implications. Although gestational hyperthyroidism is uncommon (0.2%), gestational hypothyroidism occurs in higher prevalence (2.5%) and can lead to neonatal and child neurodevelopmental deficits and maternal obstetric complications.[1,2] In addition to overt thyroid dysfunction, pregnancy may unveil subclinical hyperthyroidism and hypothyroidism. Women who have been diagnosed with thyroid gland dysfunctions are usually treated and are able to complete a normal pregnancy. Thyroid-related pathophysiologic changes aggravated by pregnancy, and some obstetric conditions, such as gestational trophoblastic disease or hyperemesis gravidarum, may affect thyroid gland function and impact maternal-fetal thyroid hormone balance. Trimester-specific reference intervals for thyroid function tests are critical for maintaining the delicate balance of thyroid hormones during pregnancy.

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Pregnancy induces complex changes in circulating maternal steroid hormones and in thyroid binding globulin (TBG) concentrations. In addition to the stimulatory effects of estrogen on TBG synthesis, a major contribution to the increased TBG concentration during pregnancy is the reduced plasma clearance of the protein caused by changes in TBG glycosylation induced by estrogen. It is commonly thought that total thyroxine (TT4) and total triiodothyronine (TT3) concentration increase in the setting of pregnancy-induced increases in serum TBG concentrations. The concentrations of TBG double by weeks 16 to 20 of gestation.[3] In addition to the 2- to 3-fold increase in serum TBG, modest decreases in both serum transthyretin and albumin are commonly found in pregnancy.[4] Free T3 (FT3) and free T4 (FT4) levels are slightly lower in the second and third trimesters. Thyroid-stimulating hormone (TSH) levels are low-normal in the first trimester, with normalization by the second trimester.

Delivery leads to a rapid reversal of this process, resulting in serum TBG, T4, and T3 concentrations returning to pregestational levels within 4 to 6 weeks. To detect abnormalities in thyroid hormone concentrations during the progression of pregnancy, it is necessary to determine their normal ranges throughout pregnancy by defining reference intervals for each of the trimesters. The reference intervals will be different when the population is iodine deficient.

Maternal overt and subclinical thyroid disorders and dysfunction are associated with complications of pregnancy and both short- and long-term consequences for the mother and child. These risks seem to be increased in women with euthyroid autoimmune thyroid disease. Hypothyroidism during pregnancy is associated with gestational hypertension and low birth weight. Women who are on thyroid replacement therapy before pregnancy may require an increase in dosage during pregnancy. Pregnant women with chronic autoimmune thyroiditis have a higher incidence of spontaneous miscarriage. Women with high TSH levels had a >3-fold increase in risk of very preterm delivery, and in some analyses, gravidas who tested positive for antithyroglobulin antibody (TgAb) at entry to prenatal care also had a >2-fold increased risk of very preterm delivery.[2,5]

Women with autoimmune thyroid disease before pregnancy are at increased risk for thyroid insufficiency during pregnancy and postpartum thyroiditis and should be monitored with TSH measurements during pregnancy.[6] Women receiving thyroxine therapy for hypothyroidism or as suppressive therapy should have their dose increased by up to 50% during pregnancy. Trimester-specific ranges of total and free thyroid hormones as well as TSH are important for appropriate treatment.

The incidence of hyperthyroidism in pregnant women has been reported to be approximately 0.2%; the leading cause is Graves disease.[7] Hyperthyroidism treatment includes anti-thyroid drugs or surgery to avoid adverse effects on the neonate, such as prematurity, intrauterine growth retardation, and fetal or neonatal thyrotoxicosis. Although hyperthyroidism in pregnancy is uncommon, effects on both mother and child are serious if untreated. The use of propylthiouracil (PTU) is recommended together with measurement of TSH receptor antibodies at 36 weeks gestation since diagnosis with radiolabeled iodine is contraindicated during pregnancy.

EFFECT OF DRUGS ON THYROID HORMONE TESTING

Women with known hypothyroidism and receiving levothyroxine (LT4) supplementation before pregnancy should plan to increase their dosage by 30% to 60% during the first few weeks of pregnancy. Numerous drugs are known to affect thyroid hormones levels. Iodine, lithium, dopamine antagonists, cimetidine, and sulfonylureas inhibit thyroid function and increase TSH levels. Glucocorticoids, propranolol, amiodarone, and PTU inhibit T4 and T3

conversion, whereas glucocorticoids, dopamine agonists, and somatostatin decrease TSH levels. Phenytoin, sulfonylureas, diazepam, furosemide, and salicylates inhibit T4 and T3 binding to transport proteins. Cholestyramine, ferrous sulfate, aluminum hydroxide, and sucralfate inhibit gastrointestinal absorption of thyroid hormones. Some of the above-mentioned drugs are contraindicated during pregnancy. The existing knowledge on the use of antidepressants, mood stabilizing agents, antirheumatic, and other drugs in pregnancy is hampered by a lack of results from randomized, controlled trials. Selected serotonin reuptake inhibitors (SSRI) and tricyclic antidepressants have not been associated with an increased risk of major malformations, but poor neonatal adaptation has been described. Benzodiazepines and glucocorticoids are not recommended for use during the first trimester.[8] Mood stabilizers, such as lithium, carbamazepine, and valproic acid (sodium valproate), are associated with an increased risk of fetal malformations.[9]

LABORATORY STUDIES AND THYROID DISEASE IN PREGNANCY

Hyperthyroidism

The diagnosis of hyperthyroidism is usually confirmed by TSH and FT4 testing. The sensitivity and specificity of these assays is particularly important in pregnant patients. Higher than normal serum TT4 immunoassay value, together with a resin T3 uptake value that is not reduced, should confirm the clinical suggestion of hyperthyroidism. Patients with Graves' disease usually have positive test results for thyroid-stimulating immunoglobulins. Women who have positive test results for thyroid peroxidase antibodies (TPOAb) early in pregnancy or shortly after delivery are at risk for developing postpartum thyroiditis (PPT) FT3 values should be measured when TSH is suppressed but the FT4 level is normal. An elevated T3 level confirms T3 toxicosis. CBC count, liver function tests, and calcium and magnesium laboratory tests should be obtained after making the diagnosis of hyperthyroidism. Elevated liver function test results, normochromic normocytic anemia, mild neutropenia, mild hypercalcemia, and hypomagnesemia can occur with hyperthyroidism.

Hypothyroidism

The diagnosis of hypothyroidism can be established readily by measuring serum FT4 and TSH levels. In patients with primary hypothyroidism, TSH levels are elevated and FT4 low. In patients with central or secondary hypothyroidism, TSH level may be normal or low and the FT4 level or FT4 index is low (hypothyroxinemia). In patients with subclinical hypothyroidism, the FT4 value is normal and the TSH level is slightly elevated. Anti-TPOAb and anti-TgAb should be measured in pregnant women with possible hypothyroidism to determine whether Hashimoto thyroiditis is the cause. Often, measuring TPOAb levels is sufficient because results are usually positive in patients with Hashimoto thyroiditis. CBC count and liver function tests should also be conducted because anemia is observed in as many as 30% to 40% of patients because of decreased erythropoiesis. Concomitant vitamin B-12 or folic acid deficiency should be considered if the anemia is macrocytic.

It is still recommended that TSH be used as marker for hypothyroidism in pregnancy (not in areas of iodine deficiency—usually evidenced by elevated serum thyroglobulin).[6] The current recommendation is that we need "more longitudinal studies of TSH during pregnancy in iodine-sufficient populations without evidence of autoimmune thyroid disease to develop trimester-specific TSH reference ranges."[6] Because immunoassays of FT4 are sensitive to abnormal binding-protein states, such as pregnancy, there is no absolute FT4 value that will define hypothyroxinemia across methods. However, new methods of analysis for FT4 have now been developed using tandem mass spectrometry without the need for

equilibrium dialysis.[10] MS/MS methods for analysis of TT4 and TT3 also are available, and trimester-specific reference intervals have been defined.[11] In comparison to FT4, TT4 changes in pregnancy are more predictable and less method-specific, but TT3 is more method dependent[11] and the correlation between MS/MS and IA for FT3 in pregnant women was only $r = 0.397$.[11]

TRIMESTER-SPECIFIC THYROID HORMONE REFERENCE INTERVALS

Immunoassays are notoriously unreliable with increasing evidence in the published literature supporting their lack of specificity.[12,13] The presence of circulating iodothyronine-binding autoantibodies that interfere with total T4 and T3 immunoassays (IAs) is a known phenomenon.[14–17] These auto-antibodies may give falsely high or falsely low values of thyroid hormone measurements depending on the assay separation method used, and often are in discordance with the clinical features.[14–18] Direct serum FT4 and FT3 measurements are technically difficult to determine because they are measured in the picomole range and, to be valid, must be free from interference by the much higher total hormone concentrations. It is, therefore, easier to measure the total thyroid hormone concentrations that are measured at nanomolar levels.

Serum TT4 and TT3 concentrations are most commonly measured by immunoassay methods. In proficiency testing of samples for different methods of measurement of both T4 and T3 the College of American Pathologists Proficiency Testing (CAP PT) Program reported that the difference in specificity of the various antibodies used in IAs can vary by a factor of two. In addition to method differences, under certain conditions, such as pregnancy, estrogen therapy or genetic abnormalities in protein-binding also have reportedly made IA methods for T4 and T3 diagnostically unreliable.[16,19]

The use of tandem mass spectrometry has been shown to be accurate, specific, reliable, simple, and fast. Using this technique, the proteins are precipitated, and both T4 and T3 are measured in the samples simultaneously. Trimester-specific reference intervals for healthy, iodine sufficient women using LC/MS/MS (using an isotopically labeled internal standard) and IA are available.[20,21] TT3 and TT4 immunoassays were compared with LC/MS/MS results during first, second, and third trimesters of pregnancy, and 1-year postpartum. The areas of disagreement between theses assays suggest that women at risk (ie, with analyte values outside of the 5–95% range) will not be detected using IA. Preliminary estimates were that 25% to 50% of such women would be missed if IA were used to assay the analyte. Trimester-specific T3, FT4, TSH, and Tg concentrations were significantly different between the first and third trimesters (all $P \leq 0.05$); second and third trimester values were not significantly different for FT4, TSH, and Tg (all $P > 0.25$), although T3 was significantly higher in the third, relative to the second trimester.[11,22,23] T4 was not significantly different at any trimester (all $P > 0.8$).[11] With 2 exceptions, analyte concentrations tended not to be correlated at each trimester and at 1-year postpartum. One exception was that T3 and T4 tended to be associated (all $P < 0.05$) at all time points except the third trimester (*rho* = 0.239, $P > 0.05$). T4 and FT4 concentrations tended to correlate positively during pregnancy (*rho* 0.361–0.382, all $P < 0.05$), but not postpartum (*rho* = 0.179, $P > 0.05$).[11,22,23] In addition to TT4 and TT3[11] trimester-specific measurements of FT4, Tg, and TSH have been conducted, means and medians are available.[22] During a normal pregnancy the stimulatory effect of hCG on the thyroid induces a partial TSH suppression below the non-pregnant range at the end of the first trimester (mean 0.89 mIU/L compared to 1.06 mIU/L postpartum).[22] Using immunoassays the reference intervals for TSH in an iodine-sufficient population without any autoimmuno-antibodies are 0.24–2.99 mIU/L for the first trimester, 0.46–2.95 mIU/L for the second trimester, 0.43–2.78 mIU/L for the third trimester, while one year postpartum TSH reference interval for the same population was 0.28–2.94 mIU/L.

The reference intervals for FT4 are 0.26–1.92 ng/dL (3.7–23.4 pmol/L) for the first trimester, 0.59–1.56 ng/dL (7.4–18.9 pmol/L) for the second trimester, 0.65–1.25 ng/dL (8.3–15.6 pmol/L) for the third trimester, while one year postpartum FT4 reference interval for the same population was 0.77–2.26 ng/dL (9.9–28.4 pmol/L).

Dashe et al[24] estimated a normal reference range for TSH during gestation in singleton and twin pregnancies. TSH decreased significantly during the first trimester, and the decrease was greater in twins (both $P < 0.001$). For singleton first-trimester pregnancies, the approximate upper limit of normal TSH was 4.0 multiples of the median, and for twins, 3.5 multiples of the median. Thereafter, the approximate upper limit was 2.5 multiples of the median for singleton and twin pregnancies. According to these studies, nomograms that adjust for fetal number and gestational age may greatly improve disease detection.

CONCLUSIONS

Physiologic changes associated with pregnancy require an increased availability of thyroid hormones by 40% to 100% to meet the needs of mother and fetus during pregnancy. Pregnancy has an effect on other thyroid functions with significant changes in iodine metabolism and clearance, serum thyroid binding proteins, and the development of maternal goiter, especially in areas with various levels of iodine deficiency. For the maternal thyroid gland to meet the demands of pregnancy, it must have sufficient supplies of iodine, be diseasefree, and capable of responding adequately. Thyroid autoimmunity is common and may contribute to miscarriages and hypothyroidism.

The fetus is totally dependent on maternal thyroxine supply during the first trimester of gestation and up to mildgestation for normal neurologic development and nervous system maturation. Because the progression of pregnancy and fetal, neonatal and child health are dependent on adequate thyroid hormone supplementation throughout pregnancy, trimester-specific reference intervals for thyroid functions can be crucial for both maternal and fetal health. Trimester-specific data determined by immunoassay and by tandem mass spectrometry are now available. In the case of multiple pregnancies, values expressed as multiples of the median may facilitate comparisons across different laboratories and populations.

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