Thyroid Hormone Changes in Pregnant and Non-Pregnant Women: A Case-Control Study

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Abstract. Pregnancy is associated with significant but reversible changes in thyroid function tests results, which are among the most profound seen as a result of a normal physiologic state. The present study was carried out to find out alterations in thyroid function tests in each trimester in normal pregnant women as compared to non-pregnant women. **Methods:** A case-control study designed with two groups of women: (1) 75 normal pregnant women randomly selected from the first (25 samples), the second (25 samples), and the third (25 samples) trimesters, and (2) 75 randomly selected non-pregnant healthy female controls. Thyroid function tests were carried out by measuring the serum levels of thyroid stimulating hormone (TSH), free and total thyroxin (FT_4, T_4), and free and total triiodothyronine (FT_3, T_3) by commercially available radio-immunoassay kits. **Results:** We found that mean T_4 increased progressively during pregnancy. Serum levels of T_3 increased in the second trimester and then declined during the third trimester compared with non-pregnant women. We also found that FT_4 strongly decreased during the third trimester. Free T_3 declined in the second and third trimesters. The mean TSH did not significantly differ in the three trimesters compared to the levels of non-pregnant women. Thyroid function tests in pregnancy should be interpreted against gestational age-related reference intervals to avoid misinterpretation of thyroid function during pregnancy.

Keywords. FT_3 • FT_4 • Pregnancy • T_3 • T_4 • TSH

Introduction

Hormone Changes. Normal pregnancy results in a number of important physiological and hormonal changes that alter thyroid function. Pregnancy is associated with significant but reversible changes in thyroid function tests, which are among the most profound seen as a result of a normal physiologic state.[1,2,3,4,5,6] Furthermore, human chronic gonadotropin can stimulate the thyroid gland during the first trimester because of its structural similarity to thyrotrophin (TSH).[7] Thyroid hormones play an important role in embryogenesis and fetal development during pregnancy.[8-10]Therefore, thyroid status is frequently assessed during pregnancy, both to evaluate suspected thyroid abnormalities, and to monitor the status of pre-existing thyroid disease. However, the usual clinical and laboratory assessment can be potentially misleading.

The findings associated with the hypermetabolic state of normal pregnancy can overlap with the clinical signs and symptoms of thyroid disease. Both normal pregnancy, and pregnancy complicated by conditions such as hyperemesis gravidarum, can be associated with thyroid function test changes that strongly suggest hyperthyroidism, in the absence of primary thyroid disease.[5, 11, 12]

Therefore, a local population reference range for thyroid hormones in pregnant women is needed.[13-18] The availability of gestational age-dependent reference intervals for thyroid hormones for local populations should help to avoid the underdiagnosis of hyperthyroidism or the overdiagnosis of hypothyroidism, with inadvertent use of thyroid hormone replacement in later pregnancy. It also allows an accurate interpretation of thyroid hormone test results in complicated pregnancies, in which abnormal thyroid function may occur, such as pre-eclampsia and hyperemesis gravidarum .[13, 15, 18]

Because of these pregnancy-related issues, we designed a cross-sectional case-control study. It involved systematic random sampling to identify alterations in thyroid function test results in each trimes-

ter in normal pregnant women as compared to non-pregnant women.

Materials And Methods

Subjects. The case group consisted of 75 pregnant women. They did not have pre-existing thyroid disease, hyperemesis gravidarum, trophoblastic disease, or preclampsia. Twenty-five blood samples were randomly selected from women who were in each of the three trimesters of pregnancy, for a total of 75 samples.

The control group was comprised of 75 randomly selected non-pregnant healthy females of childbearing age. All subjects in both groups were consuming iodide salt. Therefore, no subjects had iodide deficiencies.

Biochemical Investigation. The levels of serum thyroid stimulating hormone (TSH), total triiodothyroxine (T_3), free thyroxine (FT_4), and free triiodothyronine (FT_3) were measured by a Microparticle Enzyme Immunoassay (MEIA) on the AXSYM System (Abbott Laboratories, Abbott Park, USA), while serum total thyroxine (T_4) was measured by the Fluorescence Polarization Immunoassay (FPIA) method on the AXSYM System using standard laboratory methods.

Levels of blood glucose, total cholesterol, urea, and creatinine were determined using a fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany).

Statistical Analysis. All data were expressed as mean ± SD of number of experiments. The significance level was set at $p \leq 0.05$. The statistical significance was evaluated by Student's t-test using SPSS version 10.0 (SPSS, Cary, NC, USA).

Result

Demographic characteristics and biochemical parameters of the study population are shown in Table 1. The mean age limit of pregnant women was 28 ± 12 years and that of non-pregnant women was 25 ± 15 years. Pregnant women had a significantly increased body mass index (32 ± 6.2 kg/m^2) compared to non-pregnant women (26 ± 3.4 kg/m^2).

Diabetic subjects were defined as those with a minimum fasting blood glucose concentration >120 mg/dL. Fasting blood glucose was significantly lower in pregnant women compared to non-pregnant women. However, the total cholesterol, urea and creatinine in pregnant women and non-pregnant women did not differ significantly.

Table 2 shows the mean ± SD of the measured thyroid function test results of pregnant and non-pregnant women. We found that in the first and second trimesters, the mean TT_4 levels of pregnant women were not significantly lower. However, in the third trimester, the mean TT_4 increased significantly ($p < 0.001$) above the mean of non-pregnant women.

Table 1. Demographic characteristics and biochemical changes in pregnant and non- pregnant subjects.

Parameter	Non-pregnant women	Pregnant women
Age (years)	25 ± 15	28 ± 12
Sex-Females (%)	75 [100%]	75 [100%]
Body mass index (kg/m2)	26 ± 3.4	32 ± 6.2*
Fasting blood glucose (mg/dl)	92 ± 13	113 ± 27**
Urea (mg/dl)	21 ± 15	28 ± 17^{NS}
Creatinine (mg/dl)	0.6 ± 0.3	0.7 ± 0.4^{NS}
Total cholesterol (mg/dl)	165 ± 15	164 ± 17^{NS}

Values are given as mean ± S.D from seventy-five subjects in each group
Pregnant subjects compared with non- pregnant subjects (* p<0.05, ** p<0.01, ***p<0.001). NS-Not significant

In the first trimester, the mean TT_3 value did not significantly differ between the two groups of subjects. In the second trimester, however, the mean TT_3 value significantly increased ($p < 0.05$) compared to that of the control group. In the third trimester, the mean TT_3 of pregnant women decreased again and did not significantly differ from that of non-pregnant women.

The mean FT_4 levels in the first and the second trimesters were non-significantly lower than that of the non-pregnant subjects. But in the third trimester, the mean FT_4 significantly decreased ($p < 0.01$) relative to the mean for non-pregnant women.

The pregnant groups' mean FT_3 values declined over the trimesters. In the second and third trimesters, the mean levels of pregnant women were significantly lower ($p < 0.01$) than the mean of non-pregnant women.

In each trimester, the mean TSH levels of pregnant women were lower than the mean level of non-pregnant. In the first and third trimesters, pregnant women's lower TSH levels were not statistically sig-

Table 2. Serum thyroid hormone status in pregnant and non- pregnant subjects.

Parameters	TT_4 (nmol/L)	TT_3 (nmol/L)	FT_4 (pmol/L)	FT_3 (pmol/L)	TSH (mIU/ml)
Non-pregnant women N=75	89.75 ± 32.53	2. 95 ± 1.06	15.10 ± 11.56	6.21 ± 3.10	2.54 ± 1.32
Pregnant women N=75					
First trimester (N = 25)	80.78 ± 39.77^{NS}	2.44 ± 1.53^{NS}	14.93 ± 3.77^{NS}	7.11 ± 2.32^{NS}	1.93 ± 1.53^{NS}
Second trimester (N = 25)	93.30 ± 43.60^{NS}	3.41 ± 1.42*	12.78 ± 4.16^{NS}	4.17 ± 1.72**	2.08 ± 1.57*
Third trimester (N = 25)	127.51 ± 52.50***	2.91 ± 1.70^{NS}	7.01 ± 3.70**	3.21 ± 1.56**	2.74 ± 0.91^{NS}
Overall N = 75	107.50 ± 41.25**	3.12 ± 1.81^{NS}	11.91 ± 5.10**	5.25 ± 1.84*	1.99 ± 1.30*

Values are given as mean ± S.D from twenty-five subjects in each group.
Pregnant subjects compared with non- pregnant subjects (* p<0.05, ** p<0.01, ***p<0.001).
^{NS}-Not significant.

nificant, but in the second trimester, pregnant women's mean TSH was significantly lower ($p < 0.05$).

We also compared the mean values of each thyroid function test between pregnant and non-pregnant women (Table 2). We found the following: The mean TT_4 of pregnant women was significantly higher ($p < 0.01$), the FT_4 was significantly lower ($p < 0.01$), the FT_3 was significantly lower ($p < 0.05$), and the mean TSH was significantly lower ($p < 0.05$).

Discussion

This study showed that compared to non-pregnant women, TT_4 rose during the third trimester, whereas TT_3 increased in the second trimester. Free T_4 strongly declined during the third trimester, whereas FT_3 decreased in the second and the third trimesters. Mean TSH rose progressively through the three trimesters of pregnancy, but mean levels in the trimeters did not significantly differ from those of non-pregnant women.

The overall mean of each thyroid function test level was calculated (Table 2). The mean TT_3 did not show a significant difference between pregnant and non-pregnant women. However, the mean TT_4 significantly increased, and the mean FT_4, FT_3, and TSH were significantly lower.

Khandakar et al. performed a case-control study to learn the alterations in serum thyroid hormone levels in normal pregnant women compared to non-pregnant women in Dhaka City.[8] For this purpose, the researchers randomly selected 35 pregnant women during the third trimester and 21 non-pregnant women of childbearing age as controls. Their study showed that the mean serum TT_4 level of non-pregnant women was within the reference range. However, compared to the non-pregnant women, the pregnant women had a significantly higher TT_4 level during the third trimester. This finding was similar to ours in this study.

Khandakar et al.[8] also found that the mean TT_3 levels in the two groups were within the reference range and did not significantly differ. This result contrasts with our finding: in the second trimester, pregnant women in our study had a mean TT_3 level significantly higher than in non-pregnant subjects (Table 2).

The mean TSH level in the Khandakar et al. study was closely similar to what we found. However, a significant limitation of their investigation was a small sample size. Panesar et al. carried out a prospective study with 343 healthy pregnant women (5-41 weeks) and 63 non-pregnant controls. Their purpose was to establish gestation-related reference intervals for thyroid hormones in pregnant Chinese women.[13] They found that the FT_3 decreased during pregnancy, while the FT_4 initially increased, peaking

between 9-13 weeks. Then the FT_4 decreased, the decline becoming significant by week 21. The change in TSH level was similar to that of FT_4.

We also found declining FT_3 levels as the women's pregnancies progressed; the highest level was during the first trimester and the lowest in the third. FT_4 changes during pregnancy in our study were similar to those in the study by Panesar et al. In contrast to Panesar et al., we did not find a significant change in the mean TSH level in the first and third trimesters, but in the second trimester, the mean TSH level of pregnant women was significantly lower than that of non-pregnant women ($p < 0.05$). Also, when we calculated the mean TSH level for pregnant women for all three trimesters, it was significantly lower ($p < 0.05$) relative to the mean level of non-pregnant women.

McElduff found that the FT_4 decreased during pregnancy compared to non-pregnant women, and this resulted in the need for each laboratory to develop its own reference range for FT_4 levels in pregnancy.[15]

Our study also showed a progressive lowering of mean FT_4 levels with each trimester during the pregnancies. Kumar A et al. measured serum levels of T_3, T_4, and TSH in 124 pregnant women. The women were apparently normal, healthy young primigravidas with no known metabolic disorders and normal carbohydrate gestational intolerance test.[16] They found that the mean TT_3 increased during the second trimester and then declined in the third trimester compared to the first trimester. We found the same result in our study. Kumar A et al. also showed the mean TT_4 level rose in the second trimester and then decreased during the third trimester. But in our study, the TT_4 increased in both the second and the third trimester.

Kumar et al. also saw that the mean TSH level rose progressively through the trimesters of pregnancy.[16] Our study also showed this increase through the trimesters (Table 2).

Erem et al. investigated maternal thyroid function in 29 pregnant women with goiter and 51 pregnant women without goiter. The location of the women was the eastern black sea region of Turkey, which is an endemic goiter area.[17] They found that TT_4, FT_4, TT_3, FT_3, and thyroxine binding globulin increased during pregnancy. Erem, et al. also found that serum TSH levels declined in pregnant women without goiter compared with nonpregnant women without goiter. In our study, changes in the serum levels of TT_4, TT_3, and TSH in pregnant women were closely similar to those reported by Erem, et al. But in contrast to their findings, we found that serum levels of FT_4 and FT_3 in pregnant women increased compared to those of nonpregnant women.

Mechanisms of Thyroid Hormone Changes in Pregnancy. The etiology of increase in total circulating thyroid hormones primarily involves increased concentrations of plasma thyroxine binding globulin during pregnancy.[7]

Another proposed mechanism for the increased total thyroid hormone concentrations is production of type III deiodinase by the placenta. This enzyme, which converts T_4 to reverse T_3, and T_3 to diiodotyrosine (T_2), has extremely high activity during fetal life. Increased demand for T_4 and T_3 has been suggested to increase production of these hormones which ultimately increases the circulating concentrations of the hormones.[7] Increased sialylation, mediated by oestrogens, reduces the heptic clearance of thyroxine binding globulin, resulting in increased levels of both TT_4 and TT_3.[13] Changes in albumin and free fatty acid concentrations sustain the binding of T_4 and T_3 to carrier proteins; this lowers the blood levels of FT_4 and FT_3 as pregnancy progresses.[12,13]

Conclusion. It is important that thyroid function tests in pregnancy be interpreted against gestational age-related reference intervals. Clinical use of the results of this study could minimize the possibility of the misinterpretation of thyroid function test results of pregnant women in our research area of Coimbatore, Tamil Nadu, India.

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