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

P.Pasupathi1, V. Chandrasekar,2 and U. Senthil Kumar,3

1Department of Clinical Biochemistry, Bio Line Research Institute, Coimbatore- 641 014, Tamil Nadu, India
2Department of Microbiology, Bio Line Research Institute, Coimbatore-641 014, Tamil Nadu, India
3Department of Laboratory Medicine, Bio Line Research Institute, Coimbatore-641 014, Tamil Nadu, India

*Corresponding author: Dr. P. Pasupathi, Ph.D., Head-Department of Clinical Biochemistry, Bio Line Research Institute, Coimbatore-641 014 Tamil Nadu, India
Tel: +91 422 4270509   Fax: +91 422 4520099   drppasupathi@gmail.com

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 (FT4, T4), and free and total triiodothyronine (FT3, T3) by commercially available radio-immunoassay kits. Results: We found that mean T4 increased progressively during pregnancy. Serum levels of T3 increased in the second trimester and then declined during the third trimester compared with non-pregnant women. We also found that FT4 strongly decreased during the third trimester. Free T3 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. FT3 • FT4 • Pregnancy • T3 • T4 • 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.8,9 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>
<thead>
<tr>
<th>Parameter</th>
<th>Non-pregnant women</th>
<th>Pregnant women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25 ± 15</td>
<td>28 ± 12</td>
</tr>
<tr>
<td>Sex-Females (%)</td>
<td>75 [100%]</td>
<td>75 [100%]</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>26 ± 3.4</td>
<td>32 ± 6.2*</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>92 ± 13</td>
<td>113 ± 27**</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>21 ± 15</td>
<td>28 ± 17NS</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.6 ± 0.3</td>
<td>0.7 ± 0.4NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>165 ± 15</td>
<td>164 ± 17NS</td>
</tr>
</tbody>
</table>

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-
significant, 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 TT4 of pregnant women was significantly higher \( (p < 0.01) \), the FT4 was significantly lower \( (p < 0.01) \), the FT3 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, TT4 rose during the third trimester, whereas TT3 increased in the second trimester. Free \( T_4 \) strongly declined during the third trimester, whereas FT3 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 TT4 did not show a significant difference between pregnant and non-pregnant women. However, the mean TT3 significantly increased, and the mean FT4, FT3, 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 TT4 level of non-pregnant women was within the reference range. However, compared to the non-pregnant women, the pregnant women had a significantly higher TT4 level during the third trimester. This finding was similar to ours in this study.

Khandakar et al.\[^8\] also found that the mean TT3 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 TT3 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 FT3 decreased during pregnancy, while the FT4 initially increased, peaking

---

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TT4 (nmol/L)</th>
<th>TT3 (nmol/L)</th>
<th>FT4 (pmol/L)</th>
<th>FT3 (pmol/L)</th>
<th>TSH (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-pregnant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>women N=75</td>
<td>89.75 ± 32.53</td>
<td>2.95 ± 1.06</td>
<td>15.10 ± 11.56</td>
<td>6.21 ± 3.10</td>
<td>2.54 ± 1.32</td>
</tr>
<tr>
<td><strong>Pregnant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>women N=75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First trimester (N = 25)</td>
<td>80.78 ± 39.77NS</td>
<td>2.44 ± 1.53NS</td>
<td>14.93 ± 3.77NS</td>
<td>7.11 ± 2.32NS</td>
<td>1.93 ± 1.53NS</td>
</tr>
<tr>
<td>Second trimester (N = 25)</td>
<td>93.30 ± 43.60NS</td>
<td>3.41 ± 1.42*</td>
<td>12.78 ± 4.16NS</td>
<td>4.17 ± 1.72**</td>
<td>2.08 ± 1.57*</td>
</tr>
<tr>
<td>Third trimester (N = 25)</td>
<td>127.51 ± 52.50***</td>
<td>2.91 ± 1.70 NS</td>
<td>7.01 ± 3.70**</td>
<td>3.21 ± 1.56**</td>
<td>2.74 ± 0.91NS</td>
</tr>
<tr>
<td><strong>Overall N = 75</strong></td>
<td>107.50 ± 41.25**</td>
<td>3.12 ± 1.81NS</td>
<td>11.91 ± 5.10**</td>
<td>5.25 ± 1.84*</td>
<td>1.99 ± 1.30*</td>
</tr>
</tbody>
</table>

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.
between 9-13 weeks. Then the FT₄ decreased, the decline becoming significant by week 21. The change in TSH level was similar to that of FT₄.

We also found declining FT₃ levels as the women’s pregnancies progressed; the highest level was during the first trimester and the lowest in the third. FT₄ 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 FT₄ decreased during pregnancy compared to non-pregnant women, and this resulted in the need for each laboratory to develop its own reference range for FT₄ levels in pregnancy.[15]

Our study also showed a progressive lowering of mean FT₄ levels with each trimester during the pregnancies. Kumar A et al. measured serum levels of T₃, T₄, 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 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 FT₄ decreased during pregnancy compared to non-pregnant women, and this resulted in the need for each laboratory to develop its own reference range for FT₄ levels in pregnancy.[15]

Our study also showed a progressive lowering of mean FT₄ levels with each trimester during the pregnancies. Kumar A et al. measured serum levels of T₃, T₄, 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 TSH level 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 TSH level rose in the second trimester and then decreased during the third trimester. But in our study, the TSH 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 TSH, FT₃, FT₄, T₃, and T₄, 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 T₃, T₄, T₃, 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 FT₄ and FT₃ 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 T₄ to reverse T₃, and T₃ to diiodotyrosine (T₂), has extremely high activity during fetal life. Increased demand for T₄ and T₃ 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 TT₄ and TT₃.[13] Changes in albumin and free fatty acid concentrations sustain the binding of T₄ and T₃ to carrier proteins; this lowers the blood levels of FT₄ and FT₃ 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.

**References**

7. Fantz, C.R., Dagogo-Jack, S., Ladenson, J.H. and


