Thyroid function at the third trimester of pregnancy
in a Northern French population

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But/objectif. – Pendant la grossesse, la synthèse des hormones thyroïdiennes augmente quand la prise d’iode est suffisante. L’interprétation du dosage sérique de la T4 libre (FT4) est controversée. Nous avons évalué de façon prospective la fonction thyroïde pendant la grossesse et la pertinence du dosage de FT4. Patients et méthodes. – La fonction thyroïde de 114 femmes parisiennes enceintes et saines, avec une insuffisance modérée en iode, a été étudiée au troisième trimestre de la grossesse, ainsi que trois mois après l’accouchement pour 55 d’entre elles. Ces résultats ont été comparés aux valeurs de référence décrites pour la population nord-américaine. Résultats. – Une augmentation de la thyroxin-binding-globulin (TBG) sérique a été observée chez toutes les femmes enceintes françaises. En revanche, la sécrétion de T4 totale (TT4) et de FT4 étaient insuffisantes. Les analyses modélisées de régression linéaire ont montré une corrélation positive entre les taux de TT4 et de TBG, de TT4 et de FT4, ainsi que ceux de FT4 et de l’index de thyroxine libre (FTI). Conclusion. – L’hypothyroxinémie au troisième trimestre de la grossesse est élevée dans la population française. La carence iodée modérée a pu être responsable de l’augmentation insuffisante de TT4. Par conséquent l’incapacité de la thyroïde à établir l’équilibre exigé pourrait être corrigée par une supplémentation systématique en iode avant la grossesse. La forte corrélation entre la FT4 et le FTI suggère que la qualité du dosage de FT4 est appropriée pour estimer la sécrétion de FT4 pendant la grossesse.

Résumé : Thyroïde ; Grossesse ; Hypothyroxinémie ; Carence en iode ; Supplémentation en iode

Keywords: Thyroid dysfunction; Pregnancy; Hypothyroxinemia; Iodine deficiency; Iodine supplementation

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1. Introduction

Pregnancy results in important changes in thyroid physiology. Maternal thyroid deficiency can have several consequences for the neuropsychological development of both the fetus and the child [1–5]. Thyroxine requirement during gestation is increased for several reasons:

(i) an increase in thyroid-binding globulin (TBG) synthesis by the liver, as a result of the stimulatory effect of estrogens;
(ii) a direct stimulation of the thyroid gland by human chorionic gonadotrophin (hCG), which is explained by the close structural similarity between hCG and thyroid stimulating hormone (TSH);
(iii) an increase in the peripheral metabolism of thyroid hormone in the placenta, as a result of the action of deiodinases. Sufficient iodine intake is essential for normal thyroid function.

When iodine intake is sufficient (200–250 μg/day) during pregnancy, the thyroid machinery adjusts rapidly to reach a new equilibrium by increasing the production of total thyroxine, (TT4) which maintains stable free thyronine (FT4) levels. Normally, FT4 levels are about 10 to 15% lower at the end of pregnancy, as compared to those levels in healthy non-pregnant women [6,7].

In France, iodine intake in the general population is less than 100 μg/day, which is typical of most Western European countries [8]. This mild iodine deficiency can be responsible for excessive thyroid stimulation leading to a preferential secretion of T3, elevated serum thyroglobulin (Tg) levels and an increased thyroid volume. Consequently, the emergence of a relative hypothyroxinemia, characterized by a decrease in FT4 levels and associated with normal TSH and free triiodothyronine (FT3) concentrations, is observed [9–11]. The recommended dietary allowances of iodine, endorsed by the International Council for Control of Iodine Deficiency Disorders (ICCIDD) and the World Health Organisation (WHO), indicate an ideal iodine intake of 150 μg/day for normal adults and 250 μg/day for pregnant and lactating women [12,13].

Maternal hypothyroxinemia is potentially damaging for fetal neurosensory development. Indeed, maternal FT4 is an important determinant of early fetal brain development: only FT4 is able to cross the placenta and to locally generate FT3 in the fetal cerebral cortex during the first trimester before the onset of the fetal thyroid function [3,14]. Results from several studies have shown a significant correlation between maternal FT4 levels during the first and second trimesters of pregnancy and psychomotor development of the offspring [1,2,4,5,15].

So far, there is no specific and reliable reference range for FT4 levels during pregnancy. The Atlanta workshop in 2004 has proposed to evaluate the utility of the total thyroxine level (TT4) with a cut-off of 100 nmol/L during pregnancy [7]. Therefore, the aim of our study was to evaluate cross-sectionally the variation of thyroid function at the third trimester of pregnancy in the context of mild iodine deficiency and to evaluate if the quality of the FT4 test immunoassay is appropriate for estimating FT4 serum levels during pregnancy.

2. Patients and methods

2.1. Patients

One hundred fourteen patients at the third trimester of pregnancy consulting from July 2005 to April 2007 were enrolled at St Antoine Hospital in Paris. Patients with autoimmune thyroid disease or type 1 diabetes mellitus were excluded. None showed evidence of thyroid disease, nor were they treated with Levothyroxine®, or iodine supplementation. The fifty-five patients out of the 114 who came at their post-natal visit served as the population control, three months after delivery. None of the latter patients had developed thyroiditis following pregnancy.

2.2. Measurement of hormone serum levels

Blood samples were collected between 8 and 9 am. Serum samples were immediately prepared and stored at −20°C prior to analysis. For the study of thyroid function, serum levels of TSH, TT4, FT4, FT3, Tg and thyroxine uptake (T-Uptake) were measured using an electrocheluminescence enzyme immunoassay system (UniCel D × I 800, Beckman Coulter®, Villepinte, France). As we used the Beckman Coulter immunoassays system for all measurements, we were able to directly compare our results to the mean reference range reported by the company for the American population. Anti-thyroid peroxidase antibodies were assayed with an anti-microsomal indirect immunofluorescence assay using human thyroid sections. TBG levels were measured using a radioimmunoassay (RIA-gnost-TBG, CISbio International®, Gif-Sur-Yvette, France). In order to validate FT4, two Free T4 indices (FTI) were calculated as follows: FTI1 used the TSH assay (FTI 1 = TT4 × TSH) and FTI2 used the TBG assay (FTI 2 = TT4/TBG). Serum albumin concentrations were measured by immunonephelometry (BNII, Siemens Healthcare Diagnostics, Saint-Denis, France). Urinary iodine concentrations were measured for 24 hours in the same lab and corrected in mg/l to allow comparison with values reported in the literature.

2.3. Statistical analysis

Statistical analysis of data was performed using the STATA 8 software. Bilateral tests were used to compare hormone serum levels. Statistical significance was obtained by P values < 0.05. Linear regression models were applied to determine the correlation between the serum levels of the various hormones that were analyzed.

3. Results

The characteristics of the patients and controls are summarized in Table 1. Goiters were diagnosed by palpation in 10 patients (8.7%) and in two control patients (0.4%).
Iodine urinary concentration was 92 \pm \text{FTI2 ng/mg} 2.14 \pm \text{TUptake (%)} 28.8 \pm \text{TG ng/ml} 21.8 \text{Albuminemia g/L} 31.6 \text{TBG FT3 pmol/L} 3.9 \text{FT4 pmol/L} 7.45 \text{T4T nmol/L} 113 \text{TSH mUI/L} 1.75 \text{Goiter 10 2} \text{Average number of children 2.1 1.8} \text{Unknown 12 (10.5%) 2 (3.6%)} \text{Asia 12 (10.5%) 6 (10.9%)} \text{Africa 20 (17.5%) 10 (18.2%)} \text{North Africa 21 (18.4%) 10 (18.2%)} \text{Europa 49 (43%) 27 (49%)}

The results in this table represent the mean hormone concentrations of the pregnant women at the third trimester of pregnancy, as compared with those 3 months after delivery. Iodine urinary measurements were obtained in 62 patients at the third trimester of pregnancy. The mean iodine urinary concentration was 92 \pm 38 \mu g/L. For 39 patients it was below 100 \mu g/L, and for six patients under 50 \mu g/L.

The serum albumin concentration (31.6 \pm 3.7 \text{g/L}) was significantly decreased in the pregnant women. As expected, in the French women, the mean TBG concentration increased twofold during pregnancy. However, the mean concentration of TT4 in this cohort was found to increase only by 27.1\%, as compared to 50\% in North American pregnant women [7]. The mean concentration of TT4 in the French pregnant women (113 \pm 1.9 nmol/L) was not different from the mean reference range in the North American non-pregnant population (108 \pm 16 nmol/L). For twenty-seven of the pregnant women included in the study (20\%), TT4 concentrations were less than 100 nmol/L.

The mean concentration of FT4 was 7.4 \pm 1 \text{pmol/L} and had decreased by 24\% (P < 0.0001) during pregnancy, as compared to the concentration measured three months post delivery (9.8 \pm 1.4 \text{pmol/L}). The twenty-seven patients with low TT4 concentrations had hypothyroxinemia defined as having normal TSH concentrations, but a FT4 concentration under the lower limit of the reference range. Among the latter patients, fifteen had an iodine urinary concentration below 100 \mu g/L. The FT3 mean concentrations were 3.9 \pm 0.4 \text{pmol/L} and were found to decrease by 17\% as compared to those in the group of women three months post partum (4.7 \pm 0.5 \text{pmol/L}), whereas mean concentrations of TG did not show any variation between the two groups (21.8 \pm 19 \text{ng/ml}). The mean TSH concentrations were 1.7 \pm 0.8 \text{mUI/L} and increased significantly (P < 0.05) by 41\% during the third trimester of pregnancy, as compared to the values three months post partum (1.3 \pm 0.7 \text{mUI/L}). In contrast however, the serum TSH concentrations in pregnant women were inferior to the mean range of reference values (1.94 \text{mUI/L}), established by Beckman Coulter for the North American population.

The T Uptake concentration or FT4 fixation index that indirectly represent the concentration of unsaturated TBG was significantly (P < 0.0001) decreased by 24\% in the pregnant women (28.8 \pm 3.5\%), as compared to those in the women three months post delivery (37.8 \pm 2.8\%). However, the FTI1 value, representing the relative concentrations of FT4 measured (FTI 1 = TT4 \times T Uptake/40), did not differ between the pregnant woman and those three months post partum because the TT4 concentration did not sufficiently increase during pregnancy. However, when compared to the references values of Beckman Coulter, the FTI1 serum concentrations decreased by 33.2\%. In contrast, the FTI2 value was significantly (P < 0.0001) decreased by 30\% in the pregnant women, as compared to that three months post partum and was 50\% lower as compared to the mean range of reference values.

Results from linear regression model analyses showed a positive correlation (P < 0.001) between levels of [1] TT4 and TBG, [2] TT4 and T4L, [3] FT4 and FTI1 or FTI2, respectively (Figs. 1 and 2, Table 2). On the other hand, a negative correlation was observed between FT4 and TBG levels and no correlation between iodine and TSH or FT4 and FT3 levels.

4. Discussion

In this cross-sectional study, we compared the thyroid function of pregnant healthy Parisian women at the third trimester of pregnancy with that of controls. The patients included in the study were 55 healthy non-pregnant Parisian women. The mean age of the pregnant patients was 34 \pm 5 years, and that of the controls was 33 \pm 4.9 years (P = 0.2).

Table 1
Results of the analysis of hormone serum levels in northern French pregnant women.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Controls</th>
<th>P value</th>
<th>% variation</th>
<th>Beckman Coulter reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>34 \pm 5</td>
<td>33 \pm 4.9</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Ethnic background</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europa</td>
<td>49 (43%)</td>
<td>27 (49%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Africa</td>
<td>21 (18.4%)</td>
<td>10 (18.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>20 (17.5%)</td>
<td>10 (18.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>12 (10.5%)</td>
<td>6 (10.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>12 (10.5%)</td>
<td>2 (3.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average number of children</td>
<td>2.1</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goiter</td>
<td>10</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH mUI/L</td>
<td>1.75 \pm 0.85</td>
<td>1.3 \pm 0.7</td>
<td>&lt;0.001</td>
<td>+25</td>
</tr>
<tr>
<td>TT4 nmol/L</td>
<td>113 \pm 21</td>
<td>86.3 \pm 14</td>
<td>&lt;0.001</td>
<td>+27</td>
</tr>
<tr>
<td>FT4 pmol/L</td>
<td>7.45 \pm 1</td>
<td>9.8 \pm 1.4</td>
<td>&lt;0.001</td>
<td>+31</td>
</tr>
<tr>
<td>FT3 pmol/L</td>
<td>3.9 \pm 0.4</td>
<td>4.7 \pm 0.5</td>
<td>&lt;0.001</td>
<td>+20</td>
</tr>
<tr>
<td>TBG \mu g/ml</td>
<td>43.6 \pm 11.2</td>
<td>22.4 \pm 4.4</td>
<td>&lt;0.001</td>
<td>+90</td>
</tr>
<tr>
<td>Albuminemia g/L</td>
<td>31.6 \pm 3.7</td>
<td>42.2 \pm 2.8</td>
<td>&lt;0.001</td>
<td>–36</td>
</tr>
<tr>
<td>TG ng/ml</td>
<td>21.8 \pm 19</td>
<td>16.3 \pm 11.7</td>
<td>0.07</td>
<td>–25</td>
</tr>
<tr>
<td>TUptake (%)</td>
<td>28.8 \pm 3.5</td>
<td>37.8 \pm 2.8</td>
<td>&lt;0.001</td>
<td>–31</td>
</tr>
<tr>
<td>FTI1 nmol/L</td>
<td>81.5 \pm 17.8</td>
<td>81 \pm 14</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>FTI2 ng/mg</td>
<td>2.14 \pm 0.6</td>
<td>3 \pm 0.5</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Iodine urinary \mu g/L</td>
<td>92 \pm 38a</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a\) \(n=62\).
La grossesse.

La forte corrélation entre le FT4 et le FTI2. $p < 0.0001$ indique que la qualité de FT4 et de FTI2 a été déterminée en utilisant un modèle de régression linéaire. La forte corrélation entre le FT4 et le FTI1. $p < 0.0001$ indique que la qualité de FT4 test a été déterminée en utilisant un modèle de régression linéaire. Les corrélations sériques de FTI1 et de FT4 ont été déterminées comme décrites dans les patients et méthodes. La significativité statistique de la corrélation entre les taux de FT4 et de FTI1 a été déterminée en utilisant un modèle de régression linéaire. La forte corrélation entre le FT4 et le FTI1. $p < 0.0001$ indique que la qualité du dosage de FT4 est appropriée pour évaluer les taux sériques de FT4 pendant la grossesse.

**Fig. 1.** Correlation between FTI1 and FT4 serum concentrations. Serum concentrations of FTI1 and FT4 were determined as described in patients and methods. Statistical significance of the correlation between the serum levels of FT4 and FTI1 were determined using a linear regression model. The strong correlation between FT4 and FTI1. $p < 0.0001$ indicates that the quality of FT4 test immunoassay is appropriate for estimating FT4 serum levels during pregnancy. Correlation entre les concentrations sériques de FTI1 et de FT4. Les concentrations sériques de FTI1 et de FT4 ont été déterminées comme décrites dans les patients et méthodes. La significativité statistique de la corrélation entre les taux de FT4 et de FTI1 a été déterminée en utilisant un modèle de régression linéaire. La forte corrélation entre le FT4 et le FTI1. $p < 0.0001$ indique que la qualité du dosage de FT4 est appropriée pour évaluer les taux sériques de FT4 pendant la grossesse.

**Fig. 2.** Correlation between FTI2 and FT4 serum concentrations. Serum concentrations of FTI2 and FT4 were determined as described in patients and methods. Statistical significance of the correlation between the serum levels of FT4 and FTI2 were determined using a linear regression model. The strong correlation between FT4 and FTI2. $p < 0.0001$ indicates that the quality of FT4 test immunoassay is appropriate for estimating FT4 serum levels during pregnancy. Correlation entre les concentrations sériques du FTI2 et du FT4. Les concentrations sériques de FTI2 et de FT4 ont été déterminées comme décrites dans les patients et méthodes. La significativité statistique de la corrélation entre les taux de FT4 et de FTI2 a été déterminée en utilisant un modèle de régression linéaire. La forte corrélation entre le FT4 et le FTI2. $p < 0.0001$ indique que la qualité du dosage de FT4 est appropriée pour évaluer les taux sériques de FT4 pendant la grossesse.

**Table 2**

Significant regression results (pregnant women, $n = 114$).

<table>
<thead>
<tr>
<th></th>
<th>Coef.</th>
<th>SE</th>
<th>CI</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT4/TBG</td>
<td>0.32</td>
<td>0.13</td>
<td>0.062–0.58</td>
<td>0.016</td>
</tr>
<tr>
<td>FTI2/FT4</td>
<td>−0.02</td>
<td>0.00</td>
<td>−0.03–0.00</td>
<td>0.015</td>
</tr>
<tr>
<td>TT4/FT4</td>
<td>7.10</td>
<td>1.55</td>
<td>4.03–10.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FTI1/FT4</td>
<td>7.96</td>
<td>1.07</td>
<td>5.84–10.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FTI2/FT4</td>
<td>0.25</td>
<td>0.04</td>
<td>0.16–0.33</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

TT4: total thyroxine (mmol/L); FT4: free thyroxine (mmol/L); TBG: thyroxine-binding globulin (mg/L); FTI1: free thyroxine Index 1 (TT4 x TUptake/40); FTI2: free thyroxine Index 2 (TT4/TBG); Coef: regression coefficient; SE: standard error; CI: 95% confidence interval.

Pregnancy, having mild iodine deficiency and without evidence of thyroid disease, to those of 3 months after delivery. All serum samples were assayed using Beckman Coulter immunoassays, thereby allowing a direct comparison with values reported for the North American population, in areas with sufficient iodine intake. In USA, the mean urinary iodine excretion reported in 2001–2002 was 163 $\mu$g/L [16]. The urinary iodine excretion in the North American pregnant women, at the third trimester of pregnancy, was 66 $\mu$g/L. The American cohort population, in areas with sufficient iodine intake, had a median urinary iodine excretion of 108 $\mu$g/L [17]. However, the mean concentrations of TT4 increased only by 27% in the French pregnant women cohort instead of 50% in the North American pregnant women [7]. Moreover, TT4 serum concentrations were similar in American and French pregnant women. It is noted that TT4 concentrations for 20% of all pregnant women included in the present study were less than 100 nmol/L, which is the lower limit, as defined by Mandel et al. [7]. To accurately appreciate thyroid function during the second and third trimester of pregnancy, the TT4 concentrations measured in healthy, non-pregnant women should be adapted by multiplying this range by 1.5 fold [7]. A range of TT4 below 100 nmol/L could define a hypothyroxinaemia during pregnancy and the need for a treatment by levothyroxine. Indeed, it is admitted that FF4 concentrations decrease during pregnancy by about 10% in North American population. However, the magnitude of this decrease is often considered to be method-dependent. The methodology to measure FT4 concentrations is sensitive to TBG and albumin abnormalities both of which are characteristic of pregnancy. We therefore questioned whether the quality of FT4 test immunoassay is appropriate for estimating FT4 serum levels during pregnancy.

The reference test for evaluating FT4 concentrations is equilibrium dialysis [18]. However, this technique is long and expensive. Sapin et al. [19] have shown a large variation in FT4 mean concentration during the third trimester of pregnancy in a cohort of women from the north-eastern part of France, based on the results of nine FT4 immunoassay kits. Nevertheless, independent from the immunoassay used, all measurements showed a decrease of FT4 mean concentrations by about 20 to 30%, including the analysis in the present study, similar to those obtained with the equilibrium dialysis test [19]. Therefore, each
laboratory should establish trimester specific reference ranges for pregnant women, taking into account the different iodine intake between countries, as well as the occurrence of thyroid autoimmunity [6, 18–20]. The strong correlation between FT4 and FT11 or FT12, respectively, reported here suggests that the quality of FT4 test immunoassay is appropriate for estimating FT4 serum levels during pregnancy. Therefore, in the French women studied here, the iodine deficiency could explain the hypothyroxinemia and the insufficient increase in TT4, as thyroid gland is not able to meet the increased hormone demand over gestation, even if the TSH concentrations has increased by 41%. In the population of American pregnant women, the TSH concentrations have been reported to only increase by about 10% [7]. Our results raise the question whether a systematic iodine substitution of 150 µg/day before pregnancy should be proposed in order to restore iodine stores [21–23]. An ideal iodine intake could be 250 µg/day for pregnant and lactating women [12, 13]. This protocol is currently being used in other European countries and its implementation has not been associated with an increased risk to develop post partum thyroiditis [3], whereas no adverse effects on the fetus have been reported, if the iodine intake is under 500 µg/day [24].

However, results from a previous study have suggested that iodine substitution is not responsible for the increase in FT4 concentrations during pregnancy [25]. Therefore, further studies are needed to determine if the intelligence quotient of the child born to mothers with iodine substitution is higher than those without substitution.

5. Conclusion

In France, in a mild iodine deficiency area, the thyroid seems unable to establish the required equilibrium in pregnancy. For this reason, a systematic iodine supplementation before pregnancy should be recommended to restore iodine stores. Finally, the FT4 test immunoassay seems appropriate for estimating FT4 serum levels during pregnancy.

6. French version

A French version of this article is available at doi:10.1016/j.ando.2010.08.004.

Conflict of interest statement

The authors state that they have no conflict of interest.

References


