Gestational trimester-specific reference ranges for serum thyrotropin and free thyroxine in Japanese pregnant women

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Abstract. Thyroid diseases in pregnant and lactating women may result in adverse outcomes for both mothers and infants. A reference range for thyroid function is required in different areas; however, few studies on the gestational change or reference ranges of thyrotropin (TSH) and free thyroxine (FT4) concentrations for Japanese pregnant women have been reported. To establish the gestational trimester-specific reference ranges of serum TSH and FT4 concentrations, our previously published data on 481 pregnant women with the mean age of 30.8 years who provided serum samples as early as gestational week (GW) 6 was compiled by using their percentile values. The overall median urinary iodine concentration (UIC) during pregnancy was 201 μg/L suggesting adequate iodine intake. The prevalence of positive serum thyroid autoantibody (ThAb), i.e., antithyroid peroxidase antibody (TPOAb) and antithyroglobulin antibody (TgAb), was 11.4%. The reference ranges (2.5–97.5th percentile) of serum TSH and FT4 concentration calculated for samples with negative TgAb and TPOAb were 0.04–6.06 mIU/L in the first trimester (T1), 0.31–3.11 mIU/L in the second trimester (T2) and 0.48–3.93 mIU/L in the third trimester (T3) for TSH, and 1.10–1.87 ng/dL (T1), 0.76–1.56 ng/dL (T2) and 0.76–1.14 ng/dL (T3) for FT4. Compared to published data around the world in the 2017 American Thyroid Association (ATA) guideline, both the upper and lower limits of our TSH and FT4 reference ranges in the first trimester were higher than those in other countries. Further research is necessary in larger samples.

Key words: Thyrotropin (TSH), Free thyroxine (FT4), Thyroid autoantibody (ThAb), Reference range, Japanese pregnant women

During pregnancy thyroid hormone production increases by 50% as a consequence of increased serum thyroxine binding globulin (TBG) by estrogen stimulation and thyroid stimulatory effects of human chorionic gonadotropin (hCG). Increased activity of type 3 de-iodinase in the placenta increases the degradation of thyroxine. Total serum thyroid hormone levels increase significantly from early gestation (6–8 weeks), reach plateau levels by 20 weeks’ gestation and remain high until term, while serum free thyroxine (FT4) substantially decreases with the progression of gestation. The serum thyrotropin (TSH) level remains stable and comparable to pre-gestation levels after the transient decrease in the first trimester due to the thyrotrophic effect of high hCG secretion and the reference range of TSH is lower throughout pregnancy [1-5]. Daily iodine requirements increase during and after pregnancy. Higher glomerular filtration rates lead to a 30–50% increase in the renal excretion of iodide beginning in the late first trimester and persisting throughout pregnancy. When iodine intake is adequate, the thyroid gland is able to adapt to the demands of pregnancy. In iodine-deficient areas pregnancy may result in depletion of intrathyroidal iodine stores, which in turn may lead to hypothyroxinemia, increased TSH levels, increased thyroid volume and decreased urinary iodine excretion [2, 6].

Thyroid dysfunction may result in obstetric complications and irreversible effects on the fetus. The American Thyroid Association (ATA) and the European Thyroid Association (ETA) have published guidelines for the diagnosis and management of thyroid disease during pregnancy and postpartum in 2011, 2017 and 2014, respectively [3-5]. They recommended the determination of population-based trimester-specific reference ranges for serum TSH by including pregnant women with no known thyroid disease, optimal iodine intake, and negative antithyroid peroxidase antibody (TPOAb) status, as well as assay method-specific and trimester-specific...
pregnancy reference ranges for serum FT4. Since 2017 several studies on the reference ranges of thyroid parameters were reported in different countries [7-16]; however, in Japan limited studies have been reported [17-20]. In addition, the reference range (5–95th percentile) of TSH we reported in “Erratum” for the original paper [20] was incorrect due to a typographic error [21]. The purpose of the present study is to establish the gestational age-specific reference ranges of serum TSH and FT4 concentrations by compiling our previously reported data of the pregnant women who provided consecutive serum samples [19, 20].

Materials and Methods

Study population
An epidemiological survey on iodine nutrition and thyroid function of healthy pregnant and postpartum women without past and present history of thyroid disease was prospectively conducted in Funabashi City, Chiba Prefecture, Japan between 2005 and 2007, and the results were published elsewhere [19, 20]. Blood and urine samples were obtained at obstetric visits in the three trimesters of pregnancy. The first trimester (T1) is defined as the period from conception through gestational week (GW) 12. The second trimester (T2) is from GW 13 through GW 28 and the third trimester (T3) is from GW 29 through labor and delivery. Gestational age was confirmed by ultrasonography in the first trimester.

Three types of trimester-specific reference ranges for serum TSH and FT4 concentration were compiled using their percentile values in the three trimesters of pregnancy. In addition, the first trimester was classified into three subgroups: T1a (GW 6–7), T1b (GW 8–9) and T1c (GW 10–12), and TSH and FT4 reference ranges were calculated in each subgroup. Serum concentrations of TSH, FT4, two thyroid autoantibodies (ThAb), i.e., TPOAb and antithyroglobulin antibodies (TgAb), as well as urinary iodine and creatinine (Cr) concentrations were measured in the pregnant women.

Analytical methods
The serum TSH and FT4 concentrations were determined by electrochemiluminescence immunoassay using ECLusys TSH and FT4 (Roche Diagnostics K.K., Tokyo, Japan). The detection limit and measurable ranges given by the manufacturers were: TSH, 0.002 mIU/L, 0.005–100 mIU/L; FT4, 0.01 ng/dL, 0.023–7.77 ng/dL. The intra-assay and inter-assay coefficients of variation for TSH and FT4 were 1.2–2.5%, 1.5–3.1% and 0.9–1.6%, 2.4–3.2%, respectively. The reference ranges for Japanese defined by the manufacturers (2.5–97.5% percentile) were 0.27–4.20 mIU/L for TSH (n = 516) and 1.0–1.8 ng/dL for FT4 (n = 517). TPOAb and TgAb were measured by RIA using TPOAb Cosmic II (detection limit and range; 0.04 U/mL, 0.3–60 U/mL) and TgAb Cosmic II (0.15 U/mL, 0.3–100 U/mL) (RSR Limited, Cardiff, UK), respectively. The TPOAb and TgAb values above the manufacturer’s reference limit (0.3 U/mL) were considered positive. In the present study the values of TSH below 0.005 mIU/L which is the detection limit of measurement were treated as 0.005 mIU/L. Urinary iodine concentration (UIC) was measured by the ammonium persulfate digestion on microplate (APDM) method based on the Sandell Kolthoff reaction [22]. Cr concentration in urine was estimated by colorimetric enzymatic assay. UIC was expressed relative to creatinine excretion (μg/gCr) or as a concentration (μg/L). According to the World Health Organization (WHO)/United Nations Children’s Fund (UNICEF)/International Council for Control of Iodine Deficiency Disorders (ICCIDD)-recommended epidemiological criteria, adequate iodine intake corresponds to the median UIC values in the range 150–249 μg/L for pregnant women and ≥100 μg/L for lactating women. To convert to S.I. units: T4 μg/dL × 12.87 to nmol/L; UIC μg/L × 0.00788 nmol/L.

Statistics
Since serum TSH, FT4 concentration, UIC and UI/Cr were distributed asymmetrically and skewed, the results were presented as percentiles. The limits of the reference ranges of TSH and FT4 were calculated as median, 2.5th, 5th, 95th and 97.5th percentiles. Differences between paired data or groups were examined using one-way ANOVA with Tukey’s multiple comparison test, the Kruskal-Wallis test and Dunn’s multiple comparison test. Differences between two unmatched groups for normally and non-normally distributed data were tested using the unpaired t test and Mann-Whitney test, respectively. A p-value less than 0.05 was considered significant. Data processing and statistical analysis were performed using GraphPad Prism 8.0 from GraphPad Software Inc. San Diego, CA, U.S.A.

Ethical approval
Ethical approval was obtained in the original study [19].

Results
A total of 701 pregnant women were included in the original study [19] and after excluding the subjects with overt hypo-, hyper-thyroid or a history of thyroid disease, we evaluated serum TSH and FT4 values obtained from 481 subjects without multiple pregnancy, assisted pregnancy or obstetric complications, e.g., gestational
diabetes mellitus, hypertension or pre-eclampsia. Their mean (SD) age was 30.8 (4.2) years and the overall median UIC value was 201 μg/L (217 μg/gCr) suggesting adequate iodine intake.

There were no significant differences in the median UIC values among the three gestational trimesters (216 μg/L in T1, 206 μg/L in T2 and 216 μg/L in T3).

Serum ThAb was negative in 426 of 481 subjects (88.6%). The prevalence of subjects positive for TgAb, TPOAb or both were 5, 1.5 or 5%, respectively. Three serum samples were provided from 89 subjects and two samples from 146 subjects.

**Serum TSH, FT4 concentrations and ThAb positivity in pregnant women**

Serum TSH and FT4 concentrations were plotted along with gestational weeks as shown in the Figure (Fig. 1). There were 7 subjects with serum TSH below the lowest measurable range (0.005 mIU/L). Their FT4 values ranged from 1.2 to 2.7 ng/dL, and 2 of 7 subjects had FT4 values out of the upper reference range for non-pregnant adults (1.70 ng/dL). A TSH value of more than 5 mIU/L was observed in 9 subjects, 6 in T1 and 3 in T3. Their range was from 5.1 to 7.3 mIU/L and the FT4 values were 0.8–1.2 ng/dL. In 3 of 9 subjects the FT4 value was less than the lowest reference range (0.9 ng/dL). Serum TgAb was positive in 2 of 9 subjects with TSH above the upper reference range. There were no significant differences in the median TSH values between ThAb positive and negative pregnant women at any gestational trimester, while the median FT4 value in the positive ThAb subjects was higher than that in the negative ThAb subjects (0.98 vs. 0.93 ng/dL, \( p = 0.0178 \)).

**Gestational change of serum TSH and FT4 concentrations**

We used 481 subjects including ThAb positive subjects in order to reflect the real clinical situation for a cross-sectional study (Table 1), and selected 89 of 481 subjects for a longitudinal study (Table 2, Suppl. Fig. 1) and then 71 subjects with negative ThAb throughout the gestational period for a longitudinal study (Table 3, Suppl. Fig. 2). The mean (SD) GW of each trimester was: T1, 8.8 (1.3) weeks; T2, 27.5 (3.0) and T3, 34.3 (2.8) weeks. The mean GW in T2 was deviated closer to T3 (Tables 1–3).

The median TSH concentration was lowest in T1 and gradually increased toward T3 except in the cross-sectional study, although the differences in the TSH values were not statistically significant. The median FT4 concentration was highest in T1 then decreased and remained stable in T2 and T3 (Table 1). A similar pattern for TSH and FT4 was observed in both the cross-sectional and longitudinal studies (Tables 1–3).

![Fig. 1](https://example.com/fig1.png)  
Scattered plot of serum TSH and FT4 concentration along with gestational week. Red dotted line is the lowest measurable range for serum TSH (0.005 mIU/L). Green and blue dotted lines denote the lower and upper limit of reference range, respectively. Open circle: the subjects with negative ThAbs. Open red circle: the subjects with positive TPOAb. Closed red circle: the subjects with positive TgAb. There were no significant differences in median TSH and FT4 values between ThAb positive and negative pregnant women at any gestational trimester.
71 subjects with the mean age of 31.8 (4.7) years and FT4 concentration

89 pregnant women with the mean age of 31.7 (4.6) years, *one serum sample was excluded.

Mean (SD), GW: gestational week, T1: first trimester, T2: second trimester, T3: third trimester, n: number of serum samples
TSH: 1) vs. 2); p = 0.024
FT4: 3), 4), 5) vs. 6), 7); p < 0.0001, Kruskal-Wallis test, Multiple comparisons

Table 1  Reference ranges of serum TSH and FT4 concentrations in pregnant women by cross-sectional study

<table>
<thead>
<tr>
<th>Gestational Trimester</th>
<th>Range of GW</th>
<th>n</th>
<th>GW</th>
<th>2.5th</th>
<th>5th</th>
<th>50th</th>
<th>95.5th</th>
<th>97.5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1a</td>
<td>6–7</td>
<td>58</td>
<td>7.3 (0.5)</td>
<td>0.05</td>
<td>0.10</td>
<td>1.00</td>
<td>4.64</td>
<td>5.66</td>
</tr>
<tr>
<td>T1b</td>
<td>8–9</td>
<td>121</td>
<td>8.7 (0.5)</td>
<td>0.04</td>
<td>0.04</td>
<td>1.03(1)</td>
<td>4.29</td>
<td>5.12</td>
</tr>
<tr>
<td>T1c</td>
<td>10–12</td>
<td>45</td>
<td>10.9 (0.8)</td>
<td>0.01</td>
<td>0.02</td>
<td>0.93</td>
<td>4.20</td>
<td>5.30</td>
</tr>
<tr>
<td>T2</td>
<td>13–28</td>
<td>200</td>
<td>27.5 (3.0)</td>
<td>0.30</td>
<td>0.41</td>
<td>1.33(2)</td>
<td>3.16</td>
<td>3.31</td>
</tr>
<tr>
<td>T3</td>
<td>29–37</td>
<td>384</td>
<td>34.3 (2.8)</td>
<td>0.10</td>
<td>0.40</td>
<td>1.17</td>
<td>2.94</td>
<td>3.63</td>
</tr>
</tbody>
</table>

Table 2  Reference ranges of serum TSH and FT4 concentrations in pregnant women by longitudinal study

<table>
<thead>
<tr>
<th>Gestational Trimester</th>
<th>Range of GW</th>
<th>n</th>
<th>GW</th>
<th>2.5th</th>
<th>5th</th>
<th>50th</th>
<th>95.5th</th>
<th>97.5th</th>
</tr>
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<tbody>
<tr>
<td>T1a</td>
<td>6–7</td>
<td>21</td>
<td>7.2 (0.4)</td>
<td>0.13</td>
<td>0.13</td>
<td>1.10</td>
<td>4.70</td>
<td>4.78</td>
</tr>
<tr>
<td>T1b</td>
<td>8–9</td>
<td>47</td>
<td>8.8 (0.5)</td>
<td>0.04</td>
<td>0.05</td>
<td>1.13</td>
<td>5.40</td>
<td>7.00</td>
</tr>
<tr>
<td>T1c</td>
<td>10–12</td>
<td>18*</td>
<td>10.7 (0.7)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.78</td>
<td>3.07</td>
<td>3.07</td>
</tr>
<tr>
<td>T2</td>
<td>13–28</td>
<td>62</td>
<td>27.8 (2.7)</td>
<td>0.19</td>
<td>0.35</td>
<td>1.18</td>
<td>2.66</td>
<td>3.40</td>
</tr>
<tr>
<td>T3</td>
<td>29–37</td>
<td>118</td>
<td>34.4 (2.8)</td>
<td>0.09</td>
<td>0.43</td>
<td>1.28</td>
<td>3.42</td>
<td>3.74</td>
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</table>

Table 3  Reference ranges of serum TSH and FT4 concentrations in pregnant women with negative ThAb by longitudinal study

<table>
<thead>
<tr>
<th>Gestational Trimester</th>
<th>Range of GW</th>
<th>n</th>
<th>GW</th>
<th>2.5th</th>
<th>5th</th>
<th>50th</th>
<th>95.5th</th>
<th>97.5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1a</td>
<td>6–7</td>
<td>18</td>
<td>7.2 (0.5)</td>
<td>0.13</td>
<td>0.13</td>
<td>0.82</td>
<td>3.97</td>
<td>3.97</td>
</tr>
<tr>
<td>T1b</td>
<td>8–9</td>
<td>34</td>
<td>8.7 (0.5)</td>
<td>0.04</td>
<td>0.04</td>
<td>1.24</td>
<td>6.06</td>
<td>7.34</td>
</tr>
<tr>
<td>T1c</td>
<td>10–12</td>
<td>17</td>
<td>10.7 (0.7)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.87</td>
<td>3.07</td>
<td>3.07</td>
</tr>
<tr>
<td>T2</td>
<td>13–28</td>
<td>49</td>
<td>27.7 (2.9)</td>
<td>0.31</td>
<td>0.51</td>
<td>1.24</td>
<td>2.63</td>
<td>3.11</td>
</tr>
<tr>
<td>T3</td>
<td>29–37</td>
<td>95</td>
<td>34.4 (2.8)</td>
<td>0.48</td>
<td>0.58</td>
<td>1.30</td>
<td>3.52</td>
<td>3.93</td>
</tr>
</tbody>
</table>

Trimester-specific reference ranges for serum TSH and FT4 concentration

The reference ranges for serum TSH concentration by cross-sectional study were 0.04–5.08 mIU/L in T1, 0.30–3.31 mIU/L in T2 and 0.10–3.63 mIU/L in T3 (Table 1). In the longitudinal study the reference ranges were 0.04–5.53 mIU/L in T1, 0.19–3.40 mIU/L in T2 and 0.09–3.74 mIU/L in T3 (Table 2). In ThAb negative subjects
the reference ranges by longitudinal study were 0.04–6.06 mIU/L in T1, 0.31–3.11 mIU/L in T2 and 0.48–3.93 mIU/L in T3 (Table 3). Comparing the reference ranges of TSH among the three trimester-specific reference ranges (Fig. 2), the narrowest interval was observed in the cross-sectional study at T1 (Table 1) and in the longitudinal study on the ThAb negative subjects at T2 and T3. (Table 3).

The reference ranges for serum FT4 concentration in the cross-sectional study were 1.06–2.01 ng/dL in T1, 0.75–1.25 ng/dL in T2 and 0.76–1.21 ng/dL in T3 (Table 1). In the longitudinal study the reference ranges were 1.11–1.78 ng/dL in T1, 0.76–1.44 ng/dL in T2 and 0.77–1.17 ng/dL in T3 (Table 2). In ThAb negative subjects the reference ranges by the longitudinal study were 1.10–1.87 ng/dL in T1, 0.76–1.56 ng/dL in T2 and 0.76–1.14 ng/dL in T3 (Table 3). The narrowest interval of FT4 was observed in the longitudinal study in T1 (Table 2), in the cross-sectional study in T2 (Table 1) and in the longitudinal study on the ThAb negative subjects in T3. (Table 3, Fig. 3).

The reference ranges for serum TSH and FT4 concentration during the first trimester

Although there was no statistically significant difference in the median TSH values between the three subgroups (Tables 1–3) the median TSH was highest with the widest reference range in T1b (GW 8–9). The median FT4 value in T1a (GW 6–7) was significantly higher than that of T1c (GW 10–12) only in the longitudinal study on ThAb negative subjects (Table 3).

Discussion

In the present study we attempted to establish the trimester-specific reference ranges of serum TSH and FT4 concentration from 6 weeks gestation in pregnant women residing in an iodine-replete area. The median TSH level showed an increasing trend from the first trimester and reached plateau levels by middle gestation, and then remained stable until term, while the median FT4 level significantly decreased during the first and second trimesters. These patterns were generally comparable to the previous reports [1, 2] including ours [19, 20].

Although a longitudinal measurement on TSH and FT4 may be expected to minimize the inter-individual variation of thyroid function, the results of reported studies are conflicting [7, 23-26], and our reference ranges of TSH and FT4 in the longitudinal studies were not necessarily narrower than those in the cross-sectional study.

The establishment of gestational age-specific reference ranges for TSH and T4 (total or free) in each laboratory is recommended. If trimester-specific reference ranges are not available, the 2011 ATA guidelines have recommended the following reference ranges for TSH: first trimester, 0.1–2.5 mIU/L; second trimester, 0.2–3.0 mIU/L; third trimester, 0.3–3.0 mIU/L; however, in the 2017 guidelines these reference ranges were deleted and a cut-off value of 4.0 mIU/L was recommended as an upper reference limit in the first trimester [4]. The 2014 ETA guidelines-recommended upper limits for TSH are: first trimester, 2.5 mIU/L; second trimester, 3.0 mIU/L;
third trimester, 3.5 mIU/L. In the present study the reference range of serum TSH and FT4 calculated for samples negative for both TgAb and TPOAb were 0.04–6.06 mIU/L (T1), 0.31–3.11 mIU/L (T2) and 0.48–3.93 mIU/L (T3) for TSH, and 1.10–1.87 ng/dL (T1), 0.76–1.56 ng/dL (T2) and 0.76–1.14 ng/dL (T3) for FT4. Our upper limits for TSH were set higher than those of the 2014 ETA and ATA guidelines throughout pregnancy. According to the worldwide data described in the 2017 ATA guidelines the interval between the 2.5th and 97.5th percentiles for TSH and FT4 values during the first half of pregnancy (GW6–20) are from 0.02–0.41 to 2.15–4.68 mIU/L for TSH, and from 0.58–0.96 to 0.95–1.82 ng/dL for FT4, respectively [4]. Our 2.5th and 97.5th percentiles of TSH values in GW 6–12 were from 0.04 to 5.08–6.06 mIU/L, and those of FT4 were from 1.06–1.11 to 1.78–2.01 ng/dL. Both the upper and lower limits of our TSH and FT4 of reference ranges were higher than those in other countries. The reason for the upward shift of the reference ranges might be related to the iodine nutritional status, ethnicity of different populations and the gestational period compared (GW 6–20 vs. 6–12).

The gestation-specific reference ranges of thyroid function are influenced by many factors [27], i.e., ethnicity [28-33], inter- or intra-individual variation, body mass index [34, 35], TPOAb status [36], iodine deficiency [37], iron deficiency [38, 39], and assay methods [40-42]. FT4 reference ranges in pregnancy vary widely between methods (direct or indirect methods) and are also influenced by the iodine status of the population [4]. The international federation of clinical chemistry and laboratory medicine (IFCC) committee for standardization of thyroid function tests (C-STFT) has attempted to harmonize commercially available TSH immunoassays and also to standardize free thyroxine measurements in order to enable manufacturers to achieve more uniform reference intervals [43, 44]. Current FT4 immunoassays vary in their sensitivity to alterations in binding proteins that occur during pregnancy and re-establishment of trimester- and method-specific reference intervals is crucial for the successful implementation of standardized FT4 testing [45]. Recently in Japan the coefficients were determined for each of the TSH assay kits from 10 manufacturers who participated in the TSH harmonization study using healthy Japanese adults except pregnant or lactating women [46, 47]. This reference measurement procedure should be validated during pregnancy in future. Further studies are required to establish common reference intervals for TSH and FT4, and clinical decision limits in pregnant and lactating women.

The study was limited by the relatively small number of subjects in the selected local area and lack of measurement of serum TSH receptor antibodies levels. Therefore, subclinical hyperthyroid subjects may not be completely excluded in this study group. Ideally, the mean GWs when blood specimens were collected would be in the middle of each trimester, but in the second trimester the mean GW was shifted to the right. The strength of the study was the longitudinal measurement of serum TSH and FT4 concentrations in TgAb and/or TPOAb negative euthyroid subjects as early as 6 weeks gestation.

**Conclusion**

We established the gestational-specific reference ranges of serum TSH and FT4 concentrations for Japanese compiled according to the internationally recognized method. This value can be used as a provisional standard until a nationwide survey including regions with different iodine nutritional statuses has been conducted. More larger studies are necessary to define trimester-specific reference ranges during pregnancy in Japan.

**Acknowledgments**

The authors are grateful to Satoru Yamaguchi, M.D., the director of Yamaguchi Hospital, Chiba, for his contribution. We also express special thanks to Ms. Sheryn Mason for assistance in the preparation of the manuscript.

**Disclosure**

No competing financial interests exist.
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