Original Research

Establishment of self-sequential longitudinal reference intervals of maternal thyroid function during pregnancy

Bin Yu, Qiu-Wei Wang, Rui-Ping Huang, Fang Cao, Zi-Qiang Zhu, Da-Cheng Sun, Hong Zhou and Yi-Ming Zhang

Changzhou Women and Children Health Hospital affiliated to Nanjing Medical University, No. 26 Bo Ai Road, Changzhou 213003, Jiangsu Province, China
Corresponding author: Dr Rui-Ping Huang. Email: hrp117117@163.com

Abstract

The objective of this study is to establish self-sequential longitudinal reference intervals of thyroid function in normal pregnant women. According to the selection criteria, 301 cases were taken as the normal pregnant population to establish a normal reference range. Meanwhile, 150 healthy women were selected as the normal non-pregnant control group. To establish their own self-sequential longitudinal reference intervals, we collected samples five times in every case throughout the gestation (including first trimester, second trimester, third trimester, prenatal and postpartum), and detected the levels of thyroid stimulating hormone (TSH), free thyroxine (FT4), thyroid peroxidase antibodies (TPO-Ab), and then established the self-sequential longitudinal reference intervals. The levels of TSH, FT4 and TPO-Ab were quantified by electrochemistry immunoassay (ECL) and statistically analyzed using SPSS 13.0 software. Serum TSH of normal pregnant women was at a low level in the first trimester ($P < 0.05$) and began to rise continuously. Not until prenatal phase was it restored to the non-pregnant state ($P > 0.05$). During pregnancy, serum FT4 of normal pregnant women were consistently lower than non-pregnant levels ($P < 0.05$) and kept at low levels. Serum TPO-Ab increased significantly in the third trimester and prenatal phase ($P < 0.05$). Of normal pregnant women, 6.5% were TPO-Ab positive.

In conclusion, the reference intervals in our case will reflect the changes of thyroid function in pregnant women more realistically, resulting in a more accurate value for clinical diagnosis and therapy.

Keywords: pregnancy, thyroid hormones, reference values, self-sequential, dynamic

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Introduction

Thyroid function changes have been associated with adverse outcomes[1,2] during pregnancy and maternal thyroid dysfunction. Women with hypothyroidism or hyperthyroidism will have a higher risk of low birth weight.[3] Maternal hypothyroidism, even if very mild, can have adverse effects on cognitive and neurological development of the fetus.[4] Early diagnosis of thyroid disease during pregnancy will therefore contribute to the treatment and the improvement of maternal–fetal prognosis. Laboratory measurement of thyroid function therefore plays an important role in the assessment of maternal thyroid health. Measurement of serum thyroid stimulating hormone (TSH), free thyroxine (FT4) and thyroid peroxidase antibodies (TPO-Ab) are three common ways to assess maternal thyroid status. Their levels display physiological changes during pregnancy.[5–7] For example, the serum TSH level decreases during the first trimester, and then increases gradually. FT4 is elevated in the first trimester, and returns to the non-pregnant range in the second and third trimesters.[8] The positivity of TPO-Ab is also very often associated with a higher TSH level in gestation.[9] If a non-pregnant reference range is used, 5.6–18.3% of maternal thyroid diseases could be potentially misclassified.[10] Therefore, many studies have attempted to establish gestational-specific thyroid hormone reference intervals and use it for screening thyroid diseases during pregnancy.[11–13] However, most reference intervals are based on cross-sectional studies from different women in different stages of gestation. The self-sequential variation of reference intervals is smaller than the inter-individual variation.[14] To assess accurately the level of thyroid function during pregnancy, we designed the study to establish the self-sequential longitudinal reference intervals.

The aim of this study was to investigate the thyroid hormone during pregnancy and obtain the Chinese

population data on the reference intervals for TSH, FT4, and the cut-off for TPO-Ab. Based on the population of normal pregnant women, we collected serum continuously throughout their gestation (first trimester, second trimester, third trimester, prenatal and postnatal), then detected the serum levels of TSH, FT4 and TPO-Ab. After statistical analysis, we established the self-sequential longitudinal reference intervals. The new ranges could reduce the variation caused by sampling error from different groups, and gave more accurate values for clinical diagnosis and therapy.

Materials and methods

Patients and design

This study was designed and undertaken in the Changzhou Women and Children Health Hospital of Nanjing Medical University, Jiangsu Province, China. The cases in this study comprised pregnant women who regularly checked into our hospital for early pregnancy prenatal care from June 2008 to June 2009. Based on the research of Teng *et al.*[15] we established selection criteria as follows: (a) no past history of thyroid disease; (b) no life history in the areas of endemic goiter; (c) no other history of autoimmune diseases; (d) no medical history affecting thyroid function; (e) normal urinary iodine; (f) singleton pregnancies; (g) TPO-Ab negative ($<$50 IU/mL); and (h) after follow-up, no hyperemesis gravidarum, pregnancy-induced hypertension, gestational diabetes, premature birth or other adverse maternal–fetal outcomes.

A total of 538 cases of pregnant women received a questionnaire on their medical history and were willing to accept physical examination. According to the above selection criteria, 455 cases of normal pregnant women were included in the study. After a follow-up survey, 154 women encountered various pregnancy complications, such as hyperemesis gravidarum, pregnancy-induced hypertension, gestational diabetes, premature birth and so on. The remaining 301 cases were then taken as the normal pregnant population to establish a normal reference range. Their age range was 24.48 ± 5.31 y, while the height was 158.4 ± 9.8 cm. Meanwhile, 150 healthy women who were physically examined in our hospital, and who also followed the above selection criteria, were selected as the normal non-pregnant control group. Their age was 26.21 ± 6.35 y and the height was 157.4 ± 11.2 cm. There were no significant differences in their age and height ($P > 0.05$).

Methods

Samples collected

To establish their own self-sequential longitudinal reference intervals, we collected the sample five times in every case throughout the gestation, including the first trimester (10–14 w), second trimester (20–24 w), third trimester (30–34 w), prenatal (38–42 w) and postpartum (3–7 d). Three millimeters of blood of all the cases were collected by simple needle aspiration. The samples were centrifuged at 3000 rpm for five minutes to remove cells. The serum was stored at -86°C until assays.

Test

The levels of TSH, FT4 and TPO-Ab were quantified by electrochemistry immunoassay (ECL) using COBAS e601 automated analyzer (Roche Diagnostics, Mannheim, Germany). We observed the dynamic change of thyroid hormone over the entire gestation. We then established the database of self-sequential longitudinal reference intervals. All cases were followed by a survey of hospital medical records and telephone interviews.

The detection range was 0.005–100 mIU/L for serum TSH, 0.3–100 pmol/L for FT4 and 5–600 IU/mL for TPO-Ab.

Statistical analysis

All data were collected and statistically analyzed by SPSS 13.0 software. Results of parameters were expressed as median (M), 2.5th percentile (P2.5) and 97.5th percentile (P97.5). The limits of the reference intervals were calculated as P2.5–P97.5. Non-parametric tests were employed to compare differences in thyroid hormones (the median level); comparisons among groups with deferent gestational ages were performed with the Kruskal–Wallis test (H test) and comparisons between two deferent groups were compared with the Wilcoxon test. The Mann-Whitney (U test) was used to compare the difference between pregnant and non-pregnant groups. A P value of less than 0.05 was considered to be statistically significant.

Results

A total of 1505 blood samples were obtained from 301 normal pregnant women (5 samples/women). Their gestational age range was 10–42 weeks.

Table 1 shows the median, 2.5th percentile and 97.5th percentile for serum TSH, FT4 and TPO-Ab. The range of

Table 1 The reference intervals of thyroid function in pregnant women [M($P_{2.5} \sim P_{97.5}$)]

Group	n	TSH (mIU/L)	FT4 (pmol/L)	TPOAb (IU/mL)
Non-pregnancy	150	2.10 (0.75 ~ 5.28)	17.71 (14.35 ~ 21.69)	6.78 (5 ~ 22.42)
Normal pregnancy	301			
First trimester		1.00[a] (0.02 ~ 3.65)	15.48[a] (11.85 ~ 21.51)	6.35 (5 ~ 19.69)
Second trimester		1.26[a,b] (0.36 ~ 3.46)	12.08[a,b] (9.45 ~ 16.26)	7.05 (5 ~ 19.92)
Third trimester		1.50[a,b,c] (0.44 ~ 5.04)	12.62[a,b,c] (9.30 ~ 17.14)	8.63[a,b,c] (5 ~ 21.96)
Prenatal		2.14[b,c,d] (0.56 ~ 6.89)	12.52[a,b,c] (9.30 ~ 17.14)	8.03[a,b,c] (5 ~ 21.96)
Post-natal		2.86[a,b,c,d,e] (0.89 ~ 7.92)	12.94[a,b,c,e] (8.72 ~ 18.51)	7.59[b,c,d,e] (5 ~ 17.67)

[a]Compared with non-pregnant women, $P < 0.05$; [b]compared with first trimester, $P < 0.05$; [c]compared with second trimester, $P < 0.05$; [d]compared with third trimester, $P < 0.05$; [e]compared with prenatal, $P < 0.05$

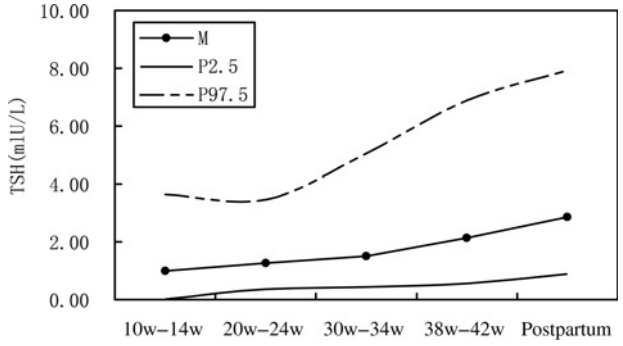

Figure 1 Fluctuations of TSH in normal pregnancy. TSH, thyroid stimulating hormone, w, week

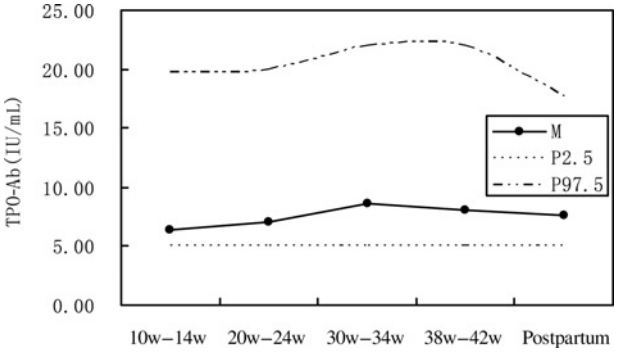

Figure 3 Fluctuations of TPO-Ab in normal pregnancy. TPO-Ab, thyroid peroxidase antibodies

P2.5–P97.5 were the self-sequential longitudinal reference intervals which we established.

Compared with normal non-pregnant women, serum TSH of normal pregnant women was at a low level in the first trimester ($P < 0.05$), and began to rise continuously, but it was always at a lower level ($P < 0.05$). Not until prenatal phase was it restored to the non-pregnant state ($P > 0.05$) (Figure 1). Its levels in the first, second and third trimesters were lower than normal non-pregnant women by 52.4%, 40.0% and 28.6%.

During pregnancy, serum FT4 of normal pregnant women were lower than non-pregnant levels consistently ($P < 0.05$), with tendency to a steady state at a low level (Figure 2). Its levels in the first, second and third trimesters were lower than normal non-pregnant women by 12.6%, 31.8% and 28.7%, respectively.

Serum TPO-Ab increased significantly in the third trimester and prenatal ($P < 0.05$) phase, and there were no significant differences in other gestational ages between non-pregnant state ($P > 0.05$; Figure 3). Among 538 pregnant women, 35 cases were TPO-Ab positive (6.5%), while six cases (4.0%) were TPO-Ab positive in 150 non-pregnant women ($P > 0.05$).

Discussion

It is well known that there is a close relationship between thyroid function and pregnancy. Thyroid hormone plays a key role in maintaining normal fetal growth and development. During pregnancy, physiological changes (hormonal, metabolic, etc.) will exert influence of maternal thyroid function. Ignorance of these changes leads to misdiagnosis and mistreatment of thyroid diseases. Stricker *et al.*[10] have reported that 5.6–18.3% of the misdiagnosis and missed diagnosis likely occur in the clinic practice due to the usage of the non-pregnant normal population reference values as a basis for diagnosis. It has been reported that the gestational age-specific reference intervals for thyroid function tests were significantly different from the non-pregnant normal reference intervals. If the non-pregnant normal reference intervals of normal group were used, the potential for misclassification was greatest in the first trimester (10.4%) for TSH, and in the second trimester (5.2%) for FT4.[10] Gilbert *et al.*[16] also drew a similar conclusion. Therefore, it is urged to establish the normal pregnancy-specific reference values of thyroid function.[11–13] However, most of the present reference range is based on a cross-sectional survey from different populations. It has a bigger variation than the self-sequential reference intervals.[14] In order to reduce the variation caused by sampling error, we collected serum from the same group (throughout the gestation including first trimester, second trimester, third trimester, prenatal and postnatal), so that we can observe clearly their own changes of thyroid hormone in every patient. After detecting the serum levels of TSH, FT4 and TPO-Ab, we established their own self-sequential longitudinal reference intervals. Our results have shown that there are significant differences in the reference range of thyroid function between pregnancy and non-pregnancy, and also in each gestational period. It appears important to select the corresponding reference intervals in clinical diagnosis and treatment of thyroid diseases during pregnancy. It is also in line with the requirements of the 'Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease' published by the US National Academy of Clinical Biochemistry.[17]

This study showed that the levels of thyroid hormones during pregnancy appeared to have a regularity of fluctuation corresponding with increase of gestational age. Figures 1 and 2 showed the curve of serum TSH and FT4 over gestation. It is consistent to previous reports.[12,13] However, when we compared the reference intervals, we found that our intervals were smaller than others.[12,13] It was probably because our multipoint observations were based on the same population. We then therefore reduced the sampling error caused by different individuals and the

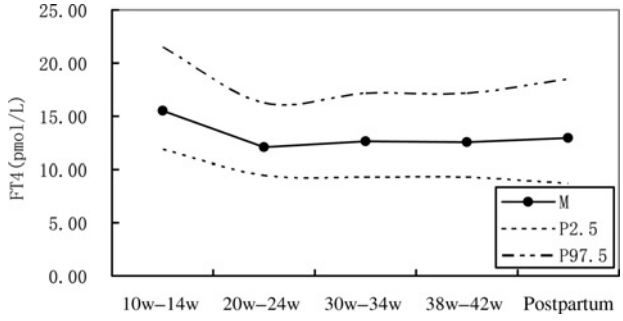

Figure 2 Fluctuations of FT4 in normal pregnancy. FT4, free thyroxine

coefficient variation of the range. The reference intervals in our case will reflect the changes of thyroid function in pregnant women more realistically, resulting in a more important value for clinical diagnosis and therapy.

There are differences in the non-pregnant reference range inconsistent to previous reports.[11–13] It was likely caused by different examination technology and different equipments or reagents. In this study, we used advanced technology (ECL) to detect serum TSH, FT4 and TPO-Ab. ECL has the characteristics of high sensitivity and high specificity, and without pollution of the environment.

TPO is one of the major enzymes in thyroid hormone synthesis. It can mediate the occurrence of autoimmune thyroid disease.[18] In addition, TPO-Ab is used to predict some autoimmune thyroid diseases, such as postpartum thyroiditis.[19] A higher TPO-Ab level during pregnancy is also the marker of the danger of the development of postpartum thyreoiditidis;[20] about 50% of TPO-Ab positive women will get thyroid dysfunction after delivery.[20,21] In the present study, we found that TPO-Ab was positive in 6.5% of pregnant women and in 4.0% of non-pregnant women ($P > 0.05$). Several studies[9,10] have shown that a higher TSH level was often associated with positivity of TPO-Ab. However, the changes of TPO-Ab are far before TSH and FT4.[22] It is the risk factor in the lack of thyroid function during pregnancy. Our results suggested that it is useful to monitor TPO-Ab in screening for thyroid function during pregnancy. We have established a primary self-longitudinal reference interval for serum TPO-Ab. It could be used to predict the occurrence of autoimmune thyroid disease.

Author contributions: R-PH carried out the assays and participated in designing the study. BY, FC, Z-QZ, D-CS and HZ carried out laboratory tests, participated in designing the study and performed the statistical analysis. R-PH, BY and Q-WW conceived the study, participated in its design and coordination and helped draft the manuscript.

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