Establishment of assay method- and trimester-specific reference intervals for thyroid hormones during pregnancy in Chengdu, China

Cheng Huang1 | Ying Wu2 | Linong Chen1 | Zhiya Yuan1 | Shuzhe Yang1 | Chenggui Liu1

1Department of Clinical Laboratory, Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China
2Department of Science and Education, Chengdu Women’s and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China

Correspondence Chenggui Liu, 1617 Ri Yue Avenue, Qingyang District, Chengdu 610091, China.
Email: lablcg@126.com

Funding information
The clinical case screening and data collection were supported by the Ministry of science and technology of the People's Republic of China (2019YFF0216501), and the Sichuan Provincial Science and Technology Department Research Foundation of China (2013FZ0080), respectively. The experiment works and statistical analysis were supported by the Sichuan Provincial Science and Technology Department Research Foundation of China (2020YFS0494), and the Chengdu Science and Technology Bureau Research Foundation of China (2015-HM01-00623-SF), respectively. The professional language editing work and other works were supported by the Health Department of Sichuan Province of China (120511).

Abstract

Background: The reference intervals of thyroid hormone will change at different stages of pregnancy because of physiological alterations. On the other hand, the reference intervals of thyroid hormone will also change in different detection systems due to the manufacturer’s methodology as well as a different race. The objective of this study was to establish the assay method- and trimester-specific reference intervals for thyroid-stimulating hormone, free thyroxine and free triiodothyronine for pregnant women in Chengdu.

Methods: A prospective, population-based cohort study involved 23,701 reference samples of pregnant women during the three trimesters and 8646 non-pregnant women with pre-pregnancy clinical and laboratory tests. The 2.5th and 97.5th percentiles were calculated as the reference intervals for thyroid-stimulating hormone, free thyroxine and free triiodothyronine at each trimester of pregnant women according to ATA Guidelines.

Results: The reference interval of thyroid-stimulating hormone in the 2.5th and 97.5th percentiles has a significant increasing trend from the first trimester, to second trimester and to third trimester, which was 0.08–3.79 mIU/L for the first trimester, and 0.12–3.95 mIU/L for the second trimester and 0.38–4.18 mIU/L for the third trimester, respectively (p < 0.001). However, the reference intervals of free thyroxine and free triiodothyronine in the 2.5th and 97.5th percentiles have significant decreasing trends from the first trimester, to second trimester and to third trimester, which were 11.87–18.83 pmol/L and 3.77–5.50 pmol/L for the first trimester, and 11.22–18.19 pmol/L and 3.60–5.41 pmol/L for the second trimester, and 10.19–17.42 pmol/L and 3.37–4.79 pmol/L for the third trimester, respectively (both p < 0.001).

Conclusion: It is necessary to establish assay method- and trimester-specific reference intervals for thyroid-stimulating hormone, free thyroxine, and free triiodothyronine because the reference intervals of these thyroid hormones are significantly different at different stages of pregnancy.
1 | INTRODUCTION

Pregnancy has a profound effect on the thyroid gland and thyroid function, which may lead to alterations of thyroid-stimulating hormone (TSH), free thyroxine (FT4), and free triiodothyronine (FT3). Early maternal thyroid insufficiency, even subclinical hypothyroidism, is associated with foetal neurodevelopment and may result in a lower-than-normal intelligence quotient (IQ) in offspring. The diagnosis and treatment of thyroid disorders in pregnant women are important to prevent adverse pregnancy outcomes (APOs) and require the establishment of trimester-specific reference intervals for TSH, FT4 and FT3 for healthy pregnant women in different areas and different immunoassay systems.

In 2011, the American Thyroid Association (ATA) published guidelines for the diagnosis and management of thyroid disease during pregnancy and the postpartum period, which recommended establishing pregnancy-specific and, ideally, trimester-specific reference intervals for all thyroid hormones, particularly for TSH and FT4. In the years that followed, some studies have investigated the trimester-specific reference intervals for thyroid hormones and found considerable variations in TSH, FT3 and FT4 levels among pregnant women in different areas and among different immunoassay assay methods. Many factors influence the establishment of reference intervals for thyroid hormones, such as ethnicity, age, parity, body mass index (BMI) and iodine status. Besides, sample size, representativeness of the reference population and the manufacturer’s immunoassay methodology also have an important impact on the reference interval.

Therefore, the ATA published the revised Guidelines for the Diagnosis and Management of Thyroid Disease during Pregnancy and the Postpartum Period in 2017, which strongly recommended establishing population-based, trimester-specific and assay method-specific reference intervals for serum TSH and FT4 using local pregnant women. In this study, we established assay method- and trimester-specific reference intervals for thyroid hormones during pregnancy according to 2017 ATA guidelines, because of the current limited availability of reference intervals for TSH, FT4 and FT3 in healthy pregnant women in Chengdu, China.

2 | METHODS

2.1 | Study participants

This study was a prospective, population-based cohort study aiming to investigate the method- and trimester-specific reference intervals of thyroid hormones during pregnancy in the region. The recruitment criteria for pregnant women in first, second and third trimesters to establish reference intervals for thyroid hormones in this study were by 2017 ATA guidelines and included the following: no personal and/or family history of thyroid diseases; no visible and/or palpable goitre; no prior use of drugs affecting thyroid function (except oestrogen); natural singleton pregnancy and no history of abortion; and gestational age ≥7 weeks. The exclusion criteria for participants included the following diseases: autoimmune diseases; liver, kidney, blood diseases and cancer. Women who were thyroid peroxidase antibody (TPOAb) and/or thyroglobulin antibody (TgAb) positive were also excluded from this study. Extreme deviation from the mean (outliers) based on the statistical analysis was also excluded from the analyses. Based on these criteria, there are 23,701 pregnant women at different stages of pregnancy (8053 first trimester, 8036 second trimester and 7612 third trimester) which were used as reference samples for the reference intervals of thyroid hormone. Another group of 8464 non-pregnant women are eugenic check-ups, and those who were receiving pre-pregnancy clinical and laboratory tests at our hospital during the same period were recruited as the controls. Pre-pregnancy clinical and laboratory tests are a comprehensive examination for every couple of childbearing age, which is provided by the government in Chengdu, China, including some free items such as thyroid hormone and fasting plasma glucose.

2.2 | Blood sample collection and measurement

Samples of blood were obtained from the study participants in the morning after a 12–15 h fast. The fresh plasma was separated by centrifugation (RCF = 1000 g) within 30 min, and the fresh sera were separated by centrifugation (RCF = 1000 g) within 60 min.

The serum concentrations of TSH, FT4, FT3, TPOAb and TgAb were quantified by chemiluminescent immunoassay (CLIA) with a Siemens ADVIA Centaur XP automatic chemiluminescence analyser with matched reagents and calibrators (Siemens Healthcare Diagnostics Inc., Tarrytown, New York, USA). The plasma concentration of fasting plasma glucose (FPG) and the serum levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and uric acid (UA) were measured by the Hitachi 7600 Automatic Biochemistry Analyzer (Hitachi High-Tech Instruments Co., Ltd., Japan) with matched commercial test kits. The high-sensitivity C-reactive protein (hs-CRP) concentration was measured by the non-competitive near-infrared particle immunoassay with a matched high-sensitivity CRP Kit (IMMAGE 800 Immunochemistry System, Beckman Coulter, Inc., USA).
2.3 Anthropometrics and lifestyle survey

Systolic blood pressure (SBP), diastolic blood pressure (DBP), body weight and height were measured with standard techniques. The BMI was calculated as body weight (kg) divided by the square of height (m). Hypertension was diagnosed when patients' SBP was ≥140 mm Hg and/or DBP ≥ 90 mm Hg. Underweight, overweight and obesity were defined as BMI < 18.5 kg/m², 24.0 to <28.0 kg/m² and ≥28 kg/m², respectively, according to the guidelines for the prevention and control of overweight and obesity in Chinese adults.

Hypercholesterolaemia and hypertriglyceridaemia, low HDL-C level and high LDL-C level were defined as TC ≥ 6.22 mmol/L and TG ≥ 2.26 mmol/L, HDL-C < 1.04 mmol/L and LDL-C ≥ 4.14 mmol/L, respectively, according to the Chinese guidelines on the prevention and treatment of dyslipidaemia in adults. Gestational diabetes was diagnosed when patients' FPG was ≥5.1 mmol/L, and/or 1-h plasma glucose (1hPG) during an oral glucose tolerance test (OGTT) ≥ 10.0 mmol/L and/or 2-h plasma glucose (2hPG) during an OGTT ≥ 8.5 mmol/L, according to the 2010 International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycaemia in pregnancy.

2.4 Data collection

The data were consecutively collected from women seen at the Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China between January and December 2018. Complete laboratory and clinical data measured by the medical staff (doctors, technicians, nurses and medical assistants) included serum concentrations of TSH, FT4, FT3, TPOAb, TgAb, TC, TG, LDL-C, HDL-C, FPG, UA and hs-CRP; height; body weight; and blood pressure. Basic information filled in by pregnant women as well as non-pregnant women included ethnicity, age, gestational age, history of illness, family history of the disease and lifestyle habits such as smoking and drinking (yes or no). Non-smoking is defined as never smoked or quit smoking for more than a month, while drinking is defined as taking any alcohol or alcoholic beverages within 30 days.

2.5 Quality control

Two levels of quality control (QC) samples (Bio-Rad Laboratories, Inc., USA, QC1: lot number 40331 and QC2: lot number 40333) for each thyroid hormone were included each day of the analysis. The intra-assay coefficients of variation (CV, n = 20) for QC1 and QC2 were 1.19% and 1.23% for TSH, 2.15% and 1.91% for FT4, 1.60% and 2.28% for FT3, 3.16% and 3.83% for TPOAb, and 5.25% and 4.69% for TgAb, respectively. The mean value and total CV of QC1 and QC2 over 1 year in our laboratory were 0.40 mIU/L and 4.40% (QC1), 28.69 mIU/L and 3.73% (QC2) for TSH; 9.20 pmol/L and 6.76% (QC1), 53.61 pmol/L and 5.20% (QC2) for FT4; 4.01 pmol/L and 4.41% (QC1), 18.79 pmol/L and 6.42% (QC2) for FT3; 147.51 IU/mL and 9.52% (QC1), 123.70 IU/mL and 7.82% (QC2) for TPOAb; and 80.85 IU/mL and 9.16% (QC1), 57.05 IU/mL and 13.21% (QC2) for TgAb, respectively.

2.6 Statistical analysis

All analyses were performed with SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were expressed as the means (standard deviations (SDs)) or medians (percentiles) according to whether they had a normal or skewed distribution, respectively. Means ± SD of more than two samples was compared with the one-way ANOVA, while medians (percentiles) of K independent samples (more than two samples) were compared with the Kruskal-Wallis H test. The intra-assay CV and the inter-assay CV were calculated by mean and SD. Aberrant values were identified using box plots; identified probable outliers were confirmed by applying Dixon’s range statistical test. The confirmed outliers, if present, were rejected from the reference sample group. The distributions of TSH, FT4 and FT3 in each trimester were examined by the histogram. The 2.5th and 97.5th percentiles (95% central interval (95% CI)) were calculated as the reference interval for each thyroid hormone at each trimester in pregnant women, according to ATA Guidelines.

3 RESULTS

3.1 Selection of reference samples and performance characteristics of the analysis system

The selection of reference samples was shown in Figure 1. A total of 30,705 pregnant women were individually screened and excluded step by step. Pregnant women were excluded if they had or self-reported a personal and/or family history of thyroid diseases (n = 215), or had palpable thyroid nodules (n = 122), or were taking endocrine and/or iodine-rich medicines (n = 283), or had multiple pregnancies or abortions (n = 204), or their gestational age was ≤7 weeks (n = 100), or laboratory test results of TPOAb (≥60 IU/mL) and/or TgAb (≥40 IU/mL) were positive, including positive results for TPOAb and TgAb (n = 2002), single positive of TPOAb (n = 991) and single positive of TgAb (n = 1076). Pregnant women were also excluded from this study if they lacked clinical information (n = 215) or had a personal history of autoimmune diseases (n = 134), or had liver, kidney, blood diseases and cancer (n = 513). Moreover, outliers based on the statistical analysis (n = 1149) were also excluded from the analyses. The performance characteristics of each thyroid hormone assay, according to the information provided by the manufacturers, are reported in Table 1.
3.2 | Comparison of principal characteristics between pregnant women and non-pregnant women

There was no significant difference between pregnant women in the first trimester, second trimester and third trimester and non-pregnant women not only in age ($F = 0.372$, $p = 0.773$) but also in prevalence of hypercholesterolaemia, hypertriglyceridaemia, high LDL-C and low HDL-C ($p > 0.05$). Prevalence of gestational diabetes, hypertension, overweight and obesity was significantly higher in pregnant women than in non-pregnant women ($p < 0.001$). However, compared to non-pregnant women, pregnant women were characterized by a decreased rate of smoking, drinking and underweight ($p < 0.001$). The comparison of principal characteristics between 23,701 pregnant women and 8646 non-pregnant women as reference samples is presented in Table 2.

3.3 | Box plots of TSH, FT4 and FT3 values for the three trimesters in pregnant women

Box plots of TSH, FT4 and FT3 values for the three trimesters, showing the medians, 25th-75th percentiles, non-outliers, outliers and extreme values, are shown in Figure 2. The median (25th-75th percentiles) of TSH was significantly lower in the first trimester compared to those in the second and third trimesters (1.36 [0.79–2.09] mIU/L vs 1.60 [1.00–2.36] mIU/L and 1.76 [1.13–2.60] mIU/L).
| Characteristics | TSH                                                                 | FT4                                   | FT3                                   | TPOAb                                | TgAb                                 |
|-----------------|---------------------------------------------------------------------|---------------------------------------|---------------------------------------|--------------------------------------|--------------------------------------|
| Method principle| Chemiluminescence immunoassay                                       | Chemiluminescence immunoassay         | Chemiluminescence immunoassay         | Chemiluminescence immunoassay        | Chemiluminescence immunoassay        |
| Assay principle | Two-site sandwich immunoassay                                        | Competitive immunoassay                | Competitive immunoassay                | Competitive immunoassay              | Competitive immunoassay              |
| Lot number of reagent (calibrator) | 68246301 (CALH CH01), 09078311 (CALH CH11), 47532312 (CALH CH12), 62052314 (CALH CH14), 87639316 (CALH CH16), 16801317 (CALH CH17), 32821319 (CALH CH19), 63380321 (CALH CH21) | 82264085 (CALA CA92), 11227087 (CALA CA93), 31885089 (CALA CA93), 48607091 (CALA CA93), 59851093 (CALA CA96), 75434094 (CALA CA96), 10953099 (CALA CA96), 30680102 (CALA CA99) | 65679219 (CALA CA92), 92272221 (CALA CA93), 30159223 (CALA CA93), 56414224 (CALA CA96), 90120226 (CALA CA96), 22470228 (CALA CA96), 28803999 (CALA CA96), 33083010 (CALA CA99) | 80572247 (CALO CO58), 05292249 (CALO CO58), 19403250 (CALO CO59), 44687252 (CALO CO59), 77266254 (CALO CO59), 04876252 (CALO CO59), 32673259 (CALO CO59), 44461261 (CALO CO61) | |
| Sample type     | Serum                                                               | Serum                                 | Serum                                 | Serum                                | Serum                                |
| Sample volume (μl) | 100                                                                | 25                                    | 50                                    | 30                                   | 40                                   |
| Sample stability | <24 h at 18-24°C                                                     | <8 h at 18-24°C                       | <8 h at 18-24°C                       | <8 h at 18-24°C                      | <8 h at 18-24°C                      |
| Sample storage  | <48 h at 2–8°C                                                      | <48 h at 2–8°C                       | <48 h at 2–8°C                       | <48 h at 2–8°C                      | <48 h at 2–8°C                      |
|                  | 2–30 days at −20°C                                                  | >48 h at −20°C                       | >48 h at −20°C                       | >48 h at −20°C                      | >48 h at −20°C                      |
| Measuring range | 0.008–150 mIU/L                                                     | 1.3–155 pmol/L                       | 0.3–30.8 pmol/L                      | 28–1300 U/ml                        | 15–500 U/ml                         |
| Precision (total CV%) | 3.18–5.51                                                          | 3.44–4.58                             | 2.76–4.05                             | 3.1–7.6                             | 3.9–6.6                              |

Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TSH, thyroid-stimulating hormone.
respectively, both \( p < 0.001 \). In contrast, the median (25th-75th percentiles) of FT4 was significantly higher in the first trimester compared to that in the second and third trimesters (14.96 [13.67-16.25] pmol/L vs 14.32 [13.03-15.48] pmol/L and 13.16 [12.00-14.58] pmol/L, respectively, both \( p < 0.001 \)). Similarly, the median (25th-75th percentiles) of FT3 was significantly higher in the first trimester compared to that in the second and third trimesters (14.96 [11.87-18.83] pmol/L vs 14.32 [11.22-18.19] pmol/L and 13.16 [10.19-17.42] pmol/L and 4.08 [3.37-4.79] pmol/L for the third trimester, respectively (both \( p < 0.001 \)). The assay method-specific reference intervals for TSH, FT4 and FT3 for the first, second and third trimesters pregnant women and non-pregnant women are shown in Table 3.

3.4 Reference intervals of TSH, FT4 and FT3 for the three trimesters in pregnant women and non-pregnant women

There were significant differences in the reference intervals for TSH, FT4 and FT3 between women in each of the three trimesters of pregnancy, non-pregnant women and the intervals provided by the manufacturer \( p < 0.001 \). The median level of TSH showed a significant increasing trend from the first trimester to the third trimester: 1.36 (0.08-3.79) mIU/L for the first trimester, 1.60 (0.12-3.95) mIU/L for the second trimester and 1.76 (0.38-4.18) mIU/L for the third trimester \( p < 0.001 \). The median levels of FT4 and FT3, however, showed significant decreasing trends from the first trimester to the third trimester: 14.96 (11.87-18.83) pmol/L and 4.59 (3.77-5.50) pmol/L for the first trimester, 14.32 (11.22-18.19) pmol/L and 4.45 (3.60-5.41) pmol/L for the second trimester and 13.16 (10.19-17.42) pmol/L and 4.08 (3.37-4.79) pmol/L for the third trimester, respectively (both \( p < 0.001 \)).

4 DISCUSSION

The levels of thyroid hormone will change during the three trimesters of pregnancy because of physiological pregnancy alterations. Different detection results may also be obtained for the same sample due to differing manufacturer methodologies for various thyroid hormone assays. Then, the thyroid hormone reference intervals provided by different manufacturers may not be the same. These differences require different biochemical interpretations between assays conducted in pregnant women and those conducted in non-pregnant women, which necessitates the establishment of specific reference intervals. However, the reference intervals for thyroid hormones are generally based on the reference intervals provided by manufacturers or other laboratory or reference literature, which usually leads to confusing results in clinical practice. Moreover,
some drugs, such as amiodarone and diphenylhydantoin, can affect the thyroid hormones production and their peripheral degradation, and they can also affect the circulating levels of thyroid hormones and TSH. Therefore, many medical associations, including the Chinese Society of Endocrinology and the Chinese Society of Perinatal Medicine, suggest that laboratory- and geography-specific reference intervals for thyroid hormones should be established by a local laboratory. The 2017 ATA guidelines have also strongly recommended that population-based trimester-specific reference intervals for serum thyroid hormones during pregnancy should be defined by a provider's laboratory and should represent the typical population for whom care is provided.

The TSH level in pregnant women is lower than that in non-pregnant women due to feedback regulation of TSH. Circulating thyroxine-binding globulin (TBG) concentrations increase after 7 weeks of pregnancy, reach a peak by approximately week 16 of pregnancy and then remain high until delivery; elevated TBG can induce increased levels of total thyroxine (TT4), which can feedback inhibit the release of TSH. Additionally, elevated maternal human chorionic gonadotropin (HCG) can directly stimulate the TSH receptor, increasing the secretion of thyroid hormone and, thereby, resulting in a subsequent reduction in serum TSH concentration. Thus, after 7 weeks of pregnancy, there is a downward shift of the TSH reference interval during pregnancy, with a reduction in both the lower and the upper limit of maternal TSH relative to the non-pregnant TSH reference interval. Studies have shown that the largest decrease in serum TSH is observed during the first trimester; thereafter, serum TSH and its reference interval gradually rise in the second and third trimesters, but they remain lower than in non-pregnant women.

Li et al. found that the median serum TSH level decreased significantly from the seventh gestational week, while the level of TSH remained stable before reaching 7 weeks of pregnancy; he proposed that the pregnancy-specific reference interval in the first trimester is suitable for 7–12 weeks of pregnancy. Liu et al. similarly reported that there was no significant difference in TSH levels between the T1-1 group (4.57–8.00 weeks of pregnancy) and the non-pregnant women group. The TSH levels in the T1-1 group were significantly higher than those in the T1-2 group (8.14–12.00 weeks of pregnancy). In 2017, the ATA guidelines recommend that the reference...
The reference intervals of TSH, FT4 and FT3 in adults provided by the manufacturer are 0.55–4.78 mIU/L, 11.5–22.7 pmol/L and 3.5–6.5 pmol/L, respectively. Subsequently, the reference intervals for TSH increased in consecutive trimesters of pregnancy (0.12–3.95 mIU/L for the second trimester and 0.38–4.18 mIU/L for the third trimester), and the reference intervals for FT4 and FT3 decreased in consecutive trimesters of pregnancy, (11.22–18.19 pmol/L and 3.60–5.41 pmol/L for the second trimester, and 10.19–17.42 pmol/L and 3.37–4.79 pmol/L for the third trimester).

**TABLE 3** The assay method-specific reference intervals for TSH, FT4 and FT3 in women with the first trimester, second trimester, third trimester and non-pregnancy

| Study group      | N    | Distribution | Percentiles
|------------------|------|--------------|-------------|
|                  |      |              | 2.5th  | 50th  | 97.5th  |
| First trimester  |      |              |        |       |         |
| TSH (mIU/L)      | 8053 | Skewed       | 0.08   | 1.36  | 3.79    |
| FT4 (pmol/L)     | 8053 | Skewed       | 11.87  | 14.96 | 18.83   |
| FT3 (pmol/L)     | 8053 | Skewed       | 3.77   | 4.59  | 5.50    |
| Second trimester |      |              |        |       |         |
| TSH (mIU/L)      | 8036 | Skewed       | 0.12   | 1.60  | 3.95    |
| FT4 (pmol/L)     | 8036 | Skewed       | 11.22  | 14.32 | 18.19   |
| FT3 (pmol/L)     | 8036 | Skewed       | 3.60   | 4.45  | 5.41    |
| Third trimester  |      |              |        |       |         |
| TSH (mIU/L)      | 7612 | Skewed       | 0.38   | 1.76  | 4.18    |
| FT4 (pmol/L)     | 7612 | Skewed       | 10.19  | 13.16 | 17.42   |
| FT3 (pmol/L)     | 7612 | Skewed       | 3.37   | 4.08  | 4.79    |
| Non-pregnancy    |      |              |        |       |         |
| TSH (mIU/L)      | 8646 | Skewed       | 0.75   | 2.31  | 5.19    |
| FT4 (pmol/L)     | 8646 | Skewed       | 13.67  | 16.13 | 19.95   |
| FT3 (pmol/L)     | 8646 | Skewed       | 3.99   | 4.82  | 5.74    |

Note: The reference intervals of TSH, FT4 and FT3 in adults provided by the manufacturer are 0.55–4.78 mIU/L, 11.5–22.7 pmol/L and 3.5–6.5 pmol/L, respectively.
ACKNOWLEDGMENTS
We would like to thank pregnant women for their assistance in obtaining the data used in this study. We also thank all funds supporting the experiment and all coauthors for devoting their time to the manuscript.

CONFLICT OF INTEREST
The authors declare no potential conflicts of interest concerning the research, authorship and/or publication of this article.

AUTHOR CONTRIBUTIONS
CH, YW and CL carried out the design of the study and coordination. CH, LC, ZY, SY and CL carried out the sample measurements, data collection and information classification. LC, ZY and SY were responsible for quality control and control. CL, YW and CH analysed the data and drafted and revised the manuscript. All authors have read and approved the final manuscript.

DATA AVAILABILITY STATEMENT
The data sets analysed during the current study are available from the corresponding author on reasonable request.

ORCID
Chenggui Liu https://orcid.org/0000-0003-3385-7441

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How to cite this article: Huang C, Wu Y, Chen L, Yuan Z, Yang S, Liu C. Establishment of assay method- and trimester-specific reference intervals for thyroid hormones during pregnancy in Chengdu, China. J Clin Lab Anal. 2021;35:e23763. https://doi.org/10.1002/jcla.23763