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REVIEW ARTICLE

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

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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.

Cheng Huang and Ying Wu contributed equally to this work.

KEYWORDS
methodology, pregnancy, reference interval, thyroid hormone, trimester-specific

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.[1] 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.[2] 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[3] and among different immunoassay assay methods.[4,5] Many factors influence the establishment of reference intervals for thyroid hormones, such as ethnicity, age, parity, body mass index (BMI) and iodine status.[6-9] Besides, sample size, representativeness of the reference population and the manufacturer's immunoassay methodology also have an important impact on the reference interval.[9,10]

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.[11] 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[11] 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 8646 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.[12]

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 serums 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/m^2, 24.0 to <28.0 kg/m^2 and ≥28 kg/m^2, respectively, according to the guidelines for the prevention and control of overweight and obesity in Chinese adults.[13,14]

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.[15,16] 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.[12]

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 (*SD*s)) 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.[17] 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,

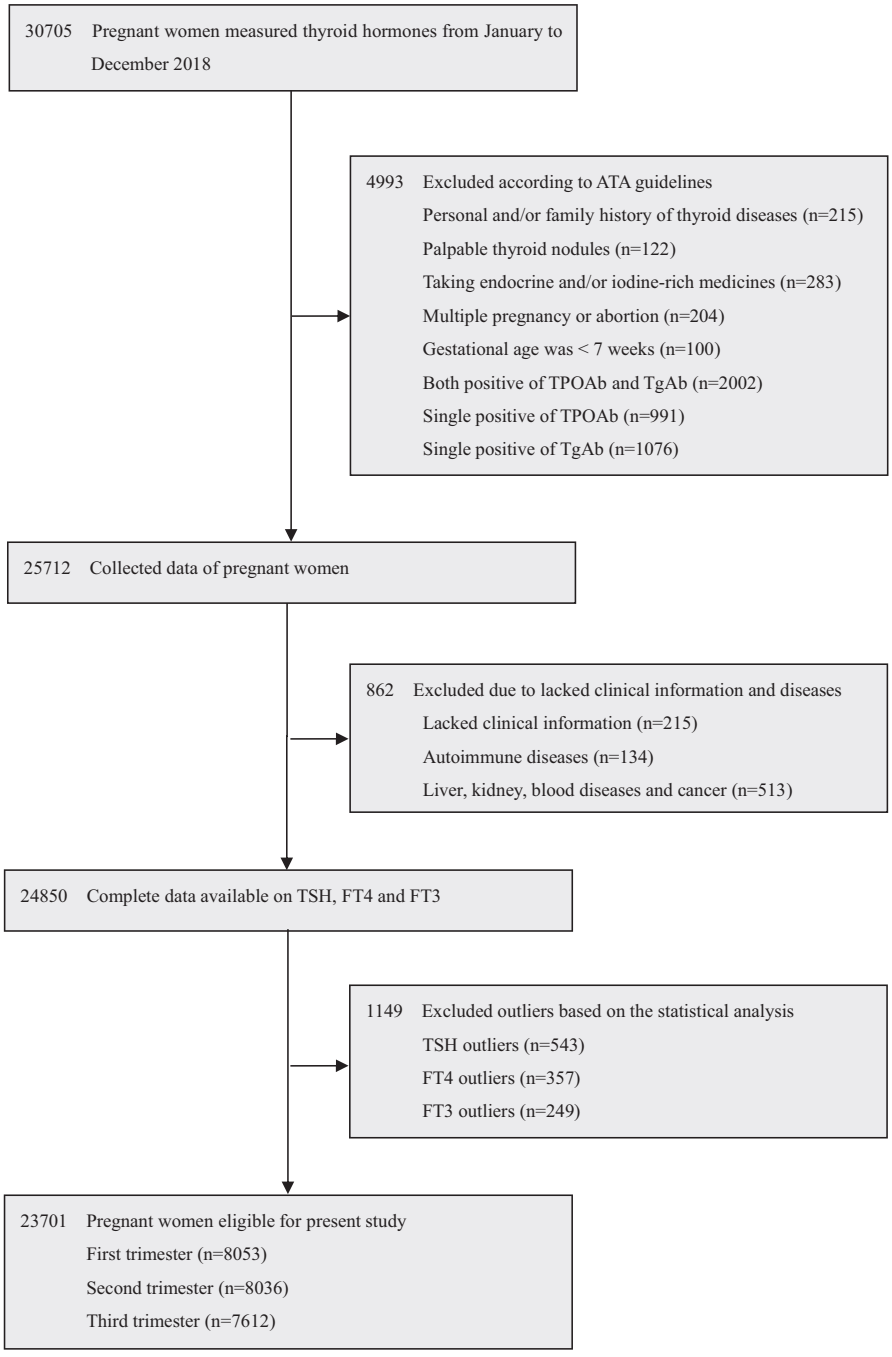

FIGURE 1 Selection of reference samples in the first, second and third trimesters of pregnancy

TABLE 1 General performance characteristics of TSH, FT4, FT3, TPOAb and TgAb assay according to information provided by the manufacturer

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)	80572247 (CALO CO58), 05292249 (CALO CO58), 19403250 (CALO CO59), 44687252 (CALO CO59), 77266254 (CALO CO59), 04876252 (CALO CO59), 32673259 (CALO CO59), 44461261 (CALO CO61)	93528294 (CALCAL1 C194), 26182296 (CALCAL1 C196), 22304298 (CALCAL1 C198), 55272299(CALCAL1 C199), 04642306 (CALCAL1 C106), 22635308 (CALCAL1 C108), 55626312 (CALCAL1 C112)
Sample type	Serum	Serum	Serum	Serum	Serum
Sample volume (μl)	100	25	50	30	40
Sample stability	<24 h at 18–24℃	<8 h at 18–24℃	<8 h at 18–24℃	<8 h at 18–24℃	<8 h at 18–24℃
Sample storage	<48 h at 2–8℃	<48 h at 2–8℃	<48 h at 2–8℃	<48 h at 2–8℃	<48 h at 2–8℃
	2–30 days at −20℃	>48 h at −20℃	>48 h at −20℃	>48 h at −20℃	>48 h at −20℃
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 (4.59 [4.30–4.90] pmol/L vs 4.45 [4.14–4.76] pmol/L and 4.08 [3.76–4.56] pmol/L, respectively, both $p < 0.001$).

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 $p < 0.001$). The assay method-specific reference intervals for TSH, FT4 and FT3 for the first, second and third trimester pregnant women and non-pregnant women are shown in Table 3.

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,

TABLE 2 Comparison of principal characteristics between pregnant women and non-pregnant women

	First trimester (n = 8053)	Second trimester (n = 8036)	Third trimester (n = 7612)	Non-pregnancy (n = 8646)	p value
Age (years)	28.27 ± 3.76	28.28 ± 3.89	28.22 ± 4.06	28.25 ± 4.05	0.773
Gestational age (weeks)	11.45 ± 1.06	14.79 ± 2.77	32.54 ± 3.16	–	<0.001
SBP (mm Hg)	126.78 ± 13.19	127.52 ± 13.97	129.28 ± 15.41	125.47 ± 12.97	<0.001
DBP (mm Hg)	81.16 ± 7.54	81.75 ± 7.95	83.04 ± 8.60	80.52 ± 7.55	<0.001
BMI (kg/m^2)	21.82 ± 2.59	22.33 ± 3.03	22.90 ± 3.89	21.64 ± 2.61	<0.001
TC (mmol/L)	4.89 ± 1.21	4.96 ± 1.32	5.04 ± 1.35	4.87 ± 1.11	<0.001
TG (mmol/L)	1.60 ± 0.72	1.68 ± 0.81	1.70 ± 0.89	1.59 ± 0.57	<0.001
LDL-C (mmol/L)	2.69 ± 0.90	2.74 ± 0.94	2.75 ± 0.99	2.64 ± 0.84	<0.001
HDL-C (mmol/L)	1.38 ± 0.30	1.47 ± 0.33	1.46 ± 0.37	1.37 ± 0.27	<0.001
FPG (mmol/L)	4.78 ± 1.26	4.95 ± 1.96	5.05 ± 2.11	4.75 ± 1.16	<0.001
UA (μmol/L)	317.78 ± 58.53	319.97 ± 59.30	321.71 ± 63.27	315.69 ± 58.11	<0.001
hs-CRP (mg/L)	3.74 (1.67–9.21)	3.93 (1.78–9.68)	4.02 (1.85–9.87)	3.67 (1.66–9.14)	<0.001
Smoking (%)	1.86	0.27	0.22	2.98	<0.001
Drinking (%)	2.89	0.36	0.32	4.87	<0.001
Hypercholesterolaemia (%)	9.02	9.13	9.37	8.21	0.051
Hypertriglyceridaemia (%)	7.93	8.45	7.83	7.75	0.349
High LDL-C (%)	8.69	8.71	8.85	7.93	0.135
Low HDL-C (%)	7.95	7.78	7.83	7.55	0.811
Gestational diabetes or diabetes (%)	6.78	7.60	9.01	3.41	<0.001
Hypertension (%)	7.93	9.17	9.34	7.52	<0.001
Underweight (%)	6.64	5.11	1.08	6.92	<0.001
Overweight and obesity (%)	14.93	18.63	25.33	14.60	<0.001

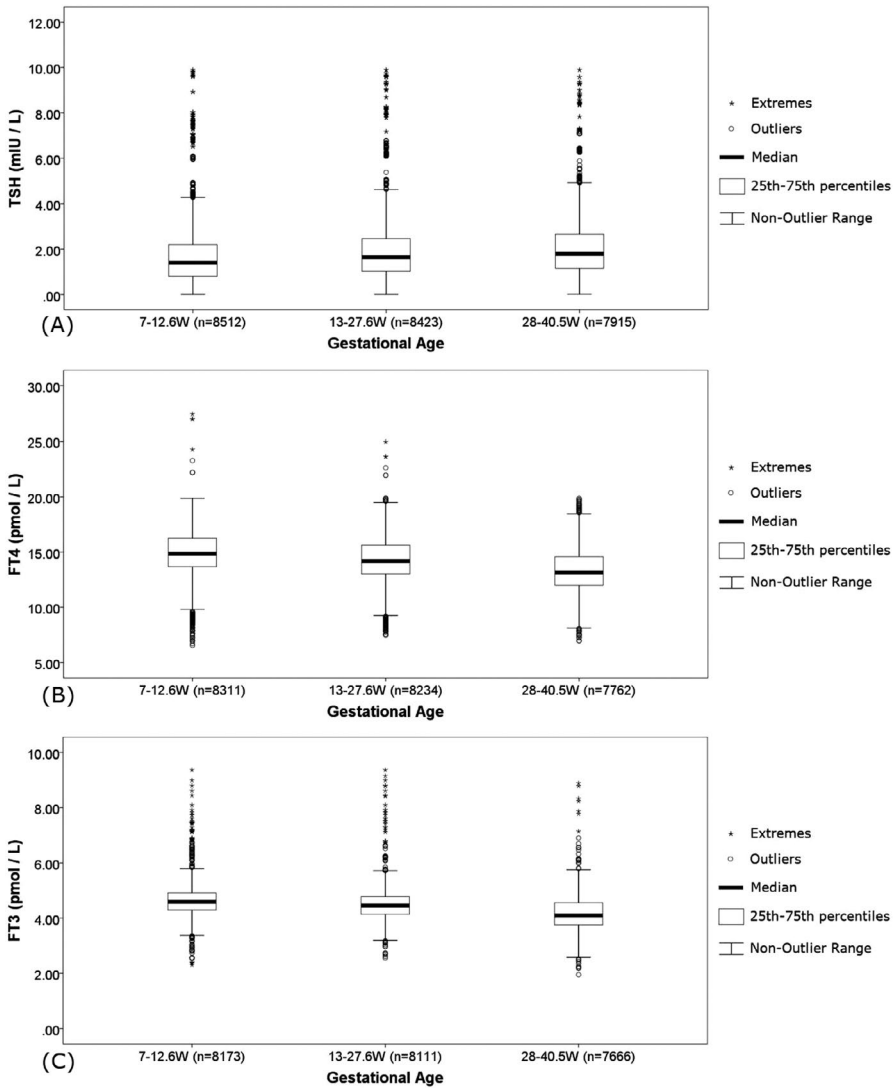

FIGURE 2 The levels of TSH (A), FT4 (B) and FT3 (C) in pregnant women. Data are presented as medians, 25th-75th percentiles (box), non-outlier range (whiskers), outliers (dots) and extremes (asterisk)

some drugs, such as amiodarone[18] and diphenylhydantoin,[19] 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.[20,21] 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.[11]

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.[22,23] 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.[23,24] 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.[11] 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.[25,26]

Li et al[27] 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[4] 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

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.

limit should generally be applied during the late first trimester of pregnancy, in weeks 7–12.[11] We excluded women from 1 to 6 weeks of pregnancy in this study, and the results showed that there was a significant difference in the reference interval for TSH between women in the three trimesters of pregnancy, non-pregnant women and the interval 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 ($p < 0.001$). This study confirmed a clear trend of increasing TSH from the first trimester to the third trimester in pregnant women.[28]

FT4 and FT3 levels also change during the three trimesters of pregnancy because of the change in TSH. The combination of TSH and thyroid cell plasma membrane receptors on the outer surface of follicles will activate adenylate cyclase, thus affecting and controlling the production of T3 and T4. On the other hand, the secretion of TSH in the pituitary is controlled by negative feedback regulation of circulating FT3 and FT4. In the present study, the levels of FT4 and FT3 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 ($p < 0.001$). Our reference intervals were consistent with the earlier report[9] and recent investigation,[29] which used the same Siemens ADVIA Centaur System as we used in the China studies. The reference intervals for FT3 and FT4 were 11.8–21.0 and 11.8–18.4 pmol/L for the first trimester, 10.6–17.6 and 11.6–17.4 pmol/L for the second trimester, and 9.2–16.7 and 9.7–15.1 pmol/L for the third trimester, respectively.

This study was valid only for the assay methods used in this study: that is, the chemiluminescent immunoassay (CLIA) methods for thyroid hormones and TSH, which used the automated platform ADVIA Centaur XP automatic chemiluminescence analyser (by Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). This was consistent with the observation from Tozzoli et al.[30] The iodine intake is an important factor that should affect the reference values for thyroid hormones and TSH in different populations. Iodine status may affect TSH levels in the general population.[30] Iodine is an important factor associated with TSH concentrations, even in a strictly selected reference population.[31] Some populations may still have some harmful effects on the thyroid due to mild or moderate iodine intake.[32] In the next phase of the project, we will include a urine iodine test.

5 | CONCLUSION

Our results demonstrated that the reference intervals calculated for TSH, FT4 and FT3 in Chengdu women in the first trimester were 0.08–3.79 mIU/L, 11.87–18.83 pmol/L and 3.77–5.50 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).

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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.

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