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 hyroid hormone will also change in different detection systems due to manufacturer's methodology as well as different race. The objective in 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 the Chengdu.

Methods: A prospective, population-based cohort study involved 23701 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 first trimester, to second trimester, and to third trimester, which was 0.08-3.79 mIU/L for first trimester, and 0.12-3.95 mIU/L for second trimester, and 0.38-4.18 mIU/L for 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 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 first trimester, and 11.22-18.19 pmol/L and 3.60-5.41 pmol/L for second trimester, and 10.19-17.42 pmol/L and 3.37-4.79 pmol/L for 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.

Background

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 is important to prevent adverse pregnancy outcomes (APOs) and requires the establishment of trimester-specific reference intervals for TSH, FT4 and FT3 for healthy pregnant women in different areas and in 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]. In addition, 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.

Methods

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, third trimesters to establish reference intervals for thyroid hormones in this study were in accordance with 2017 ATA guidelines [11], and included: no personal and/or family history of thyroid diseases; no visible and/or palpable goiter; 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. Outliers based on the statistical analysis were also excluded from the analyses. Based on these criteria, there are 23701 pregnant women at different stages of pregnancy (8053 first trimester, 8036 second trimester and 7612 third trimester) were used as reference samples for the reference intervals of thyroid hormone. Another group of 8646 non-pregnant women who were receiving a pre-pregnancy clinical and laboratory tests at our hospital during the same period were recruited as the controls. Pre-pregnancy clinical and laboratory tests is 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, fasting plasma glucose and so on [12].

Blood Sample Collection And Measurement

Blood was sampled in the morning after a 12–15 hs fast, and after the patient had been sedentary in a sitting for at least 15 min. Sampled blood was transferred, with blood flowing down the wall of the tube, never directly into the centre, to minimize mechanical disruption or turbulence, which could result in haemolysis or activation. The sampling tube was placed vertically and blocked by a tube stopper after blood collection. The fresh plasma was separated by centrifugation (3000 r/min) within 30 min and the fresh serums were separated by centrifugation (3000 r/min) within 60 min. If the serum could not be separated within 60 min, the blood was vertically placed in the refrigerator at 4°C but separated within 4 hs.

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 noncompetitive near-infrared particle immunoassay with a matched high-sensitivity CRP Kit (IMMAGE 800 Immunochemistry System, Beckman Coulter, Inc., USA).

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 mmHg and/or DBP ≥ 90 mmHg. 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].

Hypercholesterolemia and hypertriglyceridemia, 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 dyslipidemia 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 hyperglycemia in pregnancy [17].

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 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.

Quality Control

Each thyroid hormone was calibrated before each batch of sample reagent testing. 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 patients' testing results were considered acceptable when the concentrations of two QC samples were within the expected

concentration ranges. The intra-assay precision of each thyroid hormone was evaluated by measuring the QC sample 20 times within a day. 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 inter-assay precision of each thyroid hormone was evaluated once a day by measuring two levels of QC samples (QC1 and QC2) within a month. The mean value and total CV of QC1 and QC2 over one 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.

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 were 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 [18]. 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 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.

Results

Selection of reference samples and performance characteristics of analysis system

The selection of reference samples are shown in Fig. 1. A total of 30705 pregnant women were individually screened and excluded step by step. Pregnant women were excluded if they suffered or self-reported having a personal and/or family history of thyroid diseases (n = 215), or had a palpable thyroid nodules (n = 122), or were taking endocrine and/or iodine-rich medicines (n = 283), or had a multiple pregnancy or abortion (n = 204), or their gestational age was < 7 weeks (n = 100), or had positive laboratory TPOAb (> 60 IU/mL) and/or TgAb (> 40 IU/mL) results, including both positive of 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.

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 hours at 18–24℃	< 8 hours at 18–24℃	< 8 hours at 18–24℃	< 8 hours at 18–24℃	< 8 hours at 18–24℃
Sample storage	< 48 hours at 2–8℃	< 48 hours at 2–8℃	< 48 hours at 2–8℃	< 48 hours at 2–8℃	< 48 hours at 2–8℃
	2–30 days at -20℃	> 48 hours at -20℃	> 48 hours at -20℃	> 48 hours at -20℃	> 48 hours 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

TSH: thyroid stimulating hormone; FT4: free thyroxine; FT3: free triiodothyronine; TPOAb: thyroid peroxidase antibody; TgAb: thyroglobulin antibody.

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

There were 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 hypercholesterolemia, hypertriglyceridemia, high LDL-C, and low HDL-C ($P > 0.05$). The pregnant women had significantly higher prevalence of gestational diabetes, hypertension, overweight and obesity than non-pregnant women ($P < 0.001$). However, Compared to non-pregnant women, pregnant women were characterized by decreased rate of smoking, drinking and underweight ($P < 0.001$). The comparison of principal characteristics between 23701 pregnant women and 8646 non-pregnant women as reference samples are presented in Table 2.

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 (mmHg)	126.78 ± 13.19	127.52 ± 13.97	129.28 ± 15.41	125.47 ± 12.97	< 0.001
DBP (mmHg)	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
Hypercholesterolemia (%)	9.02	9.13	9.37	8.21	0.051
Hypertriglyceridemia (%)	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

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 Fig. 2. The median (25th-75th percentiles) of TSH were 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, 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 $P < 0.001$).

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 first, second and third trimester pregnant women and non-pregnant women are shown in Table 3.

Table 3
The assay method-specific reference intervals for TSH, FT4 and FT3 in women with 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
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.					

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. Moreover, the thyroid hormone reference intervals provided by different manufacturers may not be exactly 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. 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 [19, 20]. 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 increasing levels of total thyroxine (TT4), which can feedback inhibit the release of TSH [21, 22]. 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 [22, 23]. 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 still remain lower than in non-pregnant women [24, 25].

Li et al. [26] 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 to 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 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 [27].

FT4 and FT3 levels also changes 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 basically consistent with the earlier report [9] and recent investigation [28], 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.

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).

Abbreviations

1hPG: 1-h plasma glucose

2hPG: 2-h plasma glucose

95% CI: 95% central interval

APOs: adverse pregnancy outcomes

ATA: American Thyroid Association

BMI: body mass index

CLIA: chemiluminescent immunoassay

CV: coefficients of variation

DBP: diastolic blood pressure

FPG: fasting plasma glucose

FT3: free triiodothyronine

FT4: free thyroxine

HCG: human chorionic gonadotropin

HDL-C: high-density lipoprotein cholesterol

hs-CRP: high-sensitivity C-reactive protein

IQ: intelligence quotient

LDL-C: low-density lipoprotein cholesterol

OGTT: oral glucose tolerance test

QC: quality control

SBP: Systolic blood pressure

SDs: standard deviations

TBG: thyroxine-binding globulin

TC: total cholesterol

TG: triglyceride

TgAb: thyroglobulin antibody

TPOAb: thyroid peroxidase antibody

TSH: thyroid stimulating hormone

TT4: total thyroxine

UA: uric acid

Declarations

Ethics approval and consent to participate

This study was carried out in accordance with the Helsinki Declaration and was approved by the Medical Ethics Committee at Chengdu Women's and Children's Central Hospital, Chengdu, China [(protocol #: (2013)2] and with the 1964 Helsinki declaration and its later

amendments or comparable ethical standards. Participants under 18 years old were explained their participation rights and written informed consent was obtained from a parent. While participants aged 18 and over were voluntary and provided verbal informed consent who were told that their data may be used for statistical analysis and investigation of reference interval, and ensured that their privacy were not disclosed. For one thing, this study only investigated the reference intervals of thyroid hormones in healthy pregnant women in this area. For another, pregnant women do not suffer any harm because they were not intervened by medical treatment.

Consent to publish

Not applicable

Availability of data and materials

The data sets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Authors' 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 analyzed the data, and drafted and revised the manuscript. All authors have read and approved the final manuscript.

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Figures

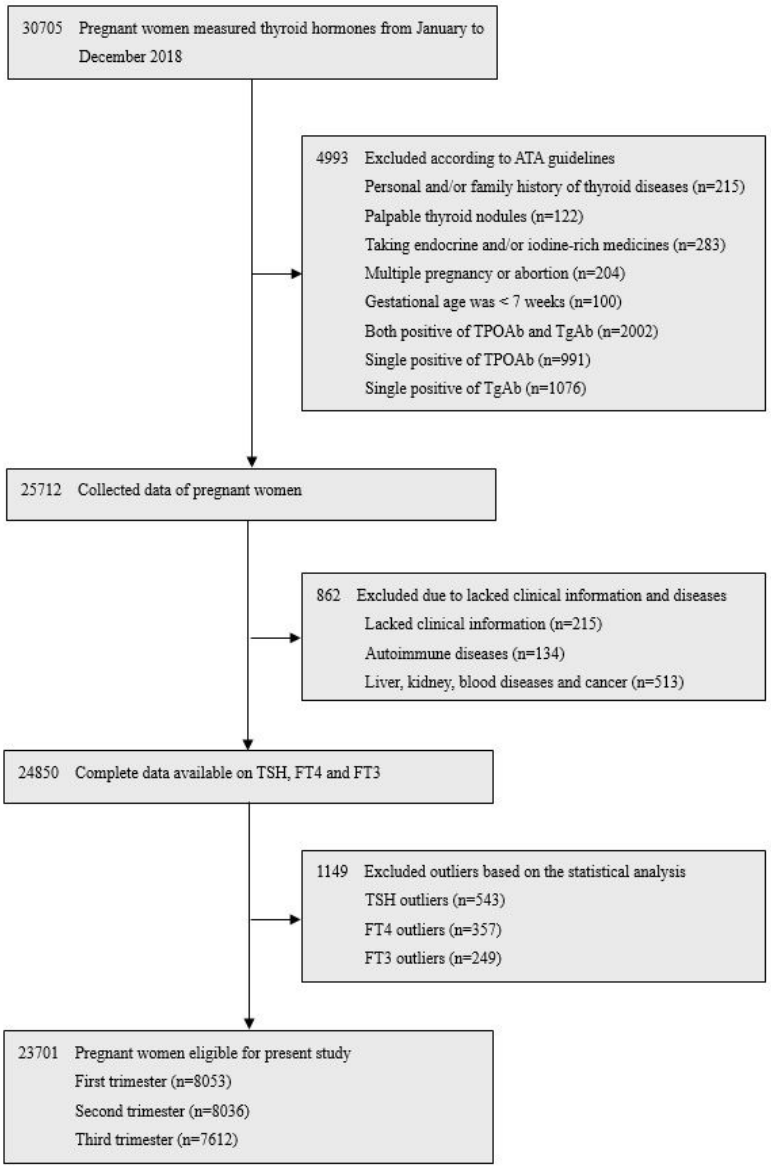

Figure 1

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

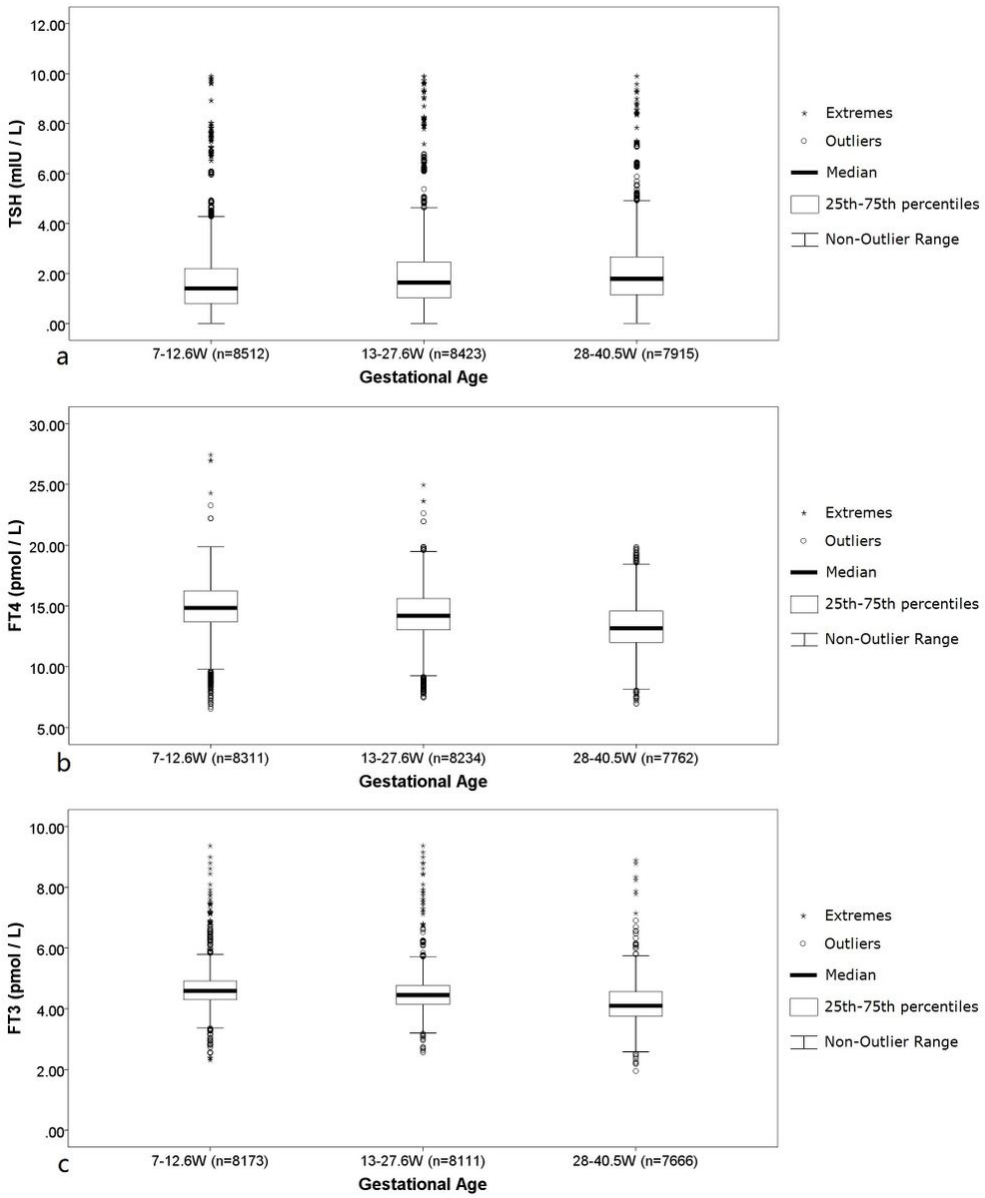

Figure 2

The levels of TSH (a), FT4 (b) and FT3 (c) in pregnant women. Data is presented as medians, 25th-75th percentiles (box), non-outlier range (whiskers), outliers (dots), and extremes (asterisk).

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