Clin. Lab. 2026;72:XXX-XXX

ORIGINAL ARTICLE

Reference Intervals for Thyroid-Stimulating Hormone and Free Thyroxine Established in Pregnant Women in Lhasa

Ni Zhen [1, *], Huihui Ren [1, *], Danzeng Zhuoga [1], Bingliang Guo [3], Gusang Zhuoma [1], Yang Jin [1], Chilie Jiumei [1], Yingying Li [2]

[*] *Ni Zhen and Huihui Ren are co-first authors and contributed equally to this paper*
[1] *Department of Laboratory Medicine, Women and Children's Hospital of Tibet Autonomous Region, Lhasa, China, Xizang Region Child Development Clinical Medical Research Center, Lhasa, China*
[2] *Department of Laboratory Medicine, West China Second University Hospital, Sichuan University, Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Chengdu, Sichuan, China*
[3] *Department of Laboratory Medicine, Lhünzê County People's Hospital, Shannan, Tibet Autonomous Region, China*

ABSTRACT

Background: **Standard reference intervals derived from non-pregnant populations are unsuitable for evaluating thyroid hormone levels in pregnancy. Each geographical region should establish pregnancy-specific thyroid hormone reference intervals that consider local population characteristics, instrumentation, and analytical methods. This research aimed to assess variations in thyroid hormone concentrations during first, second, and third trimester stages among women residing in Lhasa, Xizang. Consequently, the study developed local reference intervals for thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4) to facilitate improved management and prevention of thyroid diseases among pregnant women in this area.**

Methods: **Adhering to the National Academy of Clinical Biochemistry (NACB) guidelines, the study enrolled 1,016 healthy pregnant women undergoing routine antenatal check-ups across different pregnancy stages from October 2023 to October 2024. Additionally, 120 non-pregnant healthy women who participated in health screenings during the identical period served as the control group. Measurements of FT3, FT4, and TSH were performed utilizing the Abbott Architect i2000 automated chemiluminescence immunoassay system. Established reference intervals for each pregnancy stage were represented as medians and corresponding 2.5th to 97.5th percentile values.**

Results: **1) The derived reference intervals were: First trimester: FT3 (3.79 - 5.80 pmol/L), FT4 (10.42 - 16.43 pmol/L), TSH (0.40 - 3.41 mIU/L), second trimester: FT3 (3.36 - 5.80 pmol/L), FT4 (9.40 - 16.22 pmol/L), TSH (0.52 - 3.97 mIU/L), third trimester: FT3 (3.01 - 5.09 pmol/L), FT4 (9.52 - 15.05 pmol/L), TSH (0.69 - 4.43 mIU/L). 2) Significant differences in FT3, FT4, and TSH concentrations were identified among pregnancy stages and between pregnant participants and non-pregnant controls ($p < 0.001$). Specifically, FT3 and FT4 demonstrated a continuous decline with advancing pregnancy, while TSH exhibited a progressive increase.**

Conclusions: **For the first time, this study successfully established distinct reference intervals for TSH, FT3, and FT4 specific to healthy pregnant women in Lhasa, Xizang. These locally applicable intervals can enhance early detection accuracy for thyroid-related disorders in pregnant populations, thus mitigating risks associated with inappropriate application of general population-based reference standards.**

(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.251006)

Correspondence:
Yingying Li
West China Second University Hospital
Sichuan University
No. 1416, Chenglong Avenue
Jinjiang District, Chengdu
Sichuan 610066
P.R. China

Phone: +86 13980563522
Email: yilansuo128@163.com

Manuscript accepted October 17, 2025

KEYWORDS

plateau region, pregnancy, thyroid-stimulating hormone, free thyroid hormone, thyroid, reference interval

INTRODUCTION

The thyroid gland is an essential endocrine organ. Thyroid hormones, secreted by this gland, are primarily regulated by the hypothalamic-pituitary-thyroid (HPT) axis and play critical roles in promoting growth and development, regulating metabolism, and maintaining organ functions [1]. Upon ascending from lowland to high-altitude environments, the human body initiates compensatory regulatory responses known as "high-altitude acclimatization" to adapt to hypoxia. Current studies indicate that high-altitude hypoxia significantly activates the thyroid axis. Research by von Wolff et al. [2] demonstrated differential responses among thyroid hormones at increasing altitudes: free thyroxine (FT4) continuously increases with altitude and does not return to baseline levels after short-term acclimatization; free triiodothyronine (FT3) exhibits only a transient elevation upon initial exposure; thyroid-stimulating hormone (TSH) shows a delayed elevation only at extremely high altitudes. The endocrine adaptations triggered by high-altitude hypoxic exposure, combined with physiological fluctuations in thyroid function during pregnancy, significantly complicate the assessment of thyroid homeostasis in pregnant populations at high altitudes. However, there remains a lack of research specifically addressing thyroid function in pregnant women residing at high altitudes. The diagnostic criteria for thyroid disorders in this population often rely on guidelines established for lowland populations, potentially overlooking the unique impacts of high-altitude environments on thyroid function [3,4].

Thyroid dysfunction during pregnancy may result in multiple adverse maternal and fetal outcomes, such as miscarriage, premature delivery, fetal distress, and impaired fetal neurodevelopment [5-7]. Hypothyroidism, one of the most common endocrine disorders observed during pregnancy, is characterized by insufficient thyroid hormone synthesis, secretion, or activity, leading to a generalized hypometabolic state. Despite its frequency, hypothyroidism frequently remains undiagnosed due to its nonspecific clinical presentation. The initial 12 weeks of gestation represent a crucial developmental period for the fetal nervous system. During this interval, the fetus relies entirely on maternal thyroid hormones transferred via the placenta, as fetal hormone production has not yet commenced [8,9]. Maternal thyroid hormone deficiency during this critical window may severely impact the fetal central nervous system development [10]. After the 14th week of pregnancy, developmental damage is usually irreversible. Therefore, early and precise diagnosis of thyroid dysfunction is essential for avoiding associated negative pregnancy outcomes [11]. According to the 2019 Chinese “Guideline on Diagnosis and Management of Thyroid Diseases During Pregnancy and Postpartum (2nd Edition)”, regional and laboratory-specific thyroid hormone reference intervals for pregnancy should be established. Consequently, this study aimed to define regional reference intervals for TSH, FT3, and FT4 during different pregnancy stages in Lhasa, thus facilitating early diagnosis and timely management of thyroid disorders in pregnant women in this area.

MATERIALS AND METHODS

The study was carried out at the Women and Children's Hospital of Tibet Autonomous Region, with ethical approval provided by the Institutional Review Board.

Study subjects

This retrospective study analyzed data from women undergoing thyroid hormone assessment between October 2023 and October 2024 at the hospital. A total of 1,016 pregnant women were enrolled and categorized into groups by trimester, in accordance with National Academy of Clinical Biochemistry (NACB) guidelines: 413 participants in the first trimester (0 - 12 weeks), 343 in the second trimester (13 - 27 weeks), and 260 in the third trimester (28 - 40 weeks). Additionally, a control group included 120 healthy non-pregnant women who underwent routine health checks during the same timeframe. All participants had lived in Lhasa for at least one year. NACB-based inclusion criteria were: 1) singleton pregnancy; 2) absence of personal or familial thyroid disease history; 3) no thyroid enlargement or autoimmune disorders; 4) no recent consumption of iodine-containing or antithyroid medications, excluding estrogen; 5) age ranging from 18 to 50 years; 6) negative thyroid peroxidase antibody and thyroglobulin antibody tests; and 7) no history of adverse pregnancy events such as recurrent miscarriage or stillbirth.

Experimental methods

Serum samples were collected according to standardized protocols. A total of 5 mL venous blood was obtained from the antecubital vein, allowed to clot at room temperature for 30 minutes, and then centrifuged at 3,000 r/minute for 10 minutes. Measurements of FT3, FT4, and TSH were conducted using the Abbott Architect i2000 automated chemiluminescence immunoassay analyzer and reagents provided by Abbott Laboratories, strictly adhering to the manufacturer's guidelines to ensure data accuracy and quality.

Statistical analysis

Data were analyzed with SPSS software (version 27.0). Normal distribution of FT3 was verified by the Shapiro-Wilk test and results presented as mean ± standard deviation. Analysis of variance (ANOVA) was employed for comparing FT3 levels across pregnancy stages. FT4

and TSH values, which exhibited non-normal distributions, were presented as median and interquartile ranges [M (QL, QU)], and group differences were assessed using the Kruskal-Wallis H test. Pairwise comparisons across gestational periods utilized the Bonferroni correction method. Reference intervals for FT3, FT4, and TSH were presented as medians along with 2.5th to 97.5th percentiles. The chi-squared test compared detection rates of subclinical hypothyroidism under varying diagnostic standards, with categorical data expressed as percentages.

RESULTS

Reference intervals for thyroid function during pregnancy

Table 1 presents the established reference intervals for thyroid hormones FT3, FT4, and TSH at various stages of pregnancy, expressed using medians (M) and their corresponding 2.5th to 97.5th percentile limits (P2.5 - P97.5).

Comparison of thyroid function indicators between pregnant and control groups

The thyroid hormones FT3, FT4, and TSH were statistically compared across the four participant groups using the F/H test, revealing significant differences (all $p < 0.001$). Bonferroni-adjusted pairwise comparisons demonstrated a progressive decrease in FT3 and FT4 concentrations, accompanied by a significant rise in TSH concentration, with advancing gestational age. FT3 concentrations were notably elevated in first trimester relative to the control group but significantly lower in third trimester ($p < 0.05$). FT4 levels in the second and third trimesters were significantly reduced compared to the control group ($p < 0.05$). Additionally, TSH concentrations during third trimester were considerably elevated compared to control values ($p < 0.05$, Table 2).

DISCUSSION

This study systematically evaluated thyroid hormone variations in pregnant women residing in Lhasa, situated at an altitude of 3,650 meters, thereby elucidating distinct patterns of thyroid function in this specific population. Comparative analyses among different gestational stages and controls demonstrated that TSH concentrations initially exhibited a mild decline in first trimester, subsequently increased in second trimester, and were significantly elevated compared to controls during third trimester ($p < 0.01$). Levels of FT3 and FT4 in first trimester were slightly higher relative to the control group but markedly decreased in second and third trimester stages ($p < 0.05$). Endocrine system changes during pregnancy can alter thyroid hormone levels, thereby meeting maternal and fetal requirements. Therefore, pregnancy-specific reference intervals should be used when evaluating thyroid function [12].

The reasons and manifestations of pregnancy-specific thyroid changes vary across gestational stages. In first trimester, increased estradiol (E2) secretion stimulates hepatic synthesis of thyroid hormone-binding globulin (TBG). TBG levels begin rising at 6 - 8 weeks, peak at approximately 20 weeks, and increase 1.5 - 2 times above non-pregnant levels [13]. Increased TBG inevitably elevates total triiodothyronine (TT3) and total thyroxine (TT4) concentrations. Additionally, during first trimester, the placenta secretes high levels of human chorionic gonadotropin (hCG). Because hCG has an α-subunit similar to TSH, it binds to TSH receptors, stimulating FT3 and FT4 secretion and inhibiting pituitary TSH secretion. This mechanism results in reduced serum TSH levels in first trimester.

During second and third trimester, factors such as reduced hCG secretion, increased basal metabolic rate, and enhanced renal iodine clearance [14,15] gradually lower serum FT3 and FT4 levels. These changes stimulate pituitary secretion of TSH, increasing serum TSH in second and third trimester. Adaptive changes in thyroid function during pregnancy may be associated with significantly increased demand for thyroid hormones and iodine [16,17].

Currently, research on thyroid function among pregnant women at high altitudes remains insufficient, and specific guidelines are unavailable. International guidelines are often applied in clinical practice. Notably, the thyroid hormone reference intervals established by the present study deviate from those recommended by the American Thyroid Association (ATA), potentially impacting diagnostic precision and influencing the reported rates of subclinical hypothyroidism. According to the 2011 and 2017 ATA guidelines, this study found significant differences in the prevalence of subclinical hypothyroidism across pregnancy stages ($p < 0.05$). As shown in Table 3, the highest incidence occurred when applying the 2011 ATA guidelines. The incidence identified using the 2017 ATA guidelines was consistent with this study's findings, both being relatively low. These differences suggest that diagnostic criteria substantially influence prevalence assessments of subclinical hypothyroidism.

Specifically, the findings were as follows: 1) According to the 2011 ATA guidelines, recommended normal TSH intervals are 0.1 - 2.5 mIU/L in first trimester, 0.2 - 3.0 mIU/L during second trimester, and 0.3 - 3.0 mIU/L in third trimester, all of which are lower than the intervals determined in the current study. Adopting these ATA guidelines might consequently result in overdiagnosing subclinical hypothyroidism. 2) The 2017 ATA guidelines suggest an upper limit of 4.0 mIU/L for TSH across all pregnancy periods, closely aligning with the upper limit identified for second trimester in this research, thus yielding comparable prevalence rates. However, this study's upper TSH limit was lower in first trimester and higher in third trimester compared to the 2017 ATA guidelines. Applying the 2017 ATA

Table 1. Reference intervals for thyroid function during pregnancy.

Group	FT3 (pmol/L)		FT4 (pmol/L)		TSH (mIU/L)	
	M	P2.5 - P97.5	M	P2.5 - P97.5	M	P2.5 - P97.5
First trimester	4.70	3.79 - 5.80	12.87	10.42 - 16.43	1.49	0.40 - 3.41
Second trimester	4.64	3.36 - 5.80	11.97	9.40 - 16.22	1.66	0.52 - 3.97
Third trimester	4.03	3.01 - 5.09	11.45	9.52 - 15.05	2.21	0.69 - 4.43

M: Median.

Table 2. Comparison of thyroid function levels among four groups of patients.

Group	Cases	FT3 (pmol/L)	FT4 (pmol/L)	TSH (mIU/L)
Control group	120	4.55 ± 0.53	12.74 (11.62, 13.64)	1.54 (1.16, 2.24)
First trimester	413	4.73 ± 0.50 [a]	12.87 (11.84, 13.77)	1.49 (0.98, 2.09)
Second trimester	343	4.62 ± 0.60 [b]	11.97 (11.07, 13.00) [a, b]	1.66 (1.08, 2.26)
Third trimester	260	4.05 ± 0.51 [a, b, c]	11.45 (10.42, 12.23) [a, b, c]	2.21 (1.54, 2.91) [a, b, c]
F/H		102.201	156.640	84.982
p-value		< 0.001	< 0.001	< 0.001

Compared with the control group, [a] $p < 0.05$. Compared with early pregnancy, [b] $p < 0.05$, compared to mid pregnancy, [c] $p < 0.05$.

Table 3. Comparison of subclinical hypothyroidism diagnosed by different criteria in women at different pregnancy stages [Case (%)].

Pregnancy group	Cases	2011ATA	2017ATA	Reference interval of this study	X^2	p-value
First trimester	413	61 (14.8)	4 (1.0)	10 (2.4)	135.809	< 0.001
Second trimester	343	32 (9.3)	6 (1.7)	7 (2.0)	49.416	< 0.001
Third trimester	260	59 (22.7)	13 (5.0)	6 (2.3)	116.536	< 0.001
In total	1,016	152 (15.0)	23 (2.3)	23 (2.3)	294.585	< 0.001

Data are presented as n (%). The chi-squared test was used for comparisons.

Table 4. Comparative analysis of FT4 and TSH reference intervals in pregnant women from different regions.

Group		FT4 (pmol/L)		TSH (mIU/L)	
		M	P2.5 - P97.5	M	P2.5 - P97.5
First trimester	Plain area	15.30	12.37 - 19.09	1.43	0.08 - 4.46
	Coastal area	14.10	10.9 - 17.7	1.22	0.16 - 3.78
	Plateau area	12.87	10.42 - 16.43	1.49	0.40 - 3.41
Second trimester	Plain area	12.90	9.85 - 18.05	1.51	0.05 - 3.71
	Coastal area	12.1	9.3 - 15.2	1.31	0.34 - 3.51
	Plateau area	11.97	9.40 - 16.22	1.66	0.52 - 3.97
Third trimester	Plain area	11.59	9.12 - 14.91	1.97	0.47 - 6.29
	Coastal area	10.7	7.9 - 14.1	1.62	0.34 - 4.32
	Plateau area	11.45	9.52 - 15.05	2.21	0.69 - 4.43

Plain area: Shenyang, Liaoning Province, China; Coastal area: Hangzhou, Zhejiang Province, China; Plateau area: Lhasa (current study).

guidelines, approximately 60% of cases in first trimester would be missed, while 53.85% in third trimester would be overdiagnosed. Therefore, directly applying international guidelines may pose a risk of misdiagnosis or missed diagnosis [18]. Thus, establishing region-specific pregnancy reference intervals is crucial.

Given the inherent variability due to geographic and demographic factors, it remains crucial to establish locally appropriate reference intervals for thyroid function assessment. Currently, numerous hospitals and regions across China have set pregnancy-specific thyroid reference intervals using identical equipment and reagents employed in this study (Abbott Architect i2000 automated chemiluminescence immunoassay analyzer and original Abbott reagent kits) (Table 4). Nevertheless, even with uniform analytical methods, thyroid hormone reference ranges for pregnant women at high altitudes in Xizang exhibit variations from those identified in plains [19] or coastal regions [20]. This difference may result from the high altitude (3,650 meters), hypoxic conditions, and cold climate of the study area. Additionally, dietary habits in high-altitude regions differ significantly from other areas, such as lower seafood intake and thus reduced iodine consumption. Significant differences in thyroid hormone reference intervals among ethnic groups have also been reported [21,22]. Additional research assessing iodine nutritional status among Tibetans revealed a heightened prevalence of iodine deficiency compared to populations residing at lower altitudes [23]. Under conditions of iodine deficiency, thyroid hormone synthesis becomes inadequate, significantly elevating the iodine requirements for pregnant women relative to the general population. Therefore, establishing regional pregnancy-specific thyroid hormone reference intervals in high-altitude areas is necessary to meet local clinical needs. This approach can improve thyroid function monitoring in women at different stages of pregnancy, effectively avoiding misdiagnosis or missed diagnosis caused by applying reference intervals from plain regions. Ultimately, this will guide clinical interventions, reduce adverse pregnancy outcomes such as miscarriage and preterm birth due to thyroid dysfunction, and enhance maternal and fetal health.

In summary, this study analyzed gestational changes in thyroid hormone concentrations and, for the first time, defined reference intervals for TSH, FT3, and FT4 specific to pregnant women in Lhasa, Xizang, thereby addressing a critical regional data gap. These findings offer an evidence-based framework for accurate diagnosis and timely management of pregnancy-associated thyroid disorders at high altitudes. Results indicated that directly applying reference intervals from plain areas or international guidelines is inappropriate. Establishing region-specific reference intervals enables more accurate assessment of thyroid function in pregnant women, reduces risks of misdiagnosis or missed diagnosis, and improves maternal and fetal outcomes. Future studies incorporating larger sample sizes and multi-center collaborations that account for high-altitude environmental factors will further enhance maternal and child healthcare provision in these regions.

Source of Funds:
This work did not receive any financial support.

Declaration of Generative AI in Scientific Writing:
The authors hereby declare that no generative AI tools were used in the creation of the intellectual content, data analysis, or writing of this manuscript.

Declaration of Interest:
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References:

1. Springer D, Jiskra J, Limanova Z, Zima T, Potlukova E. Thyroid in pregnancy: From physiology to screening. Crit Rev Clin Lab Sci 2017;54:102-16. (PMID: 28102101)

2. von Wolff M, Nakas CT, Tobler M, et al. Adrenal, thyroid and gonadal axes are affected at high altitude. Endocr Connect 2018; 7:1081-9. (PMID: 30352395)

3. Gong B, Wang Y, Zhang JA, et al. Effects of altitude on thyroid disorders according to Chinese three-rung, ladder-like topography: national cross-sectional study. BMC Public Health 2024;24: 26. (PMID: 38167020)

4. Ma C, Zhong J, Zou Y, et al. Establishment of Reference Intervals for Thyroid-Associated Hormones Using refineR Algorithm in Chinese Population at High-Altitude Areas. Front Endocrinol (Lausanne) 2022;13:816970. (PMID: 35222276)

5. Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222. Obstet Gynecol 2020;135:e237-e60. (PMID: 32443079)

6. Feldt-Rasmussen U, Effraimidis G, Bliddal S, Klose M. Consequences of undertreatment of hypothyroidism. Endocrine 2024; 84:301-8. (PMID: 37556077)

7. Yap YW, Onyekwelu E, Alam U. Thyroid disease in pregnancy. Clin Med (Lond) 2023;23:125-8. (PMID: 36958843)

8. Eng L, Lam L. Thyroid Function During the Fetal and Neonatal Periods. Neoreviews 2020;21:e30-6. (PMID: 31894080)

9. Huget-Penner S, Feig DS. Maternal thyroid disease and its effects on the fetus and perinatal outcomes. Prenat Diagn 2020;40:1077-84. (PMID: 32181913)

10. Chen Z, Meima ME, Peeters RP, Visser WE. Thyroid Hormone Transporters in Pregnancy and Fetal Development. Int J Mol Sci 2022;23:15113. (PMID: 36499435)

11. Tsakiridis I, Giouleka S, Kourtis A, Mamopoulos A, Athanasiadis A, Dagklis T. Thyroid Disease in Pregnancy: A Descriptive Review of Guidelines. Obstet Gynecol Surv 2022;77:45-62. (PMID: 34994394)

12. Lee SY, Pearce EN. Testing, Monitoring, and Treatment of Thyroid Dysfunction in Pregnancy. J Clin Endocrinol Metab 2021; 106:883-92. (PMID: 33349844)

13. Lazarus JH. Thyroid function in pregnancy. Br Med Bull 2011; 97:137-48. (PMID: 21186204)

14. Chandra M, Paray AA. Natural Physiological Changes During Pregnancy. Yale J Biol Med 2024;97:85-92. (PMID: 38559455)

15. Lee SY, Pearce EN. Assessment and treatment of thyroid disorders in pregnancy and the postpartum period. Nat Rev Endocrinol 2022;18:158-71. (PMID: 34983968)

16. Bath SC. Thyroid function and iodine intake: global recommendations and relevant dietary trends. Nat Rev Endocrinol 2024;20: 474-86. (PMID: 38693274)

17. Purdue-Smithe AC, Männistö T, Reische E, et al. Iodine and thyroid status during pregnancy and risk of stillbirth: A population-based nested case-control study. Matern Child Nutr 2022;18: e13252. (PMID: 34350728)

18. Turkal R, Turan CA, Elbasan O, et al. Accurate interpretation of thyroid dysfunction during pregnancy: should we continue to use published guidelines instead of population-based gestation-specific reference intervals for the thyroid-stimulating hormone (TSH)? BMC Pregnancy Childbirth 2022;22:271. (PMID: 35361138)

19. Liu J, Yu X, Xia M, et al. Development of gestation-specific reference intervals for thyroid hormones in normal pregnant Northeast Chinese women: What is the rational division of gestation stages for establishing reference intervals for pregnancy women? Clin Biochem 2017;50:309-17. (PMID: 27916508)

20. Shen F, Xie Z, Lu S, Aw TC, Zhu B. Gestational thyroid reference intervals in antibody-negative Chinese women. Clin Biochem 2014;47:673-5. (PMID: 24631176)

21. Elebrashy I, Kamal Eldein HA, Abd-Elstar H, et al. Assessment of thyroid functions and thyroid volume in normal pregnant Egyptian females. Gynecol Endocrinol 2020;36:122-5. (PMID: 31230489)

22. Veltri F, Belhomme J, Kleynen P, et al. Maternal thyroid parameters in pregnant women with different ethnic backgrounds: Do ethnicity-specific reference ranges improve the diagnosis of subclinical hypothyroidism? Clin Endocrinol (Oxf) 2017;86:830-6. (PMID: 28346766)

23. Ning P, Ren Q, Teng D, et al. Current Iodine Nutrition Status and Prevalence of Thyroid Disorders in Tibetan Adults in an Oxygen-Deficient Plateau, Tibet, China: A Population-Based Study. Thyroid 2020;30:759-66. (PMID: 31928176)