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RESEARCH ARTICLE

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Reference intervals for thyroid-stimulating hormone and thyroid hormones using the access TSH 3rd IS method in China

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Abstract

Background: To calculate the reference intervals for thyroid-stimulating hormone (TSH) and thyroid hormones using the Access TSH 3rd IS method and evaluate the differences between age and genders in Chinese populations.

Methods: This study collected 349 serum samples of healthy subjects were from Shengli Oilfield Central Hospital in China. Subjects who tested positive for thyroid peroxidase antibody or thyroglobulin antibody were excluded. Accordingly, 313 subjects were included for establishing reference intervals for the thyroid hormones. The serum concentrations of TSH, total and free thyroxine (TT4 and FT4), and total and free triiodothyronine (TT3 and FT3) were measured using the Access TSH 3rd IS method. The 2.5th and 97.5th percentiles or mean with standard deviation were calculated as the reference interval as appropriate.

Results: The reference intervals for TSH, FT4, FT3, TT4, and TT3 calculated in present study were 0.61-4.16 mIU/L, 0.67-1.11 ng/dL, 2.63-4.33 pg/mL, 5.56-11.33 μg/dL, and 0.72-1.32 ng/mL, respectively. The FT3, TT4, and TT3 levels in males were significantly higher than in females ($P < .05$), while TSH levels in males were significantly lower than in females ($P < .05$). The levels of FT3 in subjects with the age of less than 30 years were significantly higher than other groups ($P < .05$).

Conclusion: The present study provided a valid basis for the reference intervals for TSH, FT4, FT3, TT4, and TT3 in Chinese populations. In addition, this present study indicated that age and gender should be considered in diagnostic evaluation of thyroid diseases.

KEYWORDS

Access TSH 3rd IS method, Chinese populations, reference intervals, thyroid hormones, thyroid-stimulating hormone

Li and Yu are contributed equally to this work and should be considered as co-first authors.

1 | INTRODUCTION

Thyroid disorders have emerged as the most common endocrine disease,[1] especially in China, and the prevalence and spectrum of thyroid disorders are increasing in the past 20 years.[2] However, it is usually difficult to diagnose thyroid disorders in early stages due to the lack of obvious signs and symptoms.[3] Thus, the reliable immunoassay methods and reference intervals for thyroid-stimulating hormone (TSH) measurement have great clinical relevance for detection thyroid disease. Reference intervals serve as the basis of laboratory tests and could affect the diagnostic specificity and sensitivity of laboratory tests.[4] Reference intervals are known to be method- and population-dependent, and therefore, the Clinical and Laboratory Standards Institute has recommended that each laboratory should establish its own reference intervals.[5] But, clinical laboratories commonly use insufficiently approved reference intervals given by the manufacturers of commercially available assays.

Recently, Beckman Coulter Inc developed a new immunoassay method for thyroid-stimulating hormone (TSH) measurement method named Access TSH 3rd IS, which is a new generation product of TSH tested and intended to be marketed in China.[6] Most institutions used the reference intervals for TSH and other thyroid hormones given by the manufacturers of commercially available assays. However, the available reference intervals for TSH and other thyroid hormones are established in western populations.[7] It is well known that there is significant variability between different populations, making it inappropriate to use values derived for one population to evaluate another populations.[8,9] Ideally, there should be a reference interval derived from a representative cohort of healthy subjects in Chinese populations. Therefore, the aim of present study was to calculate the reference intervals for TSH and thyroid hormones using the Access TSH 3rd IS method on Beckman Coulter UniCel DxI 800 immunoassay analyzer and evaluate the differences between age and genders in Chinese populations.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of 349 serum samples of apparently healthy subjects were collected from Shengli Oilfield Central Hospital in Shandong, China, between November 2017 and May 2019. All subjects filled out a questionnaire requesting information concerning anthropometric data, a family history of thyroid diseases, and medications.[10] Pregnant women, lactating women and subjects with personal or family history of thyroid disease, abnormal liver function, tumor, or other endocrine and metabolic disease were excluded from the study. In addition, subjects who tested positive for thyroid peroxidase antibody (TPOAb, ≥9 IU/mL) or thyroglobulin antibody (TgAb, ≥4 IU/mL) were also excluded. These criteria satisfied the recommendation from the American Thyroid Association (ATA) for setting reference intervals of thyroid function tests. The study was approved by the Institutional Research Review Board, and written informed consent was obtained from each subject.

2.2 | Laboratory methods

The blood samples were collected from healthy subjects in fasting state between 07:00 and 10:00 AM. Approximately 5 mL serum was collected from each subject into a pro-coagulant tube (Becton, Dickinson and Company, USA). Half an hour after blood collection, blood samples were centrifuged at 3500 g for 10 minutes, and then, serum was aliquoted and stored frozen at −70°C for analysis. The serum TPOAb, TgAb, free thyroxine (FT4), free triiodothyronine (FT3), total thyroxine (TT4), and total triiodothyronine (TT3) concentrations were measured using the Access TSH 3rd IS method on the UniCelTM DxI 800 Access® immunoassay analyzer (Beckman Coulter). All analyses were performed in real time.

2.3 | Statistical analysis

Statistical analyses were performed with SPSS statistics version 22.0 (SPSS Inc). Categorical variables were described by number or percentage. Continuous variables were expressed as means ± standard deviation (SD). The normality of the data distribution was tested with Kolmogorov–Smirnov test. The 2.5th and 97.5th percentiles were calculated as the reference interval for TSH and FT4 because of the skewed data distribution. Mean with standard deviation was calculated as the reference interval for TT4, TT3, and FT3. For data comparisons between genders, the independent-samples *t* test or nonparametric tests were applied as appropriate. For data comparisons between ages, the one-way ANOVA with LSD test or Kruskal–Wallis test was applied as appropriate. Differences were considered statistically significant when $P < .05$.

3 | RESULTS

3.1 | Study population

A total of 349 serum samples of apparently healthy subjects were collected from six centers. After step-by-step screening, 33 subjects were excluded from the analysis due to the TPOAb-positive and/or TgAb-positive. Finally, 313 subjects consisted of 173 (55.3%) males and 140 (44.7%) females were included for establishing reference intervals for the thyroid hormones. The age of these included subjects were 43.55 ± 13.13 years, ranging from 20 to 97 years. Besides, of the 313 healthy subjects included into the whole population, 72 were 20.0-30.0 years of age, 71 were 30.1-40.0 years, 74 were 40.1-50.0 years, 61 were 50.1-59.9 years, and 35 were older than 60 years.

TABLE 1 Reference intervals for TSH and thyroid hormones in the whole population

Hormone	Mean ± SD	Median	Min-Max	P2.5	P97.5	Reference Intervals
TSH (mIU/L)	2.00 ± 0.95	1.78	0.04-6.71	0.61	4.16	0.61-4.16
FT4 (ng/dL)	0.86 ± 0.11	0.85	0.57-1.18	0.67	1.11	0.67-1.11
FT3 (pg/mL)	3.48 ± 0.43	3.50	2.30-5.70	2.74	4.37	2.63-4.33
TT4 (μg/dL)	8.44 ± 1.47	8.41	0.75-12.42	5.70	11.36	5.56-11.33
TT3 (ng/mL)	1.02 ± 0.15	1.01	0.61-1.51	0.73	1.35	0.72-1.32

Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; Max, maximum; Min, minimum; P2.5, 2.5th percentiles; P97.5, 97.5th percentiles; SD, standard deviation; TSH, thyroid-stimulating hormone; TT3, total triiodothyronine; TT4, total thyroxine.

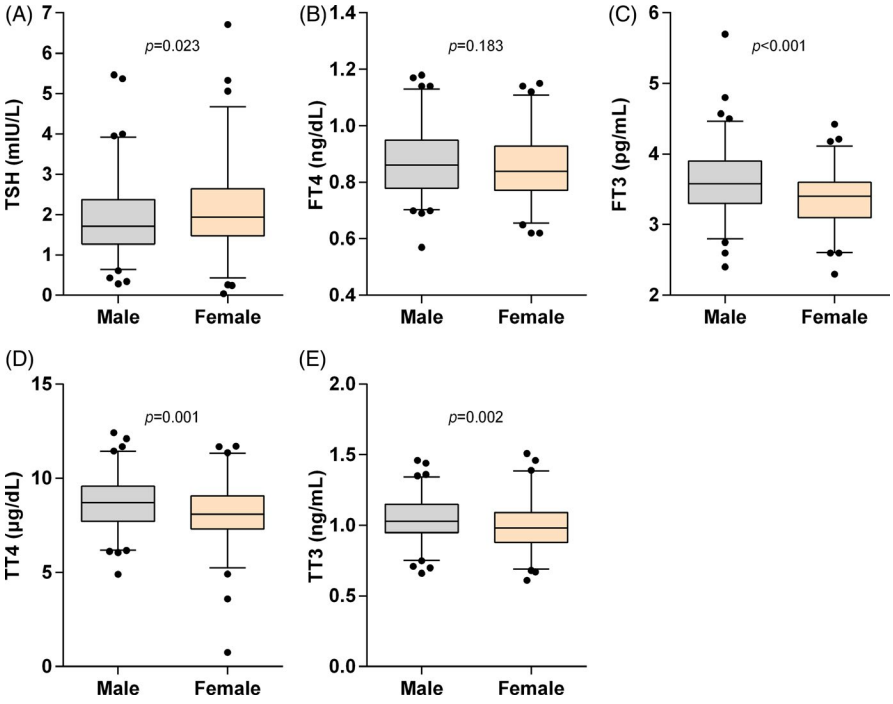

FIGURE 1 Thyroid-stimulating hormone (TSH) and thyroid hormones distribution values in males and females. Date was reported as boxes indicating the 2.5 th, 25th, 50th (median), 75th, and 97.5th percentiles. (A) Box plots of serum TSH; (B) box plots of serum FT4; (C) box plots of serum FT3; (D) box plots of serum TT4; and (E) box plots of serum TT3. FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; TT3, total triiodothyronine; TT4, total thyroxine

3.2 | Analysis of the whole population

Serum TSH and FT4 levels show a distribution approximating to a log-normal distribution ($P < .05$), while the levels of TT4, TT3, and FT3 presented normal distribution ($P > .05$). The median (2.5th percentile and 97.5th percentile) of serum TSH and FT4 levels was 1.78 mIU/L (0.61 mIU/L, 4.16 mIU/L) and 0.85 ng/dL (0.67 ng/dL, 1.11 ng/dL), respectively. The mean (SD) of FT3, TT4, and TT3 levels was 3.48 pg/mL (0.43), 8.44 μg/dL (1.47), 1.02 ng/mL (0.15). The reference intervals for TSH, FT4, FT3, TT4, and TT3 were, respectively, 0.61-4.16 mIU/L, 0.67-1.11 ng/dL, 2.63-4.33 pg/mL, 5.56-11.33 μg/dL, and 0.72-1.32 ng/mL (Table 1).

3.3 | Subgroup analysis of reference intervals

The differences in TSH, FT3, TT4, and TT3 levels between the females and males were statistically significant ($P < .05$, Figure 1). The FT3, TT4, and TT3 levels of males were significant higher than that of females (FT3:3.59 ± 0.46 vs 3.36 ± 0.36; TT4: 8.68 ± 1.32 vs 8.15 ± 1.60; TT3: 1.04 ± 0.15 vs 0.99 ± 0.16), while the TSH levels of males were significant lower than that of females (1.90 ± 0.88 vs 2.12 ± 1.01, Figure 1). No difference was found in the FT4 levels between females and males (0.87 ± 0.11 vs 0.85 ± 0.11, $P = .183$). Table 2 showed the reference intervals for female and male, respectively.

Additionally, we compared the differences in the levels of thyroid hormones in the whole population according to some age intervals. The results showed that no difference was found in the TSH, FT4, TT4, and TT3 levels among different age intervals ($P > .05$, Figure 2), while the differences in FT3 levels among different age intervals were statistically significant ($P < .05$, Figure 2). The levels of FT3 in subjects with the age of <30 years were significantly higher than other groups.

4 | DISCUSSION

It is well recognized that reference intervals are method- and population-dependent and thus should be established by each laboratory for each method and population.[11] It is important to establish reference intervals of this new method (Access TSH 3rd IS) for TSH

TABLE 2 Reference intervals for TSH and thyroid hormones in the whole population according to gender

Hormone	Mean ± SD	Median	Min-Max	P2.5	P97.5	Reference Intervals
TSH (mIU/L)						
Male (n = 173)	1.90 ± 0.88	1.71	0.28-5.46	0.65	3.92	0.65-3.92
Female (n = 140)	2.12 ± 1.01	1.94	0.04-6.71	0.43	4.67	0.43-4.67
FT4 (ng/dL)						
Male (n = 173)	0.87 ± 0.11	0.86	0.57-1.18	0.70	1.13	0.70-1.13
Female (n = 140)	0.85 ± 0.11	0.84	0.62-1.15	0.66	1.11	0.66-1.11
FT3 (pg/mL)						
Male (n = 173)	3.59 ± 0.46	3.58	2.4-5.7	2.80	4.47	2.68-4.49
Female (n = 140)	3.36 ± 0.36	3.40	2.3-4.42	2.61	4.11	2.65-4.06
TT4 (μg/dL)						
Male (n = 173)	8.68 ± 1.32	8.71	4.91-12.42	6.18	11.43	6.10-11.26
Female (n = 140)	8.15 ± 1.60	8.09	0.75-11.71	5.24	11.33	5.01-11.28
TT3 (ng/mL)						
Male (n = 173)	1.04 ± 0.15	1.03	0.66-1.46	0.75	1.34	0.76-1.33
Female (n = 140)	0.99 ± 0.16	0.98	0.61-1.51	0.69	1.39	0.68-1.30

Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; Max, maximum; Min, minimum; P2.5, 2.5th percentiles; P97.5, 97.5th percentiles; SD, standard deviation; TSH, thyroid-stimulating hormone; TT3, total triiodothyronine; TT4, total thyroxine.

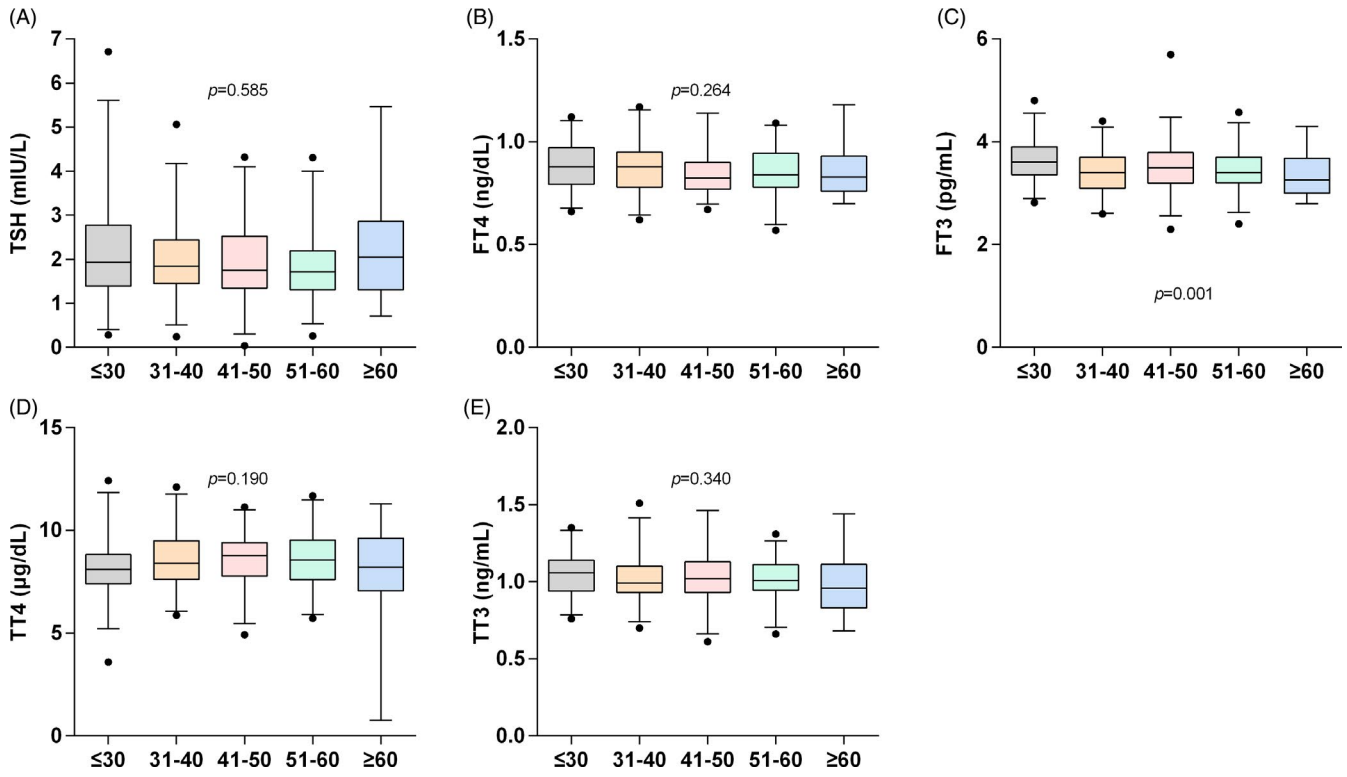

FIGURE 2 Thyroid-stimulating hormone (TSH) and thyroid hormones distribution values in different age intervals. Date was reported as boxes indicating the 2.5th, 25th, 50th (median), 75th, and 97.5th percentiles. (A) Box plots of serum TSH; (B) box plots of serum FT4; (C) box plots of serum FT3; (D) box plots of serum TT4; and (E) box plots of serum TT3. FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; TT3, total triiodothyronine; TT4, total thyroxine

and other thyroid hormones in a representative cohort of Chinese populations. To the best of our knowledge, this is the first study to report the reference intervals for TSH and other thyroid hormones using the Access TSH 3rd IS method in Chinese populations. Additionally, we compared the differences between genders and different age groups. In the present study, the reference intervals of new method (Access TSH 3rd IS) for TSH, FT4, FT3, TT4,

and TT3 were reported based on the healthy cohort of Chinese subjects.

The reference intervals for FT4 (0.67-1.11 ng/dL), FT3(2.63-4.33 pg/mL), TT4 (5.56-11.33 μg/dL), and TT3 (0.72-1.32 ng/mL) calculated in the present study are similar to that suggested by the manufacturer. Moreover, the reference interval for TSH (0.61-4.16 mIU/L) calculated in present study was narrower than that suggested by the manufacturer. The reference intervals suggested by manufacturer for TSH (0.49-4.91 mIU/L), FT4 (0.59-1.25 ng/dL), FT3 (2.14-4.21 pg/mL), TT4 (5.44-11.85 μg/dL), and TT3 (0.66-1.61 ng/mL) were calculated on a general population (n = 1316) of approximately equal numbers of male and female individuals with ages ranging from 18 to 86 years. Previously, the reference interval for TSH using new Access TSH 3rd IS assay was also verified in Italian populations, which was 0.362-5.280 mIU/L.[9] The reference interval in Italian populations is similar to that suggested by the manufacturer. Thus, we speculated that the difference in terms of TSH reference interval may be due in part to the different samples sizes, ethnicity, and alimentary habit, especially the differences in iodine intake.[12,13]

Multiple factors may affect the reference interval establishment of thyroid function tests, such as the population demographics (including age, sex, and ethnicity), iodine status, smoking status, body mass index, administration of some drugs, and methodology.[6,14,15] In the present study, we observed a significant difference in TSH, FT3, TT4, and TT3 serum levels between female and male, indicating that gender should be considered in diagnostic evaluation of thyroid diseases in Chinese populations. Furthermore, the difference values of TSH and thyroid hormones according to some age intervals were evaluated in our study. It is well kown that the relationship between TSH and age is very complex and controversial.[16,17] Unfortunately, there was no difference significance in TSH, FT4, TT4, and TT3 between different age intervals. We only found that the levels of FT3 in subjects with the age of less than 30 years were significantly higher than other groups. However, according to Clerico et al,[9] a negative linear relationship was observed between TSH throughout all interval age values and TSH values in Italian populations tend to decrease with age. These conflicting results may be explained by taking into account the different pathophysiological conditions of the enrolled individuals and the large difference between sample sizes. Given that TSH is a kind of labile hormone, only a single determination may be a unreliable representative of long-term thyroid status, especially in clinical studies including a relatively low number of individuals.[9,18] Therefore, clinical and demographic characteristics of included subjects may strongly affect the results of studies concerning the distribution of TSH values in reference populations. Thus, a large simple size clinical trial remains needed to verify the results.

The limitations of our study include the following. Firstly, the enrolled sample size was small, which may lead to a bias of our results. Secondly, this multicenter study only enrolled subjects in Shandong, which means the narrow research scope. Additional laboratories throughout China could collaborate to establish reference intervals for thyroid function tests. In short, a large simple size, national multicenter study is needed to verify our results.

In conclusion, the present study provided a valid basis for the reference intervals for TSH, FT4, FT3, TT4, and TT3 in Chinese populations. Thyroid function test reference ranges can be affected by gender and age, which indicated that age and gender should be considered in diagnostic evaluation of thyroid diseases.

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