Research Article

Reference intervals of thyrotropin, thyroid hormones, and thyroid autoantibodies in adult and older individuals according to iodine status

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Article Info

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Keywords

Reference range, age-specific reference intervals, thyrotropin, thyroid hormone, thyroid autoantibody, iodine status

Abstract

Background-Aim: Several factors, including ethnicity, age, iodine status, and assay method, can influence thyroid test results. This study aimed to establish reference intervals (RIs) for thyroid parameters in adults and older individuals, considering their iodine statuses.

Methods: A cross-sectional study at a single tertiary center was conducted. Participants underwent interviews, physical examinations, thyroid ultrasounds, thyroid autoantibody testing, and a spot urine iodine concentration analysis. The included participants were grouped into adult (age 18 – 59) and older (age ≥60) groups. The studies for 2.5th—97.5th values of thyroid parameters were committed to establishing RIs.

Results: A total of 357 individuals were screened, with 216 (112 adults, 54% women, 104 older, 50% women) were included in the analysis. The RIs for thyroid stimulating hormone (TSH) were as follows: 0.39 – 4.17 mIU/L for the overall group, 0.35 – 3.98 mIU/L in the adult group, and 0.42 – 4.83 mIU/L in the older group. The prevalence of adequate iodine intake (urine iodine level ≥ 200 µg/L) was 62.2% (186/299). Though RIs for TSH, Free T4, and Free T3 were slightly higher in the adequate iodine intake group, no statistically significant differences were noted. Positive anti-thyroglobulin antibodies were more prevalent in older participants (27.3% VS. 21.2%), as were anti-thyroid peroxidase antibodies (24.2% VS. 13.9%).

Conclusion: Older individuals exhibited significantly higher TSH levels and lower FT3/Total T3 levels, while FT4/Total T4 remained comparable to adults. All thyroid parameters and thyroid autoantibody levels showed no statistically significant differences between those with adequate iodine intake and those with iodine deficiency.

Background

Variations in thyroid hormone levels can arise from multiple factors, including ethnicity, gender, age, body mass index (BMI), habitat, and diet [1]. Additionally, variations in analytical techniques, laboratory environments, specimen collection, and transportation can further impact thyroid function test results [2]. The reference intervals (RIs) provided by the manufacturer are merely guidance; each laboratory should establish RIs tailored to its specific population. Several studies have shown population-specific thyroid hormone levels [2-4]. Notably, the large population study from the National Health and Nutrition Examination Survey (NHANES-III) in the United States identified significant disparities in mean TSH levels across racial and ethnic groups. Specifically, the results indicated that mean TSH levels were lower in Black individuals, followed by Mexican Americans, and then White individuals [4]. These results highlight the importance of considering ethnic factors in thyroid assessments.

Prior studies have shown discrepancies in thyroid function test results among different analyzers [5]. A comparative study conducted in Australia among various analyzers (Siemens Centaur, Roche, Architect, and Immulite) revealed minor differences at TSH levels less than 2 mIU/mL, approximately 1 mIU/L at TSH levels between 4 – 5 mIU/L, and about 2 mIU/L at TSH levels of 8 – 10 mIU/L. These variations can have a substantial impact on clinical decisions and patient management. The RIs of thyroid function tests among Thai individuals from the previous report used an analytic platform different from our center [6]. Consequently, it is essential to establish RIs for thyroid hormone tests in our center, which was the primary purpose of this study.

The changes in thyroid hormones associated with aging have been widely studied in multiple populations [5,7,8]. The findings from those past studies identified a significant increase in TSH levels, accompanied by only a minor change in FT4 levels as age progresses. However, the underlying pathophysiological mechanisms driving these changes remain unclear. Several hypotheses have been proposed, including reduced negative feedback effect due to altered pituitary-thyroid axis setpoints, decreased bioactivity of TSH from potential isoform modifications, diminished responsiveness of thyrocytes to TSH, and possibly epigenetic influences linked to environmental factors [4,9]. Thus, establishing age-specific thyroid hormone RIs is one of several ways to avoid inappropriate diagnosis and help improve the management of thyroid disorders in the older.

Iodine serves as a critical substrate for thyroid hormone synthesis. Individuals with iodine deficiency may exhibit clinical hypothyroidism diversely, depending on the severity of the deficiency and the age of the affected individual. The World Health Organization (WHO) recommends that at-risk populations should be screened for iodine status, and those identified as deficient should receive appropriate iodine supplementation to prevent infant mortality and enhance cognitive development. Multiple studies conducted in both iodine-deficient and iodine-sufficient regions have shown a trend of elevated TSH levels in areas with sufficient iodine status [10]. While Thailand is classified as an iodine-sufficient nation [11], assessing the relationship between iodine status and thyroid hormone levels within the Thai population remains essential.

The objective of this study was to establish RIs of thyroid function test in five parameters: thyroid stimulating hormone (TSH), free triiodothyronine (free T3), free thyroxine (free T4), total triiodothyronine (TT3) and total thyroxine (TT4), and to establish RIs of thyroid autoantibodies, including anti-thyroglobulin antibodies (Anti-Tg), anti-thyroid peroxidase antibodies (anti-TPO) and anti-thyrotropin receptor antibodies (anti-TSHr) among Thai adults and older by the laboratory method used in our center. These newly established RIs are expected to be more specific to Thai adults and older patients. The second objective is to study the association between iodine status and thyroid hormones among the Thai.

Material and Method

Study population and screening

A cross-sectional study was conducted at a single tertiary center from October 2021 to October 2022. The Faculty of Medicine, Chulalongkorn University Institutional Review Board approved this study (IRB no. 637/63) in accordance with the Declaration of Helsinki (as revised in 2013). During the COVID-19 outbreak, we used a non-face-to-face participant screening procedure to reduce visits. The initial screening tool was a QR-code-linked questionnaire distributed online through our department's social media platform. The questionnaire consisted of checklists of inclusion criteria, which were Thai individuals aged ≥18 years, and exclusion criteria, which were BMI >30 kg/m2, a recent hospitalization within the past three months, a history of receiving an iodinated contrast media during the past three months, and underlying conditions affecting thyroid diseases or having been prescribed with medications affecting thyroid functions (furosemide, heparin, non-steroidal anti-inflammatory drugs). In the next step, a phone interview was conducted to verify the adherence to inclusion and exclusion criteria among potential participants who had answered the questionnaire and to arrange a visit to enroll participants in the study.

Then, in the final screening step, selected participants underwent a physical examination and thyroid ultrasound conducted by a single endocrinologist. If the individuals were found with any of the exclusion criteria, which were enlarged thyroid gland, inhomogeneous thyroid parenchyma, multiple thyroid nodules (any size), or a single thyroid nodule, size >1 cm. In diameter, they were excluded. The enrolled participants were requested to obtain blood for thyroid function and thyroid autoantibodies tests and provide spot urine collection for urine iodine concentration

analysis. Only participants with all three negative thyroid autoantibodies will be included in the study as the reference population for normal thyroid conditions. Informed consent was obtained from all individuals included in this study. The participants were sampled and grouped by non-probability sampling method with quota selection to attain an equal balance between genders and age groups (adult group aged 18 -59 years and older adult group aged 60 years and over).

Specimen processing and Laboratory analysis

The specimen collection included two tubes of heparinized blood, two tubes of clotted blood, and a cup of urine. After the specimen was collected, all samples were processed within two hours. Two tubes of heparinized blood and a clotted blood tube were centrifuged with 1,000 g for 10 minutes and immediately analyzed for TSH, FT4, and FT3 as centrifugation was finished. The analyses for TSH, free T3, and free T4 were performed with an Abbott Alinity I analyzer (Abbott Laboratories, Chicago, Illinois) by chemiluminescence immunoassay method at the central laboratory, King Chulalongkorn Memorial Hospital. All residual plasma and sera are then portioned in different sterile vials to store at -70 Celsius degrees for further analysis. Clotted blood tubes are simultaneously processed to the Center for Medical Diagnostic Laboratories (CMDL), Faculty of Medicine Chulalongkorn University, to perform Anti-TSHr with Roche Cobas e601 (Roche diagnostics, Basel, Switzerland) by electrochemiluminescence immunoassay method. The test is performed within 3 hours. The remaining parameters, including total T3, total T4, anti-TPO, and anti-Tg, were analyzed within 30 days with -70 Celsius degree-stored sera samples thawed at room temperature for 30 minutes, removed fibrin, mixed until homogenized, and centrifuged with 1,000 g for 10 minutes. The spot urine samples collected from participants were stored at -70 Celsius degree for further analysis of urine iodine concentration with the ammonium persulfate digestion method (Sandell-Kolthoff reaction) at the Nuclear Chemistry laboratory, Faculty of Medicine, Siriraj Hospital, within 90 days.

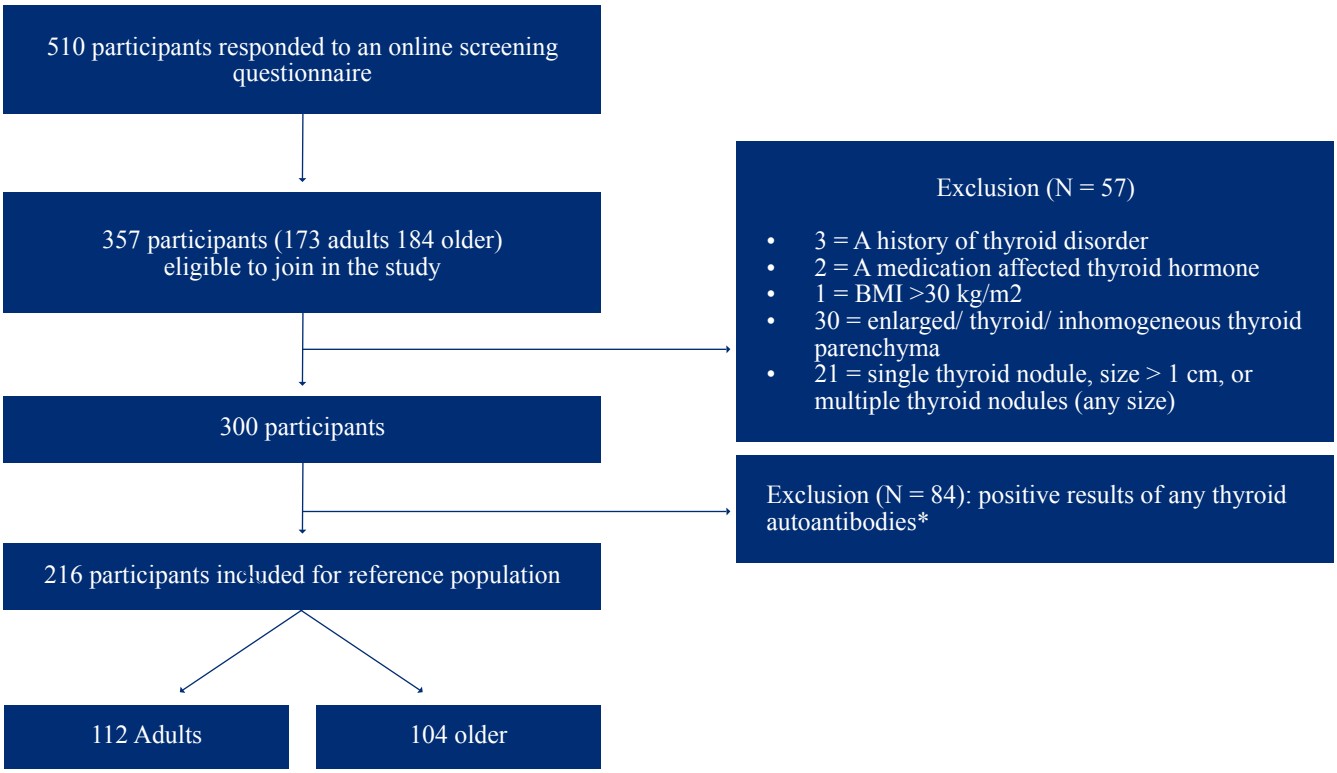
Statistical Analysis

According to the Clinical and Laboratory Standards Institute (CLSI) volume EP-28, at least 120 data were used to analyze and define the reference intervals. The percentiles of 2.5 and 97.5 were used for the lower and upper limits of the reference intervals. Each variable data was tested for normal distribution by the Shapiro-Wilk test method. In normal distributed data, mean ± SD and unpaired student t-test were used for a descriptive analysis—a non-parametric test, such as medians with IQR, and the Kruskal-Wallis test was used in non-normal distributed data. The reference range was compared among groups using the Wilcoxon rank-sum test. All analyses were a two-sided test with $\alpha = 0.05$ by the Stata Statistic program version 16.1.

Results

Five hundred and ten participants responded to the online questionnaires. After checking those answered questionnaires for the inclusion and exclusion criteria, the telephone interview was managed to respond to the eligible participants. Finally, 357 participants agreed to join an enrolment visit at our center. During the visit, six participants were revealed to meet exclusion criteria: a history of thyroid disorders in four participants, one was taking medication affecting thyroid hormone levels, and one had a BMI exceeding 30 kg/m2. During the thyroid ultrasonographic study, 51 participants were excluded due to abnormal thyroid ultrasound (30 with enlarged thyroid glands/ inhomogeneous thyroid parenchyma and 21 with thyroid nodules, size > 1 cm). The presence of thyroid nodules (any size) observed from ultrasonographic screening was 23.5% (84/357) in all, 15.6% (27/173) in the adult group, and 31.0% (57/184) in the older group. Then, three hundred participants were enrolled to collect blood samples and spot urine samples. The eighty-four participants with positive titer for any thyroid autoantibodies were further excluded, and the final number of included participants as a reference population was 216 (112 adults and 104 older) (Figure 1).

Figure 1: Flow diagram of study enrolment.



* thyroid autoantibodies: anti-thyroglobulin, anti-thyroid peroxidase, and anti-TSHr antibodies

Baseline characteristics

The positive titer of thyroid autoantibodies was defined as a higher titer than the upper reference
limit by the manufacturer (anti-Tg positive titer >4.11 IU/mL, anti-TPO positive titer >5.61 IU/mL, anti-TSHr positive titer ≥1.75 IU/L). The prevalence of positive anti-Tg, anti-TPO, and anti-TSHr antibodies was 24.3% (73/300), 19.0% (57/300), and 5.7% (17/300), respectively. There was a 21.2% VS. 27.5% prevalence of positive anti-Tg (P = 0.202) in adult and older group, respectively. The prevalence of positive anti-TPO was significantly higher in adults than in the older group: 13.9 % VS. 24.2%, P = 0.027, respectively (Figure 2). The positive titer of anti-TSHr was more likely prevalent in the adult group (8.0%) than in the older group (3.3%), with a non-statistically significant P-value (P = 0.080). Moreover, the positive anti-Tg and anti-TPO were significantly higher in women than men, with a P-significant value (Figure 3).

Among 300 enrolled participants, one did not collect samples for urine iodine analysis. The prevalence of adequate iodine intake (urine iodine level 100 – 199 µg/L) was 62.2% (186/ 299), mild iodine deficiency (urine iodine level 50 – 99 µg/L) was 24.1% (72/299), moderate deficiency (urine iodine level 20 – 49 µg/L) was 12.7%. (38/299) and severe iodine deficiency (urine iodine level <20 µg/L) was found in 1.0% (3/299). Moreover, the above-requirement iodine status (urine iodine level 200 - 299 µg/L) was 19.7% (59/299), and the excessive iodine status (urine iodine level ≥300 µg/L) was 8.3% (25/299).

Among 216 reference populations, the median age of all participants, adult, and older groups was 58.5 years, 33.5 years, and 65.5 years old, respectively. Women were 52 % in all groups, 55% in the adult group, and 50% in the older group. The mean BMI was 23.2 kg/m^2 in all and significantly higher in the older group (24.0 kg/m^2) than in the adult group (22.5 kg/m^2) with P-value <0.001. All baseline characteristics of the participants in the adult and older groups are shown in Table 1. Each laboratory parameter's analytical methods, including a limit of detection, quantification, and performances, following the CLSI EP15-A3 guideline, are shown in Supplementary Table 1. The total imprecision coefficient variation for TSH, Free T3, Free T4, total T3, total T4, anti-Tg, anti-TPO, and anti-TSHr were 1.7%, 4.7%, 2.7%, 3.3%, 1.2%, 3.0%, 2.2% and 4.0% respectively. Only samples with negative for all thyroid autoantibodies were included in the analysis of RIs.

Figure 2: Prevalence of positive thyroid autoantibodies between the adult and older groups.

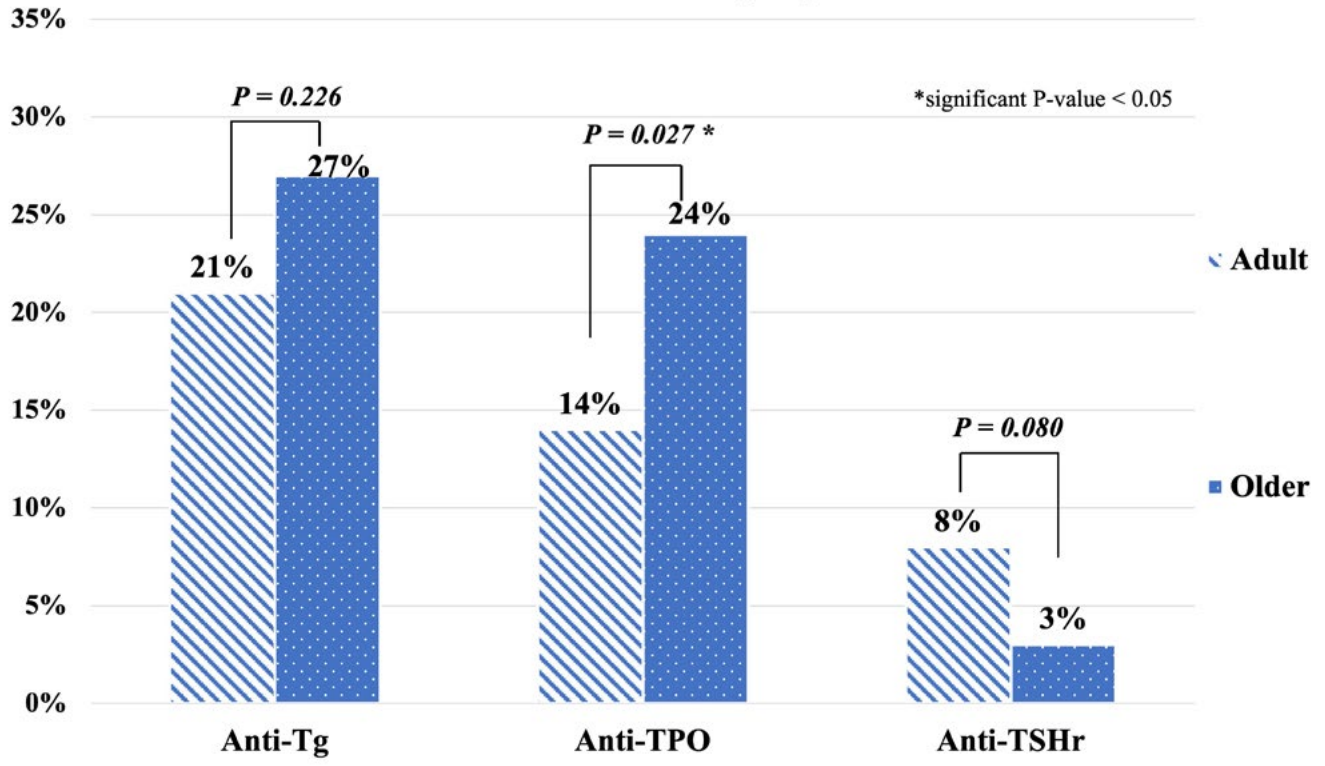


Figure 3: Prevalence of positive thyroid autoantibodies between gender.

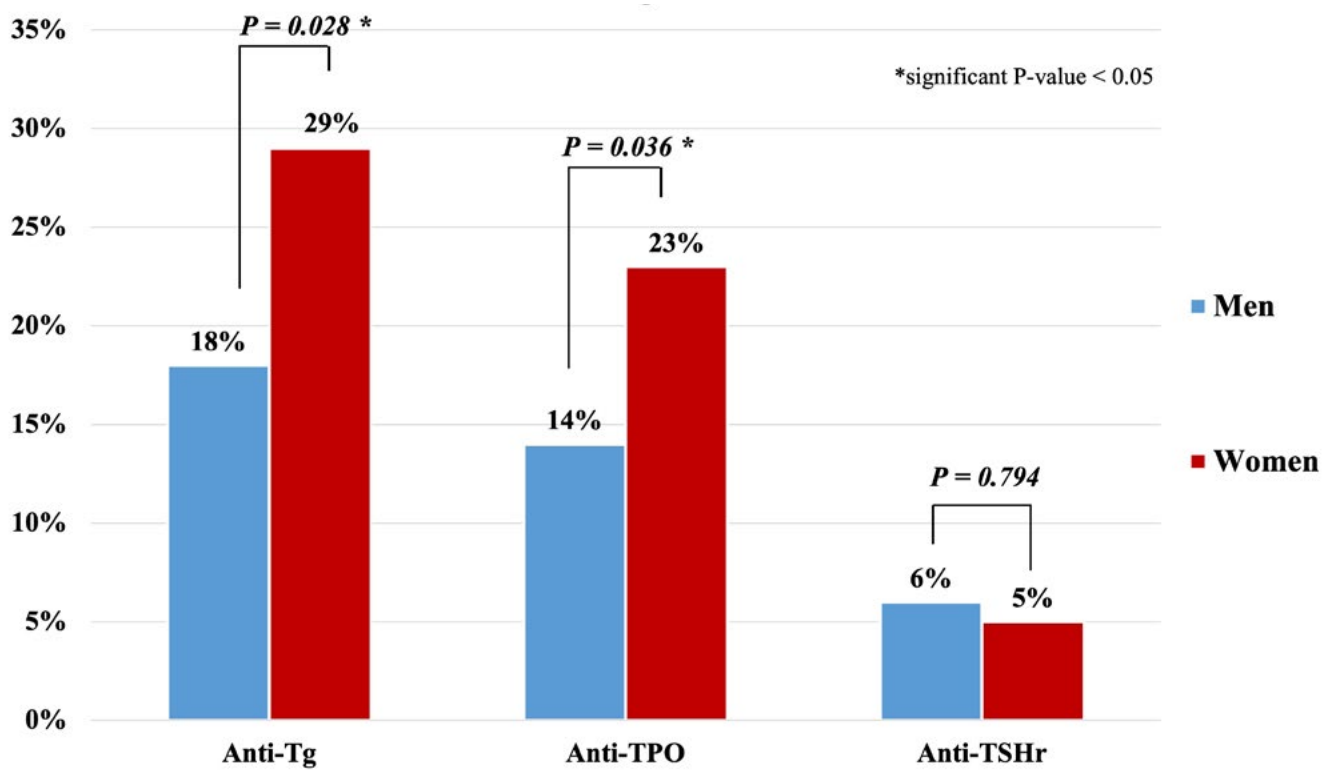


Table 1: Baseline characteristics of all included participants (N = 216).

Variables	All (N= 216)	Adults (N = 112)	Older (N = 104)	P-value
Age, year (median, IQR)	58.5 (33.0 - 65.0)	33.5 (27.0 - 47.5)	65.5 (63.5 - 68.0)	<0.001
Woman, n (%)	113 (52.3)	61 (54.5)	52 (50)	0.512
BMI, kg/m2 (mean ± SD)	23.2 ± 2.9	22.5 ± 2.8	24.0 ± 2.9	<0.001
Current smoker, n (%)	5 (2.4)	3 (2.7)	2 (1.9)	1.00
Family history of thyroid disorders, n (%)	20 (9.3)	16 (14.3)	4 (3.9)	0.006

Reference Intervals

The results of RIs of TSH in all subjects (n = 216) were 0.39 – 4.17 mIU/L, in the adult group (n = 112) were 0.35 – 3.98 mIU/L, and in the older group (n = 104) were 0.42 – 4.83 mIU/L. The upper reference limit of the TSH, free T4, total T4, free T3, and total T3 in all subjects and each group was lower than the manufacturers. Compared with the adult group, the RIs of TSH were higher, and the RIs of FT3 and TT3 were lower in the older group with statistically significant P-values. For FT4, TT4 and other thyroid autoantibodies (anti-Tg, anti-TPO, and anti-TSHr) were comparable between the adultand older groups. The RIs of TSH, free T4, total T4, free T3, total T3, and thyroid autoantibodies in each age group compared to the RIs from the manufacturer's package insert were illustrated in Table 2.

Table 2: Reference intervals of TSH, Free T4, Total T4, Free T3, Total T3, TgAb, TPOAb, and Anti-TSHr in all subjects, adult group, older group, and from the manufacturer's package insert.

Group	N	TSH (mIU/L)	Free T4 (ng/dL)	Total T4 (µg/dL)	Free T3 (pg/mL)	Total T3 (ng/dL)	TgAb (IU/mL)	TPOAb (IU/mL)	Anti-TSHr (IU/L)
All	216	0.39 - 4.17	0.75 - 1.09	4.46 - 9.38	2.10 - 3.27	59 – 115	0.35 - 3.13	0 - 2.28	0 - 1.67
Adult	112	0.35 - 3.98*	0.76 - 1.06	4.23 - 9.49	2.15 - 3.36*	60 – 118*	0.23 - 3.08	0 - 2.31	0 - 1.69*
Older	104	0.42 - 4.83	0.74 - 1.15	4.23 - 9.46	1.99 - 2.94	52 – 109	0.37 - 3.35	0 - 3.22	0 - 1.59
Manufacturer*	-	0.35 - 4.94	0.70 - 1.48	4.87 - 11.72	1.58 - 3.91	35 - 193	0 - 4.11	0 - 5.61	<1.75

*The reference interval with a statistically significant difference (P-value <0.05) between adult and older groups. (TSH: P = 0.025, Free T3: P = 0.013, Total T3: P = 0.041, antiTSHr: P<0.001)
*The reference interval is from the manufacturer's package insert. TSH, Free T4, Total T4, Free T3, Total T3, Anti-Tg, and Anti-TPO tests were performed with the Abbott Alinity I analyzer (Abbott Laboratories, Chicago, Illinois) using chemiluminescence immunoassay. The anti-TSHr test was performed with Roche Cobas e601 (Roche Diagnostics, Basel, Switzerland) using an electrochemiluminescence immunoassay.

Table 3: Reference intervals of TSH, Free T4, Free T3, Anti-Tg, Anti-TPO, and Anti-TSHr between genders.

Group	N	TSH (mIU/L)	Free T4 (ng/dL)	Total T4 (µg/dL)	Free T3 (pg/mL)	Total T3 (ng/dL)	Anti-Tg (IU/mL)	Anti-TPO (IU/mL)	Anti-TSHr (IU/L)
All	103				2.21 – 3.36*				
Men	113	0.35 – 3.72	0.76 – 1.12	3.95 – 9.04	2.00 – 2.87	57.32 – 117.07	0.27 – 3.21	0 – 3.46	0 – 1.66
Women		0.40 – 4.82	0.74 – 1.08	4.66 – 9.87		59.22 – 109.43	0.35 – 3.19	0 – 2.04	0 – 1.72
Adult	51								
Men	61	0.34 – 3.51	0.76 – 1.07	3.86 – 8.90	2.19 – 3.48*	59.48 – 121.80	0.14 – 3.07*	0 – 3.48	0 – 1.68*
Women		0.36 – 4.58	0.74 – 1.09	4.52 – 10.42	2.05 – 2.89	59.35 – 121.93	0.10 – 2.00	0.32 – 3.52	0 – 1.73
Older	52								
Men	52	0.30 – 4.57	0.73 – 1.17	3.80 – 9.31	1.90 – 3.16*	49.59 – 114.04	0.38 – 3.49	0 – 3.95	0 – 1.58
Women		0.70 – 6.08	0.73 – 1.12	3.77 – 9.69	1.98 – 2.87	50.93 – 104.68	0.35 – 3.68	0 – 4.02	0 – 1.67

*The reference interval with a statically significant difference (P-value <0.05) between men and women.

The RIs of TSH and thyroid hormones between genders were analyzed, and no statistically significant differences were observed, except for the FT3 values. The upper reference limit of the FT3 was higher in men than women in all participants, including adult and older groups, with statistically significant P-values. For RIs of thyroid autoantibodies between genders, the RIs of anti-Tg were statistically higher, and the RIs of anti-TSHr were significantly lower in adult men (Table 3).

Since one did not collect for urine iodine concentration test, thus 215 participants were categorized into 132 participants with adequate iodine intake (urine iodine ≥100 µg/L) and 83 participants with iodine deficiency (urine iodine <100 µg/L). The mean urine iodine concentration in an adequate iodine intake group was significantly higher than in the iodine deficiency group, 183.5 µg/L, and 50.8 µg/L, respectively, with P<0.001 (Table 4). The RIs of TSH, Free T4, and Free T3 in the adequate iodine intake group were slightly higher than those of the iodine deficiency group, with no statistical significance. The RIs of thyroid autoantibodies, including anti-Tg, anti-TPO, and anti-TSHr, between the adequate iodine status and iodine deficiency groups demonstrated similar results, as listed in Table 4.

Table 4: The median, 2.5th, and 97.5th percentile levels of thyrotropin, thyroxine, triiodothyronine, and thyroid autoantibodies according to iodine status.

Group	All (n = 215)[a]	Iodine deficiency (Urine iodine <100 µg/L, n = 83)	Adequate iodine intake (Urine iodine ≥100 µg/L, n = 132)	P-value[b]
UIC (µg/L)	129.7 (75 – 204.6)	59.8 (48.2 – 82.8)	183.5 (136.7 – 250.1)	<0.001
TSH (mIU/L)	1.15 (0.39 – 4.17)	1.23 (0.36 – 3.87)	1.08 (0.42 – 4.66)	0.918
FT4 (ng/dL)	0.92 (0.75 – 1.09)	0.92 (0.73 – 1.07)	0.93 (0.77 – 1.12)	0.493
FT3 (pg/mL)	2.61 (2.10 – 3.27)	2.60 (2.02 – 3.32)	2.63 (2.19 – 3.27)	0.665
Anti-Tg (IU/mL)	0.95 (0.35 – 3.13)	0.85 (0.31 – 3.05)	1.04 (0.35 – 3.23)	0.074
Anti-TPO (IU/mL	0.56 (0 – 2.28)	0.53 (0 – 3.39)	0.56 (0 – 2.16)	0.717
Anti-TSHr (IU/L)	0.98 (0 – 1.67)	1.0 (0 – 1.64)	0.95 (0 – 1.68)	0.750

UIC: urine iodine concentration, reported as median (IQR), the other data reported as median (2.5^{th} – 97.5^{th} percentile) value; one missing value of urine iodine level in a total of 216 participants; [b] P-value <0.05 as significant value.

Discussion

This study showed a comprehensive evaluation of establishing the reference intervals (RIs) of thyroid hormones in adults and the older. The method for recruitment of the reference population was extensive to enroll subjects with the most likely normal thyroid status, according to the National Academy of Clinical Biochemistry (NACB) guideline [12]. The study enrolment included history taking, physical examination, thyroid ultrasonographic study, serologic testing for thyroid autoantibodies, and spot urine iodine concentration examination. Our RI results of adults' and the older's TSH, thyroid hormones, and thyroid autoantibodies were narrower than those from the manufacturer's. The RI of TSH among the older group was higher and had a wider range than in the adult group, with a significant P-value (P-value = 0.025). The thyroxine hormones (FT4 and Total T4) were comparable between the adult and older groups, whereas the RIs of the FT3/Total T3 were lower in the older group with statistically significant P-value. The RIs of TSH and FT4 were similar between genders, but the FT3 level showed a statistically significant lower in women, corresponding to the previous results in the Thai population [6]. This study also demonstrated a comparable range of RIs of TSH, thyroid hormones, and thyroid autoantibodies between the adequate iodine intake group and the iodine deficiency group.

Several past studies, including a meta-analysis study [10], found variable TSH ranges that could be affected by several factors, including study population and analytical assay methods. The study population of disease-free individuals without exclusion for positive thyroid autoantibodies tended to have a higher TSH level [13]. The study, excluding unknown thyroid disease with either thyroid autoantibodies or thyroid ultrasound, resulted in a narrower range of TSH [13-16]. The RI studies reported by different analytical platforms from various ethnicities are listed in Table 5.

Table 5: Comparison of TSH and Free T4 reference intervals, reported by previous studies from various ethnicities and laboratory methods.

Study author Country, year	Thyroid-Ab testing[a]	Thyroid USG[b]	Sample size	TSH range (mIU/L)	Analyzer platform	Analytical method
This study Thailand, 2024	Yes	Yes	216[a]	0.39 – 4.17	Alinity I, Abbott	CLIA
Lu Y [14] China, 2023	Yes	Yes	1,114[a]	0.70 – 4.93	Architect, Abbott	CLIA
Hickman [15] Australia, 2017	Yes	No	1,177	0.43 – 3.28	Architect, Abbott	CLIA
Kim [13] Korea, 2015	No Yes	No Yes	18,043 7,686[a]	0.62 – 7.20 0.72 – 6.80	DiaSorin S.p.A.	CLIA
Kratzsch [16] Germany, 2005	Yes	Yes	453a	0.40 – 3.77	ELECSYS, Roche	ECLIA
Sriphrapradang [6] Thailand, 2014	Yes	No	2,545	0.34 – 5.11	Cobas e411, Roche	ECLIA

[a] study population with the exclusion of abnormal thyroid autoantibodies, [b] study population with the exclusion of abnormal thyroid ultrasound. ECLIA: electrochemiluminescence immunoassay, CLIA: chemiluminescence immunoassay.

Most thyroid parameters analyzed in our study showed no significant differences between genders, except for a statistically significant higher FT3 level in men than women. In China, Lu et al. identified higher TT3 in men than women, and higher TSH levels were observed in women [14]. The study of the Korean population clearly showed that women exhibited higher levels of TSH [13]. However, some studies indicated only minor differences in TSH levels between genders [4,17]. Additionally, higher levels of thyroid autoantibodies were found in women than in men, according to our study's results and some other studies [13]. Thus, gender-specific RIs will likely differ among various ethnic groups, highlighting the importance of developing tailored RIs that apply to specific subpopulations.

A trend of higher TSH levels with increasing age was observed in our study, which aligns with findings from previous reports [6-8, 18]. However, the rise in TSH levels among the older participants from our study was less pronounced than in an earlier study conducted on the Thai population. This discrepancy may be because most participants in the older group were aged 60 to 70, with only 17% (18 out of 104) being over 70. This limitation highlights that our older participants may only partially represent the broader population of older individuals.

Dietary iodine is necessary for thyroid hormone synthesis. It is absorbed and transported to the thyroid gland, where it undergoes oxidation and organification, ultimately contributing to the synthesis of T3 and T4. In most adults, TSH secretion is increased if iodine intake falls below 100 µg/day [19]. The thyroid gland has autoregulatory mechanisms to handle iodine intake involving the sodium iodide symporter. When dietary iodine is adequate, the thyroid typically absorbs less than 20% of it. However, this absorption rate may increase significantly, exceeding 80% during chronic iodine deficiency [19]. The compensation mechanism for low iodine levels is increasing the thyroid gland's size and activity to capture more iodide.

A spot urine specimen for measuring urine iodine concentration is a standard method for assessing iodine status in the population, according to WHO/UNICEF/ICCIDD [20]. This method is easy to collect and affordable. The limitation of this method is the falsely low iodine status if subjects had too little fluid ingestion [20]. In this study, we found the prevalence of adequate iodine intake in two-thirds (62.2%, 186/299), the prevalence of iodine deficiency was 37.8% (113/299), which was primarily mild to moderate iodine deficiency level, and only three individuals (1%) with severe iodine deficiency. However, among the adequate iodine intake group, almost half of them, 45.2% (84/186), were above the requirement of iodine intake.

The result of this study shows that the median levels of TSH are higher in individuals with iodine deficiency than those with adequate iodine intake group. However, the 2.5th and 97.5th percentile levels are higher in the group with adequate iodine intake, although these differences are not statistically significant. Our study's other thyroid parameters, including FT4, FT3, Anti-Tg, Anti-TPO, and Anti-TSHr antibodies, also demonstrated no significant difference among different iodine status groups. In contrast, the results from the Danish and Northeastern German populations with iodine deficiency found a significantly lower trend of TSH levels in the lower iodine status group [21,22]. The other study showed no difference in TSH and thyroid hormone levels among different iodine statuses, as shown in the results of this study [23]. The observed discrepancies may arise from differences in the reference populations and varying levels of iodine deficiency in each study. Moreover, the RIs provided by

manufacturers were primarily derived from healthy individuals in iodine-sufficient areas. Of note is that the manufacturers did not evaluate for iodine status among their reference populations. Therefore, established specific RIs for subpopulations in an iodine-deficient area should be considered.

The prevalence of positive titer of any thyroid autoantibodies from this study was 28% (84/300), which corresponded to the results from the previous report in the Thai population that found about 23.5% of positive titer of any thyroid autoantibodies [6]. In China, Zhai et al. reported a lower prevalence of anti-Tg and anti-TPO positive in 12.7% (1,889/14,985) and 11.5% (1,728/14,985), respectively [24]. In Caucasians, the report by the Rotterdam study in the Netherlands [4] showed a comparative prevalence to the result from Chinese, with an anti-TPO positive in 12.1% (1136/9402). By gender, the more prevalent positive anti-Tg and anti-TPO antibodies were observed in women of our study in both the adult and older groups. For anti-TSHr, the positive rate was found to be similar.

The limitation of our study is the relatively small number of reference population. The sample size in each age group did not reach the recommended number of 120, partly due to the unexpectedly high positive rates of thyroid autoantibodies in participants who had to be excluded. The RIs for thyroid function tests established in this study are derived from a specific single-center population and cannot be generalized to other populations. However, they serve as a reliable reference for Thai individuals at centers utilizing the same analytical methods.

Conclusion

This study provided established reference intervals for the full panel of thyroid testing, including TSH, Free T4, T4, Free T3, T3, anti-thyroglobulin (Anti-Tg), anti-thyroid peroxidase (Anti-TPO), and thyrotropin receptor antibody (anti-TSHr) in adult and older individuals among Thai. Compared to adults, the RIs in the older showed higher TSH levels, lower FT3/TT3 levels, and comparable levels of FT4/TT4. Lastly, there were no statistically significant differences among RIs of TSH and thyroid hormones between subjects with discrepant iodine status.

Conflict of interest

Abbott Laboratories provided most of the reagents for the thyroid hormone analysis in this study, except for the anti-TSHr and urine iodine concentration tests. However, the laboratory company was not involved in any study methods, protocols, analyses, or manuscript writing.

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Supplementary Table 1.

Parameter	Instruments	Method	Unit	Measuring Interval	Total imprecision		LOD	LOQ
					%CV (Conc)	%CV (Conc)		
TSH	Alinity I (Abbott)	CMIA	mIU/L	0.0083 - 100	1.7% (0.642)	2.7% (24.739)	0.0036	0.0083
Free T3	Alinity I (Abbott)	CMIA	pg/mL	1.5 - 20	4.7% (2.02)	2.1% (10.01)	0.95	1.25
Free T4	Alinity I (Abbott)	CMIA	ng/dL	0.42 – 5.00	2.7% (0.93)	3.8% (2.99)	0.28	0.42
Total T3	Alinity I (Abbott)	CMIA	ng/dL	40.0 – 600.0	3.3% (72.64)	2.9% (257.42)	5.0	30.0
Total T4	Alinity I (Abbott)	CMIA	µg/dL	3.00 – 24.00	1.2% (6.93)	2.6% (13.89)	0.55	2.17
Anti-TG	Alinity I (Abbott)	CMIA	IU/mL	3.00 – 1000.00	3.0% (11.8)	4.4% (45.87)	0.11	0.33
Anti-TPO	Alinity I (Abbott)	CMIA	IU/mL	3.00 – 1000.00	2.2% (15.40)	2.0% (54.57)	0.03	0.21
Anti-TSHr	Cobas e601 (Roche)	ECLIA	IU/L	0.8 – 40.0	4.0% (4.42)	2.2% (18.1)	0.80	1.10

LOD: Limit of detection, LOQ: Limit of quantification, CMIA: Chemiluminescent microparticle immunoassay, ECLIA: Electrochemiluminescence immunoassay method

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