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Original Article

Biochemistry Section

Establishment of Reference Interval of Thyroid Hormones and Autoantibodies: A Cross-sectional Study

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ABSTRACT

Introduction: The accurate diagnosis of thyroid disease relies on the sensitivity and specificity of laboratory tests, which require specific reference intervals. Thyroid hormone levels can be influenced by factors such as age, gender, iodine intake and geographical region. Therefore, it is recommended for clinical laboratories to establish their own reference intervals.

Aim: To establish reference intervals for Thyroid Stimulating Hormone (TSH), Free Thyroxine (FT4), Anti-thyroglobulin antibody (anti-TG), and Anti-Thyroid Peroxidase Antibody (anti-TPO) in males and and females.

Materials and Methods: A cross-sectional study was conducted in the Department of Biochemistry, Amrita Institute of Medical Sciences (AIMS), Kochi, India, from December 2021 to July 2022. The study included 217 apparently healthy subjects aged between 18 years and 60 years. TSH, FT4, anti-TPO, and anti-TG levels were analysed to establish reference values for males and females in the two age groups: 18-40 years and 41-60 years. Reference intervals for thyroid parameters were expressed as mean±Standard Deviation (SD), median, 2.5th and 97.5th percentiles. The Mann-Whitney U test was used to assess the statistical significance of thyroid parameters between gender and age groups.

Results: The mean age in the female population was 38.24±11.64 years, while in the male population, it was 39.24±10.98 years. The reference intervals and cut-offs for TSH, FT4, anti-TG and anti-TPO in females were determined as 0.80-4.12 µIU/mL, 0.99-1.57 ngm/dL, <85.33 IU/mL and <45.13 IU/mL, respectively. In males, the reference intervals and cut-offs for TSH, FT4, anti-TG and anti-TPO were determined as 0.51-5.09 µIU/mL, 1.03-1.79 ng/dL, <87.09 IU/mL and <33.88 IU/mL, respectively. There was a significant difference in anti-TPO (p-value=0.017) in males between the two age groups. A significant difference was also observed in FT4 (p-value=0.010) and anti-TPO (p-value=0.034) between males and females.

Conclusion: The study successfully established reference intervals and cut-off values for TSH, FT4, anti-TG, and anti-TPO. It was found that anti-TPO levels decrease significantly with age progression. Additionally, the reference interval for TSH was wider in the 41-60 years age group compared to the younger age group.

Keywords: Anti-thyroglobulin, Anti-thyroid peroxidase, Free thyroxine, Subclinical thyroid disorders, Thyroid stimulating hormone

INTRODUCTION

Thyroid hormones play a vital role in maintaining various bodily functions, including the development of the nervous system, normal body growth, gastrointestinal tract mobility, regulation of muscle strength, gluconeogenesis, lipolysis, protein synthesis, protein degradation, oxygen consumption, breathing rate, heart rate, and body temperature. These hormones also impact the state of consciousness [1-4].

The thyroid gland produces two major hormones: Triiodothyronine (T3) and Thyroxine (T4). The production of thyroid hormones is regulated by TSH from the anterior pituitary gland. TSH secretion is induced by Thyrotropin Releasing Hormone (TRH) from the hypothalamus. Negative feedback of thyroid hormones regulates the circulating levels of T3 and T4. When hormone levels are excessively high, they inhibit the production of TSH and TRH, thereby regulating their own production. Dysfunctions in thyroid hormone levels can lead to various disorders [1].

Common thyroid disorders include hypothyroidism, hyperthyroidism, and subclinical thyroiditis. Symptoms of these disorders are often vague and non specific, and they are more commonly observed in elderly individuals [5,6]. Studies have shown that elderly people have higher TSH levels, with a prevalence of hypothyroidism of about 12-18%, often coexisting with other conditions [2]. Early diagnosis of thyroid disorders is crucial for effective treatment. TSH, which regulates T4 and T3, and T4, the primary hormone secreted by the thyroid gland, are the first-line investigations for diagnosing thyroid disorders. T3 is not routinely used as it is found in circulation due to the peripheral conversion of T4 to T3 [1].

Accurate diagnosis of thyroid disorders relies on sensitive and specific laboratory tests. However, there is a scarcity of studies providing standard reference intervals for thyroid parameters based on the current iodine status of the population. Currently, laboratories often rely on reference intervals provided by reagent manufacturers. The International Federation of Clinical Chemistry (IFCC) and the Clinical Laboratory Standards Institute (CLSI) recommends that laboratories establish to their own reference interval as concentration of thyroid hormones is influenced by factors such as age, gender, iodine intake and geographical region [7-10].

Therefore, the present study was aimed to establish reference intervals for thyroid hormones (TSH and FT4) and thyroid autoantibodies (anti-TPO and anti-TG) in males and females within two age groups: 18-40 years and 41-60 years.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Biochemistry, Amrita Institute of Medical Sciences and Research Centre (AIMS), Kochi, India, from December 2021 to July 2022. Approval was obtained from the Institutional Ethics Committee, AIMS (ECASM-AIMS-2022-086), and written informed consent was obtained from all participants.

Inclusion criteria: Apparently healthy euthyroid subjects aged between 18-60 years, irrespective of gender, were included in the study.

Exclusion criteria: Subjects with known endocrinological disorders, history of malignancy, family history (only 1st-degree relatives) of thyroid illness, acute or chronic autoimmune disorders, acute or chronic infections, renal disorders, smokers, alcoholics, pregnant or lactating females, subjects under medication, heart diseases, and diabetes mellitus were excluded from the study. Patients with positive anti-TPO and anti-TG results were also excluded to establish reference intervals for thyroid parameters.

Sample size calculation: Samples were collected from healthy euthyroid donors who visited the Department of Transfusion Medicine during the study period. A total of 252 samples were collected, of which 242 samples met the inclusion and exclusion criteria. These samples were analysed for TSH, FT4, anti-TPO, and anti-TG. Samples with abnormal anti-TPO and anti-TG results were excluded from the study [7,8]. Therefore, a total of 217 participants were included in the further analysis.

Study Procedure

Approximately 5 mL of venous blood samples were collected between 8 am and 10 am under aseptic precautions and transported to the clinical biochemistry laboratory without delay. The samples were stored at -20ºC in the Clinical Biochemistry Laboratory. TSH, FT4, anti-TPO, and anti-TG levels were analysed using the Roche Cobas 8000 Auto Analyser with the Electrochemiluminescence (ECLIA) method after performing quality checks.

The subjects were randomly grouped into two age groups [9]:

Group I: 18 years to 40 years

Group II: 41 years to 60 years

The reference intervals recommended by the manufacturers of the reagent assay kit were as follows:

TSH: 0.27-4.2 µIU/L

FT4: 1.0-1.7 ng/dL (males), 1.0-1.6 ng/dL (females)

Anti-TG: <115.0 IU/mL

Anti-TPO: <34.0 IU/mL

In the present study, the reference intervals for TSH, FT4, anti-TPO, and anti-TG are presented as the 2.5th to 97.5th percentile [7,8].

STATISTICAL ANALYSIS

The data was analysed using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) software version 20.0 (SPSS, Inc, Chicago, USA). The reference intervals for thyroid parameters according to gender and age groups were expressed as mean±SD, median, 2.5th percentile and 97.5th percentile. The Mann-Whitney U test was used to assess the statistical significance of the differences in median values of thyroid parameters between gender and age groups. A p-value of <0.05 was considered statistically significant, and the reference intervals were calculated accordingly.

RESULTS

A total of 217 samples, including 111 males and 106 females, were analysed for TSH, FT4, anti-TPO, and anti-TG in the age group of 18-60 years. The subjects were further divided into two groups based on age (group I: 18-40 years, group II: 41-60 years). The mean age in female subjects was 38.24±11.64 years, while in males it was 39.24±10.98 years.

In females, the mean TSH level was 2.23±0.93 with a reference interval of 0.80-4.12 µIU/mL (2.5th percentile-97.5th percentile) and a median of 2.01 µIU/mL. The mean FT4 level was 1.28±0.16 ng/dL with a median of 1.28 ng/dL. The reference intervals for anti-TG and anti-TPO were 21.03-85.33 IU/mL and 5.31-45.13 IU/mL (2.5th percentile-97.5th percentile), respectively, with medians of 31.08 IU/mL and 9.17 IU/mL, respectively.

In males, the mean TSH level with a mean±SD of 2.11±0.95 µIU/mL with a reference interval was estimated as 0.51-5.09 µIU/mL (2.5th percentile-97.5th percentile) and a median of 1.89 µIU/mL. The mean FT4 level was 1.34±0.18 ng/dL with a median of 1.89 µIU/mL. FT4 had a mean value of 1 .34±0.1 8 and median were 1.35 ng/dL. The 2.5th percentile and 97.5th percentile was 1.03 and 1.79 ng/dL, respectively. The anti-TG and anti-TPO were estimated as 21.48-87.09 IU/mL and 5.41-33.88 IU/mL with a median of 30.48 IU/mL and 10.09 IU/mL (2.5th percentile and 97.5th percentile), respectively [Table/Fig-1].

Parameters	Mean±SD	Median	2.5th-97.5th percentiles
Females (n=106)			
TSH (µIU/mL)	2.23±0.93	2.01	0.80-4.12
FT4 (ng/dL)	1.28±0.16	1.28	0.99-1.57
Anti-TG (IU/mL)	36.06±15.08	31.08	21.03-85.33
Anti-TPO (IU/mL)	14.61±22.96	9.17	5.31-45.13
Males (n=111)			
TSH (µIU/mL)	2.11±0.95	1.89	0.51-5.09
FT4 (ng/dL)	1.34±0.18	1.35	1.03-1.79
Anti-TG (IU/mL)	35.23±15.20	30.48	21.48-87.09
Anti-TPO (IU/mL)	12.14±7.04	10.09	5.41-33.88

[Table/Fig-1]: Reference interval of thyroid parameters (TSH, FT4, anti-TG, anti-TPO) with respect to gender.

In the 18-40 years age group, the mean TSH level was 2.20±0.89 µIU/mL with a reference interval of 0.69-4.39 µIU/mL (2.5th percentile-97.5th percentile) and a median of 2.09 µIU/mL. The mean FT4 level was 1.35±0.19 ng/dL with a median of 1.32 ng/dL. The reference intervals for anti-TG and anti-TPO were 21.46-84.05 IU/mL and 5.57-44.57 IU/mL (2.5th percentile-97.5th percentile), respectively, with medians of 31.15 IU/mL and 9.26 IU/mL, respectively.

In the 41-60 years group, TSH level with a mean of 2.15±1.02 reference interval was estimated as 0.65-4.99 µIU/mL (2.5th percentile-97.5th percentile) with a median of 1.88 µIU/mL FT4 had a mean of 1.30±0.16 and median were 1.30 ng/dL the 2.5th percentile and 97.5th percentile was 1.00 and 1.59 ng/dL, respectively. The anti-TG and anti-TPO were estimated as 20.71-85.41 IU/mL and 5.41-32.36 IU/mL (2.5th percentile and 97.5th percentile) with a median of 30.36 IU/mL and 10.11 IU/mL, respectively [Table/Fig-2].

Age groups	Mean±SD	Median	2.5th-97.5th percentiles
18-40 years (group I)			
TSH (µIU/mL)	2.20±0.89	2.09	0.69-4.39
FT4 (ng/dL)	1.35±0.19	1.32	1.00-1.85
Anti-TG (IU/mL)	34.54±13.48	31.15	21.46-84.05
Anti-TPO (IU/mL)	11.72±8.25	9.26	5.57-44.57
41-60 Years (group II)			
TSH (µIU/mL)	2.15±1.02	1.88	0.65-4.99
FT4 (ng/dL)	1.30±0.16	1.30	1.00-1.59
Anti-TG (IU/mL)	36.05±15.80	30.36	20.71-85.41
Anti-TPO (IU/mL)	12.35±7.01	10.11	5.41-32.36

[Table/Fig-2]: Reference interval of thyroid parameters (TSH, FT4, anti-TG, anti-TPO) with respect to age 18-40 years (n=112) and 41-60 years (n=105).

On age group comparison among females, thyroid parameters such as TSH, FT4, anti-TPO, anti-TG were found to be not statistically significant [Table/Fig-3]. In males, on comparison of thyroid parameters, anti-TPO showed a statistically significant difference with a p-value of 0.017. The comparison of other thyroid parameters, TSH, FT4 and anti-TG were not statistically significant between both the age groups among males [Table/Fig-4]. On comparison of thyroid parameters in age group 18-40 years between males and females, TSH, anti-TPO and anti-TG was not statistically significant. The comparison of FT4 showed statistically significant difference with a p-value of 0.010 [Table/Fig-5]. On comparison of

thyroid parameters in age group 41-60 years between males and females, TSH, FT4 and anti-TG were not statistically significant. The comparison of anti-TPO showed statistically significant difference with a p-value of 0.034 [Table/Fig-6].

Parameters	Age group		p-value
	18-40 years Median (*Q1-Q3)	41-60 years Median (*Q1-Q3)	
TSH	2.22 (1.61-2.82)	1.90 (1.47-3.08)	0.368
FT4	1.28 (1.18-1.42)	1.20 (1.10-1.39)	0.733
Anti-TG	31.27 (27-36.93)	30.71 (25.96-44.97)	0.930
Anti-TPO	9.30 (7.44-13.77)	8.98 (7.79-13.16)	0.798

[Table/Fig-3]: Comparison of thyroid parameters between the age group in females.
Mann-Whitney U test (*Lower quartile, Upper quartile)

Parameters	Age group		p-value
	18-40 years Median (*Q1-Q3)	41-60 years Median (*Q1-Q3)	
TSH	2.00 (1.56-2.61)	1.81 (1.49-2.50)	0.585
FT4	1.35 (1.23-1.46)	1.33 (1.18-1.44)	0.185
Anti-TG	30.58 (26.93-34.34)	30.02 (25.96-39.81)	0.960
Anti-TPO	9.13 (8.12-10.62)	10.65 (8.87-15.07)	**0.017**

[Table/Fig-4]: Comparison of thyroid parameters between the age groups in males.
Mann-Whitney U test (*Lower quartile, Upper quartile)

Parameters	Gender		p-value
	Males Median (*Q10-Q3)	Females Median (*Q1-Q3)	
TSH	2.00 (1.57-2.62)	2.20 (1.58-2.70)	0.600
FT4	1.35 (1.24-1.46)	1.27 (1.75-1.41)	**0.010**
Anti-TG	30.58 (26.73-33.66)	31.27 (27-35.81)	0.430
Anti-TPO	9.06 (8.01-10.66)	9.37 (7.51-13.77)	0.639

[Table/Fig-5]: Comparison of thyroid parameters between gender in the age group 18-40 years.
Mann-Whitney U test (*Lower quartile, Upper quartile); The p-value in bold font indicates statistically significant values

Parameters	Age group		p-value
	Males Median (Q1-Q3)	Females Median (Q1-Q3)	
TSH	1.81 (1.49-2.50)	1.88 (1.46-2.95)	0.756
FT4	1.33 (1.18-1.44)	1.29 (1.18-1.40)	0.308
Anti-TG	30.02 (25.96-39.81)	30.71 (25.96-44.80)	0.775
Anti-TPO	10.65 (8.87-15.07)	8.90 (7.74-12.72)	**0.034**

[Table/Fig-6]: Comparison of thyroid parameter between gender in the age group 41-60 years.
Mann-Whitney U test (*Lower quartile, Upper quartile)

DISCUSSION

The purpose of the present study was to establish reference intervals for thyroid parameters (TSH, FT4, anti-TPO, and anti-TG) in females and males of two different age groups. The subjects were further divided into two age grouped based on the age. Existing literature on the topic points out that people above 60 years of age showed several biases in the concentration of thyroid parameters and these were found to influence the study results [9,11]. Hence, individuals between the age group of 18 years and 60 years included in the study. The mean age for females was 38.24±11.64 years and for males was 39.24±10.98 years.

Most existing studies have not established reference interval for thyroid parameters separately in males and females [8,12]. Therefore, in the present study, the authors categorised samples and analysed thyroid parameters (TSH, FT4, anti-TG, anti-TPO) in males and females.

The reference range for TSH in males in the present study was 0.51-5.09 µIU/mL and for females was 0.80-4.12 µIU/mL. Study by Kutluturk F et al., showed that the 97.5th percentile of TSH (4.12 µIU/mL) was similar to 97.5th percentile of TSH in females of our study [13]. A study by Sriphrapradang C et al., estimated 97.5th percentile as 5.11 µIU/mL which was similar to that of males in the present study (5.09 µIU/mL) [8]. However, in the present study, the age group reference interval for 18-40 years (0.69-4.39 µIU/mL) and the 41-60 years (0.65-4.99 µIU/mL) were different from previous study [9,13]. The difference could be because of the larger sample size in their study (N=870) when compared to the present study, which included 224 study subjects [13]. In this study the reference values for TSH was found to be wider in males when compared to females i.e., the 2.5th percentile was lower 97.5th percentile was higher in males than females. The reference interval between the age groups was close to each other in this study. However, the comparison of TSH between gender and age groups showed no statistically significant difference.

In the present study, FT4 had a narrow range of reference interval in females (0.99-1.57 ng/dL), and males (1.03-1.79 ng/dL) and also between age groups, however, in a study by Abbas R et al., the reference interval of FT4 was wider (0.92-1.75 ng/dL) [4]. In group I (18-40 years) it was 1.00-1.85 and in group II it was 1.00-1.59. The 97.5th percentile of FT4 in a study by Sriphrapradang C et al., was similar to that of males in the present study (1.79 ng/dL) but 2.5th percentile in their study was higher for males, as compared to the present study [8].

The parameters, anti-TPO and anti-TG were analysed in females and males and positive samples were excluded for calculating the reference range for TSH and FT4. The TSH values were normal in majority of the subjects. Similar findings were observed by Mariotti S et al., that thyroid parameters were preserved until the eighth decade of life in healthy subjects without changes in the TSH values [14]. In a study by Bjoro T et al., the anti-TPO values were more in males and females aged over 40 without any history of thyroid diseases [15].

The cut-off values for anti-TPO and anti-TG in a study by Mirjanic-Azaric B et al., showed that the cut-off for anti-TPO and anti-TG <18.02 mIU/mL and <98.00 IU/mL, respectively [12]. Similar result for anti-TG was seen in the study by Pandav CS et al., study [16]. Also, in the present study, the cut-off for anti-TPO was 80.65 IU/mL. In the current study, the cut-off for anti-TG in females ((21.03-84.91 IU/mL) was similar to that in group I (21.46-84.05 IU/mL). The cut-off for anti-TPO was lower in the present study (anti-TPO in group II was <32.36 IU/mL and in group I was <44.57 IU/mL) than in other studies [12,16-18]. This could be an indication of the changes in the thyroid parameters as the age progresses. The observed differences in the level of anti-TPO across the studies may be attributed to the difference in the assay techniques, reagent kits and the instruments used for analysis [19].

On comparison of thyroid parameters between the age groups in males, there was a statistically significant difference for anti-TPO with a p-value of 0.017. Additionally, the comparison of anti-TPO between males and females in the 41-60 years age group was also statistically significant with a p-value of 0.034. However, anti-TG did not show statistical significance in either comparison. These findings are consistent with previous studies [20-22].

The reference intervals for TSH and FT4 in the present study were similar to the intervals recommended by the manufacturers of the reagent assay kit. The manufacturer's reference intervals for TSH were 0.27-4.2 µIU/L, and for FT4, it was 1.0-1.7 ng/dL in males and 1.0-1.6 ng/dL in females. In the current study, the reference intervals for TSH were 0.80-4.12 µIU/mL in males and 0.51-5.092 µIU/mL in females. The reference intervals for FT4 in group I were 0.69-4.39 µIU/mL and 1.00-1.85 ng/dL, respectively. In group II, the intervals were 0.65-4.99 µIU/mL and 1.00-1.59 ng/dL for TSH and FT4, respectively.

Regarding the cut-off values for autoantibodies, the present study observed lower cut-offs than those provided by the manufacturer. The manufacturer's cut-offs for anti-TG were <115.0 IU/mL and for anti-TPO were <34.0 IU/mL. In the present study, the cut-offs for anti-TG were <85.33 IU/mL for females and <87.09 IU/mL for males. The cut-offs for anti-TPO were <45.13 IU/mL for females and <33.88 IU/mL for males. In group-I, the cut-offs for anti-TPO and anti-TG were <44.57 IU/mL and <84.05 IU/mL, respectively. In group II, the cut-offs were <32.36 IU/mL for anti-TPO and <85.41 IU/mL for anti-TG.

Overall, the findings in the present study for TSH and FT4 were similar to or contrasted with previous literature. This discrepancy could be attributed to the use of different assays in different populations.

Limitation(s)

Limitations of the present study were that, it only included subjects attending the tertiary care centre and small sample size.

CONCLUSION(S)

In the present study, reference intervals and cut-off values for TSH, FT4, anti-TG, and anti-TPO were established. Age was found to have a significant effect on the levels of anti-TPO, with decreasing levels observed as age progresses. The reference interval for TSH was also found to widen with age. Based on the findings, the authors conclude that each laboratory should establish population-specific and method-specific reference intervals to effectively diagnose and treat thyroid disorders, ranging from subclinical to overt thyroid diseases. Large population studies are recommended to establish reference intervals for thyroid parameters. Furthermore, the authors suggest including physical examinations of subjects and ultrasonography of the thyroid gland to avoid missing thyroid nodules or small goitre which could not be performed in the present study.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Apr 04, 2023
- Manual Googling: Jun 15, 2023
- iThenticate Software: Oct 02, 2023 (11%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

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