

Thyroid hormone reference intervals and the prevalence of thyroid antibodies

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Aim: To establish new reference intervals for thyrotropin (TSH), free thyroxine (FT4), and free triiodothyronine (FT3) on the Beckman Coulter DxI 800 in healthy Turkish adults and assess the prevalence of laboratory evidence of thyroid antibodies in this population.

Materials and methods: Specimens were obtained from 619 healthy volunteers (338 females, 281 males). The study was conducted with health care professionals and their relatives, The mean age of all the subjects was 34.7 and the median was 31 years, with ages ranging from 15 to 84. Moreover, the subjects had no evidence of thyroid autoimmunity or a history of drug use, both of which might interfere with thyroid function.

Results: After exclusion of samples positive for thyroid antibodies (Antithyropoxidase; TPOAb, Antithyroglobulin; TgAb), the final sample size for the evaluation of TSH, FT3, and FT4 reference ranges was 509 (254 females, 255 males) and the age range was 15-80 (mean 34.1, median 30). The prevalence of antithyroid antibodies was 17.8% in total and positive thyroid antibodies were more common among females (13.6%) than males (4.2%). We established the reference intervals for the healthy adults as 0.41-4.25 mIU/L for TSH, 0.61-1.06 ng/dL (7.85-13.64 pmol/L) for FT4, and 2.62-3.84 pg/mL (4.02-5.90 pmol/L) for FT3.

Conclusion: The reference intervals of thyroid hormones vary considerably from those of other populations and the intervals recommended by the manufacturer. This underlines the need for population-specific reference ranges.

Key words: Reference intervals, thyroid antibodies, thyrotropin, free thyroxine, free triiodothyronine

Tiroid hormon referans aralıkları ve tiroid antikor prevalansı

Amaç: Bu çalışmanın amacı sağlıklı Türk yetişkinlerinde Beckman Coulter DxI 800 analizöründe tirotropin (TSH), serbest tiroksin (FT4) ve serbest triiodotironin (FT3) için yeni referans aralıklarını ve bu popülasyondaki tiroid antikorlarının prevalansını belirlemektir.

Yöntem ve gereç: Örnekler tiroid fonksiyonlarını etkileyebilecek ilaç kullanımı, tiroid otoimmunitesi hikayesi bulunmayan, yaşları 15-84 (ortalama 34,7, medyan 31) arası olan sağlıklı 619 (338 kadın, 281 erkek) gönüllü sağlık çalışanı ve yakınlarından elde edildi.

Bulgular: Tiroid antikorları (Antitiroperoksidaz; TPOAb, Antitiroglobulin; TgAb) pozitif olan örnekler çalışma dışı bırakıldıktan sonra, TSH, FT3, FT4 referans aralıkları yaşları 15-80 (ortalama 34,1, medyan 30) arasında olan 509 (254 kadın, 255 erkek) örnekle değerlendirildi. Antitiroid antikorların varlığı kadınlarda (% 13,6) erkeklerden (% 4,2) daha yüksek olmak üzere toplamda % 17,8 olarak tespit edildi. Sağlıklı yetişkinlerde yeni referans aralıkları TSH için 0,41-4,25 mIU/L, FT4 için 0,61-1,06 ng/dL (7,85-13,64 pmol/L) ve FT3 için 2,62-3,84 pg/mL (4,02-5,90 pmol/L) olarak bulundu.

Sonuç: Bu çalışmada, tiroid hormonlarının referans aralıkları bir popülasyondan diğerine göre ve üretici firmanın önerdiği değerlere göre farklılık göstermiştir. Bu da popülasyona özgü referans aralıklarının belirlenmesinin gerekli olduğunun önemini belirtir.

Anahtar sözcükler: Referans aralıkları, tiroid antikorları, tirotropin, serbest tiroksin, serbest triiodotironin

Received: 25.06.2009 – Accepted: 19.02.2010

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Introduction

Reference ranges vary considerably from one laboratory to another and are dependent on the population, diet, methodology, and selection of reference group or needs of the clinics (1). For the determination of reference ranges, the most important stage is the selection of the reference group and the standardization of preanalytical factors (2,3). The Clinical Laboratory Standards Institute (CLSI, formerly known as NCCLS) recommends non-parametric methods, while the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommends both non-parametric methods and parametric methods for the determination of reference ranges (1,2,4).

Biochemical examination of the thyroid function by using the measurement of thyrotropin (TSH) and thyroid hormones (free thyroxine (FT4) and free triiodothyronine (FT3)) in serum is an everyday task in most clinical biochemical laboratories. Thyroid hormone levels are known to be influenced by a variety of factors, including diet, genetics, and the presence of thyroid autoimmunity. Reference intervals provided by assay manufacturers are representative data and may not always be appropriate for different locations and patient demographics.

According to the results from the National Health and Nutrition Examination Survey (NHANES) studies on regular thyroid function (5), the National Academy of Clinical Biochemistry (NACB) proposed that when establishing new TSH reference intervals only euthyroid healthy volunteers be included, who should be free from detectable autoantibodies against thyroid peroxidase (TPOAb) or thyroglobulin (TgAb) and any personal or family history of thyroid dysfunction (6).

The aims of the present study were: 1) to establish thyroid hormone reference intervals in healthy Turkish adults without evidence of thyroid autoimmunity on the DxI 800 instrument, and 2) to assess the prevalence of laboratory evidence of thyroid antibodies in this population.

Materials and methods

Subjects

According to the CLSI C28-A standard, we prepared a questionnaire for determining exclusion criteria and preanalytic factors (7). Specimens were obtained from healthy volunteers, all of whom were healthcare professionals and their relatives. The subjects were adults with no visible goiter, no personal or family history of thyroid dysfunction, and on no medication that might interfere with thyroid function. Furthermore, their fasting blood glucose, urea, creatinine, AST, ALT, total cholesterol triglyceride, HDL and LDL cholesterol, complete blood count, and sedimentation levels were normal. The study was approved by the local ethical committee.

Assay methods

Samples were collected at 0900-1000 after an overnight fast into vacutainer tubes with gel. Centrifugation was performed within 1 h at 1500 × g for 10 min and samples were analyzed immediately.

Analyses of TSH, FT3, FT4, TPOAb, and TgAb were performed on an automated immunoassay system (DxI 800, Beckman Coulter) using a direct chemiluminescence detection system according to the manufacturer's instructions. The TSH assay is a 2 site immunometric (sandwich) assay whereas the FT3 and FT4 assays are competitive assays with FT4 being measured in 2-step free-analyte assay format and FT3 in an analog-type free analyte assay format.

The measurements were made using the same reagents and the same instruments for all patients. TPOAb and TgAb levels above the 9 and 4 IU/mL respectively were regarded as positive according to the manufacturer's data.

Assay imprecision was assessed by the use of commercial quality-control materials, Liquicheck levels 1-3 (Bio-Rad) and Mass Liquimmune levels 1-3 (Microgenics). Each control material was analyzed in duplicate per run. Daily runs were performed 5 days a week over 3 weeks for a total of 30 replicates for each control.

Statistical analysis

Reference values were determined in accordance with the protocols published by the Expert Panel on the Theory of Reference Values of the IFCC and the CLSI (2,7,8).

TSH, FT3, and FT4 distributions were calculated by using Kolmogorov-Smirnov tests. Reference intervals as well as reference limits were estimated via percentiles of distributions if distribution (or its transformation, e.g. via log-function) was not normally distributed. In that case, lower and upper 2.5th and 97.5th percentiles of distribution were used to estimate reference limits. If distributions (or its transformation, e.g. via log-function) did not deviate from normal distribution, normal distribution (position of cumulative probabilities) was used to calculate reference limits. For this analysis, both approaches were used depending on the properties of distribution. The Dixon and Block procedures were used to identify outliers (9,10). Confidence intervals were calculated according to binomial distribution (11).

Gender dependency of thyroid hormone concentrations was confirmed by the Mann-Whitney U test. P values < 0.05 were defined as statistically significant. Gender specific reference limits and confidence intervals were examined. The relationship between the thyroid hormones and TPOAb and TgAb were examined by correlation analysis. All calculations were performed with MedCalc 9 software.

Results

In total, 619 specimens were included; the age range was between 15 and 84 (mean 34.7, median 31): 338 female, 281 male. Of these 110 specimens (17.8%), 84 females and 26 males (age range 18-84; mean 37.4, median 35) were excluded from the study because TPOAb and/or TgAb values were positive.

Elevated TgAb and TPOAb frequencies according to the gender are shown in Table 1 and distributions according to age are shown in Figure 1.

There was no association between TSH and a positive TgAb alone; however, a significant correlation was found between TPOAbs and TSH concentrations (r: 0.394, P: 0.000).

Figure 2 shows the prevalence of thyroid antibodies across TSH intervals. The prevalence of the lowest thyroid antibodies (<20.7%) was seen in a TSH range between 0.42 and 3.50 mIU/L. The prevalence of thyroid antibodies progressively increased with TSH above 2.00 mIU/L, approaching 80% when TSH was above 5 mIU/L.

The final sample size for the evaluation of FT3 and FT4 was 509 and the age range was 15-80 (mean 34.1, median 30); 254 females, 255 males.

For TSH 4 outliers (above the 4.95 mIU/L) were removed by the Block procedure and the study continued with 505 specimens. Neither original values nor log-transformed values were normally distributed (Kolmogorov-Smirnov test: P < 0.0001).

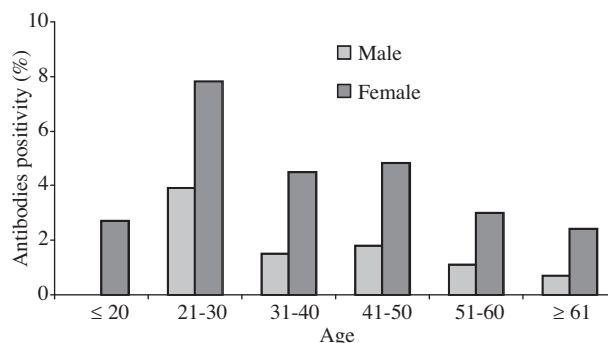


Figure 1. TgAb, TPOAb distributions according to age.

Table 1. Elevated thyroid antibodies distributions according to gender.

Antibodies	Gender		Total
	Male	Female	
TgAb	7 (1.1%)**	29 (4.7%)*	36 (5.8%)**
TPOAb	14 (2.3%)**	29 (4.7%)**	43 (6.9%)**
TgAb and TPOAb	5 (0.8%)**	26 (4.2%)**	31 (5%)**
TgAb or TPOAb	26 (4.2%)**	84 (13.6%)**	110 (17.8%)**

* % within gender, ** % within total

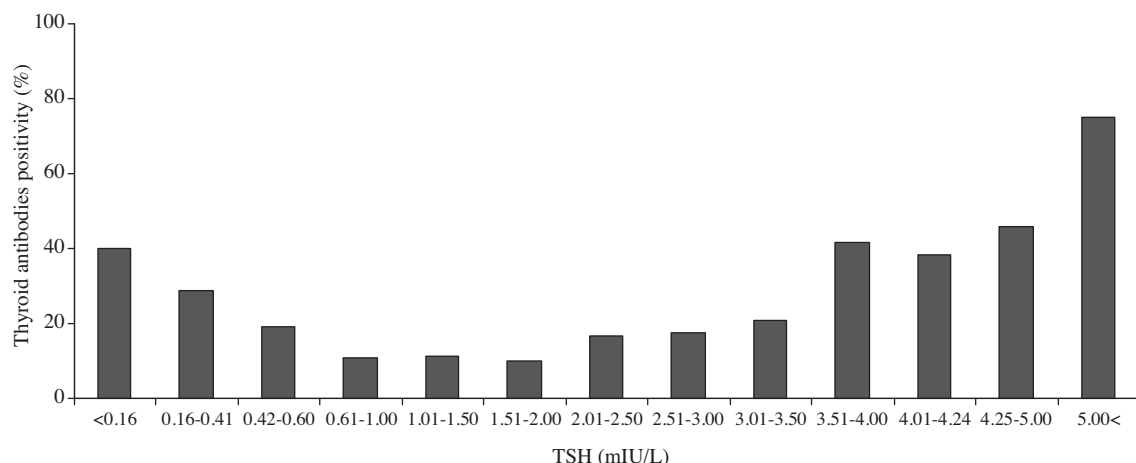


Figure 2. The prevalence of thyroid antibodies across TSH intervals.

For FT3 and FT4 there were no outliers according to either the Dixon or the Block procedure. FT3 and FT4 distributions for all subjects were log-normal.

The 97.5th and 2.5th percentile values of upper and lower reference limits of TSH, FT4, and FT3 together with the 90% confidence intervals are shown in Table 2.

There was a correlation between the FT4 and TSH ($r: -0.096, P: 0.031$) and FT3 ($r: 0.184, P: 0.000$), and also between age and TSH ($r: -0.168, P: 0.000$) and FT3 ($r: -0.231, P: 0.000$).

There were no gender specific differences between the FT4 serum concentrations. Nevertheless, there were significant differences in the TSH ($P: 0.000$) and FT3 serum concentrations ($P: 0.000$). There were gender specific differences in both the upper and lower reference limits of TSH and FT3 and these are shown in Table 3. The confidence intervals of the gender specific reference limits overlap.

Intra and interassay imprecisions ($n = 20$) were $<6.4\%$ for TSH (concentrations, 0.24 mIU/L, 13.5 mIU/L, and 21.8 mIU/L), $<4.7\%$ for FT3 (concentrations, 1.54 pg/mL, 2.86 pg/mL, and 5.01

Table 2. Descriptive analysis and reference limits of thyroid function tests.

Variable	TSH (mIU/L)	FT4 (ng/dL)	FT3 (pg/mL)
Sample size	505	509	509
Lowest value	0.04	0.4900	2.2400
Highest value	4.95	1.3800	4.2300
Geometric mean	1.4620	0.7996	3.1720
Median	1.5400	0.8000	3.1700
Coefficient of Skewness	-1.1882 ($P < 0.0001$)	0.0172 ($P = 0.8726$)	-0.0649 ($P = 0.5462$)
Coefficient of Kurtosis	3.9435 ($P < 0.0001$)	0.5286 ($P = 0.0357$)	0.3088 ($P = 0.1705$)
Significance test for normal distribution	$P < 0.0001^*$	$P = 0.1087^{**}$	$P = 0.3259^{**}$
Method of 95 th reference limits (double sided)	non-parametric	parametric	parametric
Lower limit	0.41	0.61	2.62
90% Confidence interval	0.22 to 0.48	0.60 to 0.62	2.59 to 2.65
Upper limit	4.25	1.06	3.84
90% Confidence interval	3.87 to 4.46	1.04 to 1.07	3.79 to 3.88

*reject normality ** accept normality

Table 3. TSH and FT3 reference intervals according to gender.

Variable	TSH (mIU/L)		FT3 (pg/mL)	
	Male	Female	Male	Female
Sample size	252	253	255	254
Lowest value	0.04	0.09	2.24	2.37
Highest value	4.54	4.95	4.23	3.89
Geometric mean	1.31	1.63	3.26	3.08
Median	1.37	1.69	3.25	3.10
Coefficient of Skewness	-1.41 (P < 0.0001)	-0.98	-0.30 (P: 0.0537)	-0.02 (P: 0.8769)
Coefficient of Kurtosis	4.93 (P < 0.0001)	2.82	0.76 (P: 0.0393)	0.22 (P: 0.4176)
Significance test for normal distribution	P < 0.0001*	P < 0.0001*	P: 0.0186*	P: 0.7114**
Method of 95 th reference limits (double sided)	non-parametric	non-parametric	non-parametric	parametric
Lower limit	0.32	0.44	2.69	2.60
90% Confidence interval	0.11 to 0.48	0.19 to 0.65	2.41 to 2.80	2.56 to 2.64
Upper limit	3.38	4.45	3.89	3.66
90% Confidence interval	3.15 to 4.05	4.14 to 4.84	3.82 to 4.03	3.60 to 3.72

*reject normality ** accept normality

pg/mL), <5.2% for FT4 (concentrations, 0.45 ng/mL, 1.27 ng/mL, and 1.98 ng/mL), <5.7% for TgAb (concentrations, 54.7 IU/mL, 183 IU/mL, and 448 IU/mL), and <6.8% for TPOAb (concentrations, 5.20 IU/mL, 57.6 IU/mL, and 115 IU/mL).

Discussion

This study provides new reference intervals for TSH, FT3, and FT4 in healthy euthyroid Turkish adults free from detectable autoantibodies using the Beckman DxI platform. Clinical reference intervals in a population are necessary in order to accurately assess potential thyroid disease. Common practice in Turkey, both in hospitals and research laboratories, is to use the manufacturer's ranges for a given clinical laboratory assay system. Many of these assay systems are procured from Europe or the United States and use reference values based on their populations, which may not be representative of the Turkish population. A small number of publications address reference values in Turkey (12,13), but these are limited in scope to thyroid function parameters.

This study found that the prevalence of antithyroid antibodies (TPOAb and/or TgAb) was higher in Western Anatolia than in Europe and the United States (5,14-20). In another study, from the Eastern Black Sea Region in Anatolia (21), Baştemiş et al. demonstrated

athyroid antibody prevalence similar to our study. However, the positive antibody group had a high frequency of hyperthyroidism, whereas in our study the positive antibody group had more hypothyroidism. Some of the differences in thyroid function are related to differences in iodide intake. In the Eastern Black Sea region, iodide insufficiency was common (they frequently eat collard); because of this iodide supplementation is given as a preventative therapy and since then an increase in thyroid antibody prevalence has been seen. In Western Anatolia, nutrition habits are different and depend more on olive oil. Since increased iodine intake may enhance thyroid autoimmunity, regional data may be helpful as a guide for public health policy about iodide usage; however, prospective studies will be needed to confirm this. Erdoğan et al. evaluated the current nationwide iodine status in Turkey by determining urinary iodine concentrations (UIC) and household salt iodine content. They showed that moderate to severe iodine deficiency still exists in 27.8% of the Turkish population (median UIC was 147 µg/L in urban and 42 µg/L in suburban and rural areas), which is much better when compared with previous years. Iodine deficiency has been eliminated in most of the urban population, but it is still an important problem in rural areas and in particular geographical regions. Therefore, we think that iodine deficiency does not exist in our region (22).

In contrast to other studies that found the prevalence of antithyroid antibodies increased with increasing age, we found antibody prevalence higher in a younger age group, in which females and males were between 21 and 30 years old. However, we determined higher antibody levels in females than males and this agreed with other studies. These results may be explained by differences in ethnicity, population, or geographic derived covariates such as lifestyle, salt iodination, and nutrition.

Both the 2.5th and 97.5th percentiles of our new reference intervals for FT3 and FT4 were slightly different from the previous studies (23-25). No significant differences were observed for the concentration of FT4 in males and females, whereas TSH and FT3 levels exhibited significant differences between females and males. However, because of the overlapping confidence interval of the reference limits, gender specific reference limits were not used.

The reference interval for TSH (0.41-4.25 mIU/L) found in this study was different from the ranges suggested by Beckman Coulter (0.34-5.60 mIU/L) and from some previously published studies (18,23,26). The NHANES III study reported a TSH reference interval of 0.45 to 4.12 mIU/L (5). TSH serum levels showed a skewed distribution with a relatively long 'tail' towards higher TSH levels. It has been argued that subjects belonging to this 'tail' (with TSH levels 2.5 mIU/L) still have subclinical thyroid disease, which might have been detected by the use of thyroid ultrasound and more sensitive assays of thyroid antibodies; thus the 'true' upper normal limit of TSH would be 2.5 mIU/L (27). While our subjects were screened for the presence of a visible goiter, no palpation for the presence of a goiter was carried out, nor were any thyroid ultrasound studies performed; this may be a limitation of this study. However, specimens with positive TPOAb and/or TgAb were excluded from the study. In Germany, 453 healthy blood donors with ultrasonographically assessed normal thyroid glands had TSH reference interval of 0.40-3.77 mIU/L (26). In a Danish study with 3174

participants, a TSH reference range of 0.40-3.6 mIU/L was found (28), and a recent large Danish study defined the upper normal limit of TSH as 4.5 mIU/L (29). Interestingly, in our study we established 20% positivity of thyroid antibodies when TSH levels were below 0.42 mIU/L and above 3.01 mIU/L and 40% positivity when TSH levels were below 0.16 mIU/L and above 3.51 mIU/L. The possible consequences of the use of a TSH concentration < 4.5 mIU/L for therapeutic interventions to prevent clinical manifestation of hypothyroidism are still being discussed (30-32). There is still dispute about the benefits of treating subclinical hypothyroidism with levothyroxine when the serum TSH is below 10 mIU/L (33), and there are no accurate data about the effect of levothyroxine when serum TSH levels are in the range of 2.5 and 4.0 mIU/L (34). Lowering the upper limit of normal serum TSH levels from 4-5 mIU/L to 2.5 mIU/L will also substantially increase the risk of thyroxine overtreatment, resulting in the well-known and evidence-based risks of subclinical hyperthyroidism (31). As a result, we suggest follow up of patients according to their clinical status who have TSH values between 3.0 and 4.5 mIU/L and may possibly have the very earliest stage of hypothyroidism.

In conclusion, we have established reference intervals for TSH, FT4, and FT3 and found the prevalence of antithyroid antibodies in sera in our population. There is no indication for gender dependence for TSH, FT3, and FT4 reference intervals in healthy individuals. We suggest that there is a need for country-specific reference ranges.

Acknowledgement

We thank Dr Sherry Fare (MRCPATH EMEAI Scientific Manager Immunoassay) for reviewing the manuscript; Dr Thomas Keller (ACOMED statistik, Germany) for helping with the statistical analysis, and Aylin Tin (Beckman Coulter Turkey) for technical assistance.

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