

Biological Variation and Reference Change Values of TSH, Free T3, and Free T4 Levels in Serum of Healthy Turkish Individuals

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Aim: Thyrotropin (TSH), thyroxine (T4) and triiodothyronine (T3) levels in serum are important for diagnosis of thyroid dysfunction. Thyroid function tests have considerable biological variations. Evaluation of the components of biological variation is essential to assess the usefulness of reference values and to evaluate significance of changes in serial results from an individual. Published estimates may show disagreement about the values of biological variations. Not many studies exist at present that evaluate the reference change values (RCV) of these parameters. The aim of this study was to determine the biological variations of TSH, free T3, and free T4 and to calculate their RCV values in serum of healthy individuals in our population.

Materials and Methods: The study group included 46 healthy volunteer subjects (mean age 33.5 ± 9 years). During six consecutive weeks, four blood samples were obtained for each subject at two-week intervals. Serum TSH, free T3, and free T4 levels were measured by an immunoluminometric assay on a random-access analyzer.

Results: The intra-individual/inter-individual biological variations (defined in terms of percent coefficient of variation, CV) for serum TSH, fT3, and fT4 were 37.21/37.6%, 22.32/2.43%, and 13.26/7.47% respectively. The RCVs for TSH, fT3, and fT4 were 104.0%, 63.1%, and 38.8%, respectively ($P < 0.05$).

Conclusions: TSH, fT3, and fT4 showed a significant intra-individual biological variation. The utility of population-based reference intervals for TSH would be of limited use. During evaluation, especially when monitoring thyroid functions, the estimates of biological variations and the RCV values should be considered.

Key Words: Biological variation, TSH, free T3, free T4

Sağlıklı Türk Bireylerde TSH, Serbest T3, Serbest T4 Düzeylerinin Biyolojik Varyasyonu ve Referans Değişim Değerleri

Amaç: Serum TSH, T4, T3 düzeyleri, tiroid fonksiyon bozukluklarının tanısında önemli bir yere sahiptir. Tiroid fonksiyon testleri dikkate değer düzeyde biyolojik varyasyona sahiptir. Biyolojik varyasyonun bileşenlerinin incelenmesi, topluma dayalı referans aralığın kullanılabilirliğinin değerlendirilebilmesi için gereklidir. Çeşitli yayınlarda, biyolojik varyasyon değerleri farklı bildirilebilmektedir. Biz bu çalışmada; TSH, T4, T3 düzeyleri için biyolojik varyasyon ve referans değişim değerlerini araştırmayı amaçladık.

Yöntem ve Gereç: Gönüllü, 46 sağlıklı birey (yaş ortalaması 33.5 ± 9 yıl) çalışmaya alındı. Altı hafta boyunca, ikişer hafta aralıklarla, her bir bireyden toplamda dörder tane serum örneği toplandı. Serum TSH, serbest T3, serbest T4 düzeyleri immünoluminometrik test ile bir otoanalizörde ölçüldü.

Bulgular: TSH, serbest T3, serbest T4 için birey-içi / bireyler-arası biyolojik varyasyonlar sırasıyla % 37.21/37.6, % 22.32/2.43, % 13.26/7.47 idi. Referans değişim değerleri sırasıyla % 104.0, % 63.1, % 38.8 bulundu ($P < 0.05$).

Sonuç: TSH, serbest T3, serbest T4 anlamlı düzeyde birey-içi biyolojik varyasyon göstermektedir. TSH için topluma dayalı referans aralığın kullanılabilirliği sınırlı değere sahiptir. Tiroid fonksiyonlarının takibi, incelenmesi durumunda biyolojik varyasyonun dikkate alınması gerektiği unutulmamalıdır.

Anahtar Sözcükler: Biyolojik varyasyon, TSH, serbest T3, serbest T4

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Introduction

Thyroid dysfunction is common (1). Symptoms and signs of hyperthyroidism and hypothyroidism are often non-specific and vague if present (2) and measurement of thyrotropin (TSH), thyroxine (T4) and triiodothyronine (T3) in serum is important for diagnosis of overt and subclinical thyroid dysfunction (3).

A test result in an individual is usually compared to a laboratory reference range. Such a reference range is often defined by a probability distribution including 95% of test results in healthy individuals in a population. Hence, the variation in the population determines the width of the population-based reference range (4).

Large variations exist in TSH and thyroid hormones in serum (4). The components of variation include pre-analytical, analytical and biological variation. Biological variation is divided into intra(within)-(CV_i) and inter(between)-(CV_g) individual variation (5). The former is characterized by rhythmic aberrations of multiple frequencies (6) and the latter is caused by different set-points around which each individual varies (7).

Data on the biological variations of analyte concentrations or other quantities have important uses in clinical chemistry, including judging the usefulness of conventional population-based reference ranges (8), assessing the true significance of changes in results obtained for serial specimens from a single patient, and determining the standards of performance, or analytical goals, required to facilitate optimal patient care (9). Thus, for serial results to be significantly different, the difference in numerical results must be greater than the combined variation inherent in the two results. This value is traditionally referred to as the critical difference but is better called the reference change value (RCV) (5).

Some studies on the biological variations of free (f)T3, fT4 and TSH concentrations in serum have been reported. These have been done on different numbers of individuals, over different time periods, with different assay methods and in different countries. The published estimates may be discrepant regarding the values of biological variations, and not many studies have evaluated the RCV of these parameters.

In this study, we evaluated the biological variations and RCVs of TSH, fT3, and fT4 in serum of healthy individuals in our population.

Materials and Methods

The study lasted from 2 March 2006 until 14 April 2006 (6 consecutive weeks). Forty-six healthy subjects (mean age 33.5 ± 9 years) participated in the study; 23 males and 23 females (pre-menopausal). They had an average body mass index of 24.8 ± 3.86 kg/m², range 18.5-33.6. All subjects were volunteers and were selected from among laboratory personnel and their friends. All subjects provided written informed consent in accordance with the ethical standards of the local ethical committee. Inclusion criteria were no chronic disease (diabetes mellitus, hypertension, thyroid disease, coronary artery disease, anemia, etc.) and a stable settled lifestyle. Exclusion criteria for subjects were: major medical illnesses (e.g. endocrine or autoimmune disorders); use of any medication, including oral contraceptive drugs; a past history of psychiatric or personality disorders; heavy tobacco or alcohol use; and substance use or abuse. None of the women was pregnant during the study span.

We determined biological variations by blood sampling on 4 occasions at 2-week intervals (5,10). Blood collections were performed in standardized conditions in order to minimize sources of pre-analytical variation. Venipuncture was performed after an overnight fast, between 8-10 a.m. in the antecubital vein of the subjects. All blood samples were collected from seated subjects by three experienced investigators (3 medical doctors) using conventional venipuncture with minimal stasis. Each subject always had blood sampling carried out by the same investigator. The samples were allowed to clot before separation by centrifugation at 3000g for 15 min. All serum samples were stored frozen at -20°C until testing at the end of the collection period as recommended or as described in the literature. Each sample from one individual was assayed in duplicate and after removing outliers, the average value of the individual's duplicate measurements was used for statistical procedures. Outliers were determined as those exceeding $\pm 3SD$ (11). After exclusion of any outliers, 35 subjects for TSH, 42 subjects for fT3, and 41 subjects for fT4 were included in final statistical analysis.

All assays for each parameter were performed by the same analyst. Using this standardized procedure, pre-analytical variation was considered negligible.

We measured serum TSH, fT3, and fT4 levels by an immunoluminometric assay on a random-access analyzer (Architect i2000; Abbott Diagnostics Division). Biological principle of the procedure was chemiluminescent microparticle immunoassay. The reference intervals used in our laboratory for TSH, fT3, and fT4, respectively, are (0.35-4.94 uIU/ml), (1.71-3.71 pg/ml), and (0.7-1.48 ng/dl).

The within-run (intra-assay) analytical variation was minimized by assaying all of the samples with the same lots of reagent on the same day. Furthermore, all samples from a subject were analyzed in random order in the same assay to eliminate inter-assay variation. We determined the coefficients of analytical variation (CV_A) from analyses of several serum control pools running concurrently with assays of the study group. For each assay, we used two lyophilized pools obtained commercially. For each pool, we determined the mean and standard deviation and then calculated CV_A with the following formula: $CV_A = (SD/mean) \times 100$. Because the CV_A values for different quality control sera were similar, we used the average CV_A for the control serums (10-15).

Statistical Data

Biological variation was defined as the mean CV. The total variation, the CV, expressed in terms of percent CV, was calculated for each individual using the formula: percent coefficient of variation = (standard deviation/mean) $\times 100$.

For each subject, we calculated the mean, SD and variation of the TSH, fT3, and fT4 values for the four visits by means of a commercially available statistics program (SPSS 10 and Microsoft Excel). From these data, the mean and SD for the entire population were calculated. The total variation in the measurements for four specimens from one subject is composed of the biological variation and the analytical variation. The intra-individual biological variations were calculated by means of the following formula (5,14):

$$(CV_I)^2 = (\text{Total CV})^2 - (\text{Analytical CV})^2$$

One-half of the mean CV_I is proposed as the quality specification for imprecision, i.e., $CV_A < 0.5 CV_I$.

The inter-individual variance (SD_G^2) reflects the difference between the means of the individuals and excludes intra-individual biological and analytical

variation. To determine SD_G^2 , the total variance (SD_T^2) was calculated by use of all of the individuals' data sets and transformed to relative SD (CV_T) by use of all of the overall means. The inter-individual biological variation (CV_G) was calculated by means of the following formula described by Fraser and Harris:

$$CV_G = (CV_T^2 - CV_I^2 - CV_A^2)^{1/2}$$

From the data generated, RCV was calculated using this formula:

$$RCV = 2^{1/2} * Z * (CV_A^2 + CV_I^2)^{1/2}$$

The number 2 in the formula is a constant for two measurements. RCV should be adjusted to local laboratory standards. In usual practice, we want to know about change in general and thus apply bidirectional Z-scores. 95.5% probability is conventionally regarded as significant. In consequence, generally 1.96 is the appropriate Z-score to use. The RCV provides the limit for a change attributable to random variation with probability specified by the Z value (5,14).

Results

Parametric means and absolute ranges for serum fT3, fT4 and TSH measurements for the subjects are shown in Figures 1, 2, 3.

Evaluation of means of serial serum measurements of individuals (mean, median, SD, minimum and maximum) are given in Table 1.

Results of calculations of TSH, fT3, and fT4 for CV_A , CV_I , CV_G , RCV, and index of individuality (CV_I / CV_G) are given in Table 2.

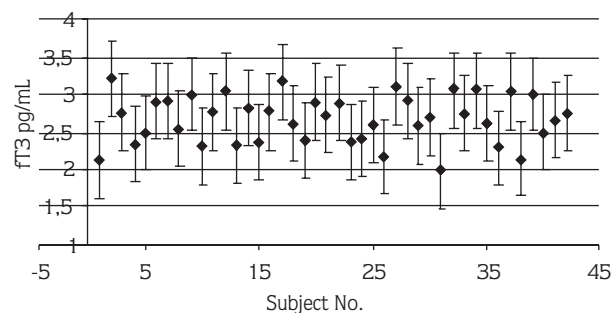


Figure 1. Parametric means and absolute ranges for serum fT3.

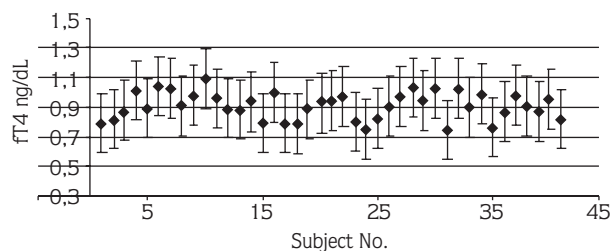


Figure 2. Parametric means and absolute ranges for serum fT4.

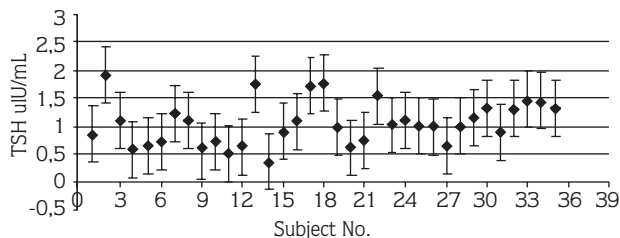


Figure 3. Parametric means and absolute ranges for serum TSH.

Table 1. Evaluation of means of serial serum measurements of individuals.

	Mean	SD	Median	Min-Max
fT3 (pg/ml)	2.66	0.310	2.69	1.99-3.17
fT4 (ng/dl)	0.91	0.092	0.93	0.75-1.09
TSH (uIU/ml)	1.04	0.398	0.99	0.36-1.91

Table 2. Calculated components of variance.

	CV _A %	CV _I %	CV _G %	RC _v %	CV _I / CV _G
fT3	4.60	22.32	2.43	63.17	9.1
fT4	4.57	13.26	7.47	38.87	1.77
TSH	4.91	37.21	37.60	104.04	0.98

Discussion

The major findings of this study are that (i) the serum concentrations of TSH, fT3, and fT4 show a significant intra-individual biological variation, (ii) the intra-individual variability of these parameters is not much lower than the inter-individual variability, and (iii) RCV, the critical difference for a change between two consecutive concentrations, is considerably high for all.

The ratio of intra-(CV_I) to inter-(CV_G) individual variation, also known as the index of individuality (I-index), is important in determining the use of population-based reference intervals in detecting changes in disease status of individuals (4,15). When the I-index is low (<0.6), the individual results stay within a narrow range compared with the population reference interval. Hence, a low index suggests the utility of evaluating serial changes in analyte values in an individual, and population-based reference intervals would be of limited use. A high index (>1.4) suggests that the reference interval is

appropriate (16). It is generally pronounced that this ratio is low for serum TSH, T3, and T4, indicating that laboratory reference ranges are relatively insensitive to aberrations from normality in the individual. However, the results of the present study for fT3 and fT4 are not similar to published estimates (2,13). I-indexes for fT3 and fT4, by our estimation, are higher than 1.4.

The present study found a similar ratio (I-index=0.98) for serum TSH when compared with the ratios reported by Ricos et al. (19.3/19.7 = I-index = 0.97) (17), Andersen et al. (I-index = 0.49) (18), Maes et al. (29.3/48.4 = I-index = 0.60) (6), and Browning et al. (16.2/31.7 = I-index = 0.60) (13). The ratio of intra- to inter-individual variation was low for serum TSH in all studies (4,13,18). This implies that some individuals with TSH within the population-based reference range have a TSH outside the individual reference range. This is illustrated in cross-sectional population studies in which participants with serum TSH close to the outer limits of

the laboratory reference range have a higher frequency of thyroid abnormalities (4). As found by others, for TSH, population-based reference range is an insensitive measure in the large majority of individuals.

The optimal level of thyroid hormones and TSH in serum to attain physical and mental well-being has not been established, but the trend these years is to narrow the range of serum TSH regarded as optimal. Detrimental effects on the cardiovascular system have been reported for suppressed and particularly elevated serum levels of TSH, and follow-up studies have shown an increase in risk of development of overt thyroid dysfunction in subjects with high normal serum TSH levels (19,20).

In practice, population-based reference ranges for TSH are necessary, but it is important to recognize their limitations for use in individuals. Serum TSH responds with amplification to minor alterations in T4 and T3 (21,22). The importance of TSH in diagnosis and monitoring of thyroid dysfunction is indicated. Furthermore, it implies that subclinical thyroid disease may be defined in purely biochemical terms. Under clinical circumstances such as pregnancy, where normal thyroid function is of importance for fetal brain development, subclinical thyroid disease should be treated. Even TSH within the reference range may be associated with slightly abnormal thyroid function in an individual. The clinical

importance of such small abnormalities in thyroid function in small children and pregnant women for brain development remains to be elucidated (4).

Knowledge of the details of predictable biological cyclical rhythms is required for correct clinical interpretation of laboratory data (23). Inherent biological variation affects all quantities examined in laboratory medicine and must be taken into account in both the generation and the application of reference values (23).

In the present study, CV_I and CV_G values for TSH and fT3 were not comparable with some of the previous reports (see Table 3). Our values were higher than some of the others. This may be explained by the season (March – April) in which our study was undertaken. It is reported that TSH and fT3 show significant yearly variations, characterized by annual, biannual and trimonthly rhythms (6).

There is a need for validation of the results of this study with further researches.

In conclusion, we report the importance of biological variation of serum TSH when evaluating the levels of TSH and thyroid hormones. Quality specifications in laboratory medicine are best based on calculations involving biological variation.

Table 3. Published data on biological variations of fT3, fT4 and TSH.

	fT3		fT4		TSH	
	CV_I %	CV_G %	CV_I %	CV_G %	CV_I %	CV_G %
Present study	22.3	2.4	13.2	7.4	37.2	37.6
Reference (14)	7.9	-	7.6	12.2	19.7	27.2
Reference (6)	-	-	7.1	9.1	29.3	48.4

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