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Determination of biological variation components and quality specifications for serum thyrotropin

Serum tirotropin için biyolojik varyasyon bileşenleri ve kalite spesifikasyonlarının belirlenmesi

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Abstract

Purpose: Serum thyroid hormone levels are used in the diagnosis and follow-up of thyroid disorders. The present study aims calculation and evaluation of biological variation (BV) measures for serum thyrotropin (TSH).

Materials and methods: Blood samples were collected from ten volunteers (10) (five women, five men; ages ranging between 20 to 35) weekly throughout five weeks. Serum TSH level was measured on Architect ci8200 immunoassay system and BV estimates were obtained using nested ANOVA test according to the method of Fraser.

Results: Calculated analytical variation (CV_A), within-subject BV (CV_I), between-subject BV (CV_G), index of individuality (II) and reference change value (RCV) for serum TSH were 5.4%, 26.2%, 26.9%, 0.99 and 74.2%, respectively. Desirable performance goals for imprecision, bias and total allowable error (TE_A) were <13.1%, <6.7% and <28.3% respectively.

Conclusion: When we compared our results with the results from BV database (CV_I=19.3% and CV_G=24.6%) for serum TSH we found out higher CV_I and CVG values. Calculated low II meaning marked individuality indicates that population-based reference values are less useful for the evaluation of results while monitoring serum TSH.

Key words: TSH, biological variation, intra-individual variation, inter-individual variation, reference change value.

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Özet

Amaç: Serum tiroid hormon düzeyleri tiroid hastalıklarının tanı ve takibinde kullanılır. Bu çalışma serum tirotropin (TSH) için biyolojik varyasyonu (BV) hesaplanması ve değerlendirilmesini amaçlamaktadır**.**

Gereç ve yöntemler: Kan örnekleri, on (10) gönüllüden (beş kadın, beş erkek; yaşları 20-35 arasında değişen) beş hafta boyunca, haftalık olarak alındı. Serum TSH seviyeleri Abbott Architect ci8200 immunassay cihazında ölçüldü ve Biyolojik varyasyon (BV) hesaplamaları Fraser'ın yöntemine göre nested ANOVA testi kullanılarak yapıldı.

Bulgular: Serum TSH için hesaplanan analitik varyasyon (CV_A), birey içi BV (CV_I), bireylerarası BV (CV_G), bireysellik indeksi (II) ve referans değişim değeri (RCV) değerleri sırasıyla %5,4, %26,2, %26,9, 0,99 ve %74,2 idi. Belirsizlik, bias ve izin verilen toplam hata (TE_A) için istenen performans hedefleri sırasıyla <%13,1, <%6,7 ve <%28,3 idi.

Sonuç: BV veri tabanındaki (CV_I=%19,3 ve CV_G=%24,6) sonuçlar ile karşılaştırdığımızda serum TSH için elde ettiğimiz CV_I ve CV_G değerlerinin daha yüksek olduğunu tespit ettik. Düşük bireysellik indeksi bireyselliğin belirgin olduğunu ve toplum bazlı referans değerlerin TSH takibi sırasında sonuçların değerlendirilmesi için daha az kullanışlı olduğunu gösterir.

Anahtar kelimeler: TSH, biyolojik varyasyon, birey-içi biyolojik varyasyon, bireyler arası biyolojik varyasyon, referans değişim değeri.

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Introduction

Thyrotropin (TSH) is a pituitary hormone which stimulates the production and release of thyroxine and triiodothyronine hormones from thyroid gland. It is a glycoprotein with a molecular weight of approximately 28,000 daltons which is consisted of two subunits, alpha and beta. Release of TSH is regulated by secretion of TSH-releasing hormone (TRH) from hypothalamus. Levels of TSH and TRH are inversely related with thyroid hormone levels. When there is an increase in blood thyroid hormone concentration, less TRH is released by hypothalamus, so less TSH is secreted by the pituitary gland. The opposite is also true for the decreased thyroid hormone concentrations in blood. This downregulation is named as negative feedback and is responsible for the maintenance of blood thyroid hormone levels [1].

Thyroid diseases are common in general population and cause serious public health issues. Laboratory testing is necessary for the diagnosis and management of these diseases. Serum TSH measurement is the first step laboratory test for a patient with suspected thyroidal illness. Generally a TSH level within the reference interval is an important finding to exclude hypo- or hyperthyroidism [2]. Just like the other tests analyzed in the clinical laboratory serum TSH also has three sources of variation including preanalytical, analytical and biological variation [3-5]. Biological variation has both intra-individual and inter-individual components. The term CV_I is used for intra-individual, while CV_G is used for inter-individual variation. Intra-individual variation is defined as the random fluctuations seen around the homeostatic setting point of an analyte in an individual while inter-individual variation is caused by the variations seen among the different equilibrium points of different individuals [6]. There are several important applications of BV data commonly used in clinical practice. Setting quality goals for analytical performance, evaluation of the usefulness for population based reference values and interpretation of difference between two consecutive results of an individual are examples of these applications [7]. Therefore, estimation of CV_I and CV_G values are quite important for clinical laboratories.

Aims of the present study are to obtain and evaluate biological variation parameters of serum TSH in healthy individuals.

Materials and methods

Study subjects and specimen collection

The study was initially planned to include 13 healthy participants, but 3 of them were excluded from the study during follow-up due to discontinuing sample collection. Afterwards the study was continued with ten volunteers (five women, five men; ages ranging between 20 to 35). The inclusion criteria were not having concomitant autoimmune or autoinflammatory disease, acute or chronic infection, malignancy, systemic diseases, previously known thyroid diseases, pregnancy or being in postpartum 6 months. During the study period, the participants were asked to maintain their stable settled lifestyles. The study protocol was approved by the local ethics committee and written informed consent was obtained from all participants.

Blood samples were collected on the same day of 5 consecutive weeks. Venous blood samples were collected between 09.00 and 09.30 a.m. in the sitting position after 8 h fasting with minimal stasis by the same skilled phlebotomist to minimize procedural variations. Blood specimens were taken into vacuum tubes containing separator gel [BD Vacutainer SST II advance (Becton, Dickinson and Company Franklin Lakes, NJ, USA)] and were centrifuged at 1500g for 10 minutes. Separated serum samples were stored at -80 °C until the time of analysis.

TSH measurement

Serum TSH level was analyzed with a two-step Chemiluminescent Microparticle Immunoassay (CMIA) on Architect ci8200 immunoassay analyzer. To minimize inter-batch analytical variation, all samples from the same volunteer were assayed in the same batch. Each serum sample was analyzed duplicately. Two levels of internal quality control material were run on the study day. Same lot numbers of calibrator, reagent and quality control materials were maintained and analyses were done by the same technician during the study in order to avoid variability.

Statistical analyses

Statistical data was processed using Analyse it for Microsoft Excel® 4.0 (Analyse-it Software, Ltd. Leeds, UK). Normality assessment for serum TSH results of each individual was performed separately using Shapiro-Wilk test. The Cochran test and the Reed test were applied to exclude outlying values. Outlier values of two subjects were excluded from the estimations. After exclusion of outliers, data belonging to 8 individuals were used to calculate components of biological variation. CV_A, CV_I, CV_G and the confidence intervals of these components were estimated using Nested ANOVA test. The index of individuality (II) was calculated using the CV_I/ CV_G ratio. And RCV was calculated using the following formula:

$2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}$ (Z=1.96 at 95% confidence interval, CI).

Results

Results assessed for CV_A, CV_I, CV_G, II and RCV from serial measurements of serum TSH in healthy subjects are shown in table 1.

Table 2 presents the calculated desirable quality goals for imprecision, bias and total analytical error. Mean values of TSH results are shown in Figure 1.

Discussion

The present study obtained the biological variation estimates of serum TSH measured by CMIA at weekly intervals throughout 5 weeks in 8 healthy subjects. High within and between individual biological variation values with low individuality index were found according to the results.

There are previous studies in the literature related with BV parameters of TSH (Table 3). These studies have reported similar CV_I and CV_G values with our results. Maes et al conducted a study including 26 (13 male, 13 female) volunteers for 12 months, using IRMA for TSH measurement [8]. In a study by Ankrah-Tetteh et al 10 (4 M, 6 F) subjects were enrolled and competitive immunoassay method was used [9]. Erden et al. included 46 subject and results for CV_I and CV_G were 37.2% and 37.6%, respectively [10]. Ricos et al reported 19.3% and 19.7% for CV_I and CV_G respectively from a study including 15 healthy subjects and using competitive immunoassay [11]. In the study by Browning et al. which included 12 volunteers CV_I and CV_G were estimated as 16.2% and 31.7% respectively [5]. Limited number of subjects and sampling period were the disadvantages of our study. However Fraser and Harris suggested that components of variation could be assessed

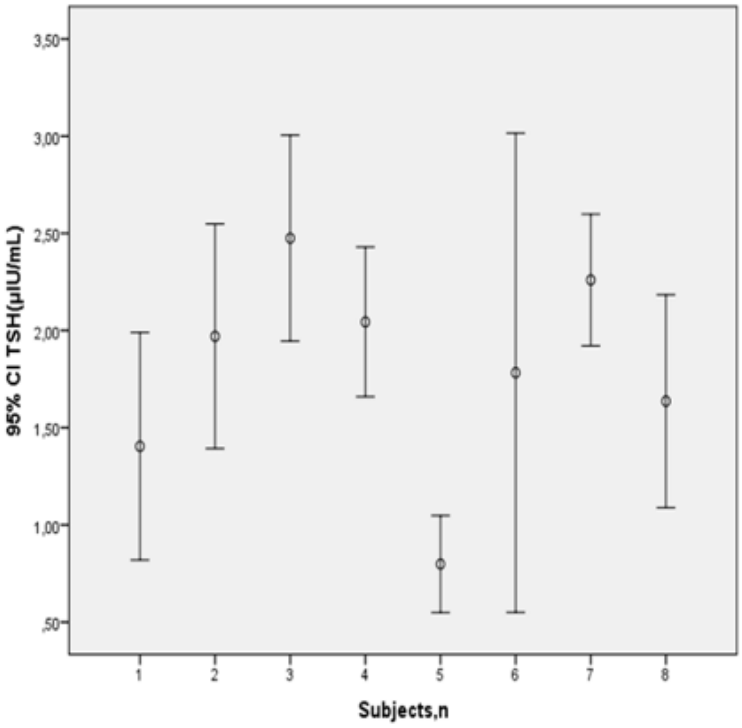

Figure 1. Mean values and absolute range of TSH concentrations of study subjects.

Table 1. Variance components for TSH measurement and estimates (II and RCV) derived from data on BV.

TSH (µIU/mL) (Mean±SD)	CV_A% (95% CI)	CV_I% (95% CI)	CV_G% (95% CI)	II	RCV%
1.69±0.61	5.4 (4.4-7)	26.2 (22.1-33.8)	26.9 (14.3-63.5)	0.99*	74.2**

*$(CV_A^2 + CV_I^2)^{1/2} / CV_G$ (used if $CV_A < CV_I$)

**$2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}$ (Z = 1.96 at 95% CI).

Table 2. Desirable quality specifications for TSH measurement.

	Imprecision, %	Bias,%	TE_A%
Desirable	<13.1	<6.7	<28.31

TE_A: total allowable error

Desirable imprecision $CV_A < 0.50\ CV_I$, Bias $< 0.250\ (CV_A^2 + CV_G^2)^{1/2}$, $TE_A < 0.250\ (CV_A^2 + CV_G^2)^{1/2} + 1.65\ (0.5\ CV_I)$

Table 3. Previously published data on BV of TSH.

	CV_A%	CV_I%	CV_G%	II
Erden et al. [10]	4.91	37.2	37.6	0.98
Maes et al. [8]	5.8	19.7	27.2	0.72
Ankrah-Tetteh et al. [9]	13.5	25.1	36.9	0.68
Browning et al [5].	7.5	16.2	31.7	0.52
Ricos et al [11]	4.7	19.3	19.7	0.97
Present study	5.4	26.2	26.9	0.97

from a relatively small number of specimens collected from a small group of subjects over a reasonably short period of time [12].

While setting performance targets for analytical imprecision desirable goal was defined as half of the CV_I [11]. The present study and all the previous studies with the exception Ankrah-Tetteh et al's met this target.

The Index of individuality (II) is helpful in determination of whether population based reference intervals are appropriate or not in the evaluation of a subject test result. A value of II <0.6 reflects strong individuality for the test of interest and comparison of consecutive results is recommended instead of using population based reference intervals. Conversely in the case of a high II especially >1.4 it has been suggested that reference intervals will be more useful. When the II is less than 1, two consecutive results of an individual may be still within the population based reference interval but between difference of the results being outside the RCV [13]. In this case the reference interval is limited especially for deciding whether a significant change has occurred. Vey few analytes have an II greater than 0.6 most of them are <1.0 [13]. In the present study II was found 0.97 which means that reference interval has limited utility for serum TSH. This finding was supported by the previous studies in the literature. For the interpretation of significant

difference between serial results RCV is the recommended parameter derived from CV_A and CV_I. In the present study we found a RCV value of 74.2%. In the studies of Erden et al and Tankrah-Tetteh et al [9, 10] RCV was reported as 104.04% and 78.94% respectively.

Since clinical laboratory has an increasing effect on clinical decision making appropriate quality specifications are needed in order to evaluate the analytical performance objectively [13]. In the present study desirable analytical goals for imprecision, bias and total error were calculated based on the components of biological variation data (Table 2). The desirable performance goals for imprecision, bias and total allowable error were <13.1%, <6.7% and <28.31% respectively.

In conclusion, serum TSH in healthy subjects displayed high within- and between-subject biological variations, with an II lower than 1 in agreement with the previous studies. Therefore, RCV which was found as 74.2% may be more useful while interpretation of results and clinical decision making instead of population-based reference intervals.

Applications of BV data are helpful for the quality improvement in clinical laboratories via setting objective analytical goals, calculation of RCV and evaluation of the utility of population based reference intervals for the analytes assayed.

Conflict of interest: No conflict of interest was declared by the authors.

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Ethics approval: The study was approved by Harran University Faculty of Medicine Ethics Committee's,decision dated 11.02.2019 and number: HRU 19.02.09