Research Article

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Biological variation and reference change value data for serum copper, zinc and selenium in Turkish adult population

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Abstract

Objectives: Calculation of biological variation (BV) components is very important in evaluating whether a test result is clinically significant. The aim of this study is to analyze BV components for copper, zinc and selenium in a cohort of healthy Turkish participants.

Methods: A total of 10 serum samples were collected from each of the 15 healthy individuals (nine female, six male), once a week, during 10 weeks. Copper, zinc and selenium levels were analyzed by atomic absorption spectrometer. BV parameters were calculated with the approach suggested by Fraser.

Results: Analytical variation (CV_A), within-subject BV (CV_I), between-subject BV (CV_G) values were 8.4, 7.1 and 4.3 for copper; 4.2, 9.1 and 13.7 for zinc; 7.6, 2.5 and 6.9 for selenium, respectively. Reference change values (RCV) were 30.46, 27.56 and 22.16% for copper, zinc and selenium, respectively. The index of individuality (II) values were 1.65, 0.66 and 0.36 for copper, zinc and selenium, respectively.

Conclusions: According to the results of this study, traditional reference intervals can be used for copper but we do not recommend using it for zinc and selenium. We think that it would be more accurate to use RCV value for zinc and selenium in terms of following significant changes in recurrent results of a patient.

Keywords: biological variation; copper; reference change value; selenium; zinc.

ÖZET

Amaç: Biyolojik varyasyon (BV) bileşenlerinin hesaplanması, bir test sonucunun klinik olarak önemli olup olmadığını değerlendirmede çok önemlidir. Bu çalışmanın amacı, sağlıklı Türk katılımcılardan oluşan bir kohortta bakır, çinko ve selenyum için BV bileşenlerini analiz etmektir.

Gereç ve Yöntem: 15 sağlıklı bireyin (9 kadın, 6 erkek) her birinden 10 hafta boyunca haftada bir olmak üzere toplam 10 serum örneği alındı. Bakır, çinko ve selenyum seviyeleri atomik absorpsiyon spektrometresi ile analiz edildi. BV parametreleri Fraser tarafından önerilen yaklaşımla hesaplandı.

Bulgular: Birey içi BV (CV_I), analitik varyasyon (CV_A), bireyler arası BV (CV_G) değerleri bakır için sırasıyla 8.4, 7.1 ve 4.3; çinko için sırasıyla 4.2, 9.1 ve 13.7; selenyum için sırasıyla 7.6, 2.5 ve 6.9 idi. Bakır, çinko ve selenyum için referans değişim değerleri (RCV) sırasıyla % 30.46, % 27.56 ve % 22.16 idi. Bakır, çinko ve selenyum için bireysellik indeksi (II) değerleri sırasıyla 1.65, 0.66 ve 0.36 idi.

Sonuç: Bu çalışmanın sonuçlarına göre geleneksel referans aralıklarının bakır için kullanılmasını ancak çinko ve selenyum için kullanılmaması öneriyoruz. Çinko ve selenyum için hastanın tekrarlayan sonuçlarındaki önemli değişiklikleri takip etmek açısından RCV değerinin kullanılmasının daha doğru olacağını düşünüyoruz.

Anahtar Kelimeler: biyolojik varyasyon; referans değişim değeri; bakır; çinko; selenyum.

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Introduction

Trace elements are minerals that make up less than 0.01% of the total body weight, which must be taken with diet to maintain health as well as maintaining the continuity of physiological events such as growth and development [1, 2]. Trace elements such as copper, zinc and selenium act as a cofactor for many vital enzymes, enabling the conversion of substrates to specific products. They also play a role in reduction and oxidation reactions, inflammatory and immune responses [3–5]. As a result of inadequate intake, metabolic functions are impaired and the incidence of chronic diseases increases, the recovery time of diseases prolong and early deaths occur. In addition, pathological conditions such as mental health impairment, insufficient growth and development, infections, cancers, cardiovascular and neurological diseases may appear. Excessive intake of trace elements causes toxic symptoms and damage to various organs, especially the liver [6–8].

The amount of trace elements such as copper, zinc and selenium in the human body is homeostatically regulated. Under ideal conditions, the levels of these elements are expected to fluctuate within narrow limits [9, 10]. Although the use of a reference interval is rational for this purpose, what the normal is and the fluctuation around these values are not exactly known. Reference interval studies were conducted in different societies, in different age and gender groups [11–13]. Most commonly used methods for copper, zinc and selenium in laboratories are flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS) and inductively coupled plasma mass spectrometry (ICP-MS) [14]. Since metal analyzes are difficult, time-consuming, methods are open to contamination and they require relatively large sample volumes depending on the number of metals to be looked at in a person, the number of studies recently conducted to give faster and more accurate results with less samples and analytical quality specifications in metal analysis determination has become very important [15, 16].

Biological variation (BV) is a very important parameter in the interpretation of laboratory results. With BV studies, information about the physiological changes of within individual and between individual values of the substance analyzed is obtained. The reference change value (RCV), which allows deciding whether a clinically meaningful change is based on a person's repeated test results, can be calculated. In addition, analytical quality specifications such as precision and bias can be determined [17–19].

The aim of the present study is to determine the BV of serum copper, zinc and selenium. Despite the clinical significance of trace element analysis, there have not been enough studies in the field of BV. The absence of a common and standardized methodology and terminology in BV studies is an important problem, and different research groups have tried to enlighten the issue with their publications [17, 20]. In the literature, a trace element and biological variation study in accordance with the comprehensive and current methodology is very few [21]. For this reason, we believe that our study will provide valuable data in this area.

Method

The study group included 15 healthy volunteers, among the hospital staff (nine female and six male; aged between 20 and 45) from the Central Anatolia Region. The local ethics committee approved the study (Ref number: 26379996/174, date: 2017). Participants gave their written informed consent for participating in the study. Inclusion criteria were that the participants were completely healthy and the body mass index (BMI) was within the normal range: 18.5–24.9. For ensuring that all of the participants were providing healthy lifestyles, interviews and health questionnaires were conducted. Routine blood and biochemistry tests were controlled. The exclusion criteria were had an acute or chronic diseases, taking any medical and herbal treatment, pregnancy or being in breastfeeding period for female, taking any alcohol, tea, or tobacco products. During the study time, all subjects kept their normal lifestyles.

To reduce preanalytical variables, venous blood samples were drawn according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI), Document GP41 (Collection of Diagnostic Venous Blood; Approved Guideline, seventh edition) [22]. After overnight fasting and without any morning exercises, venous blood was collected in a serum trace element testing tube with clot activator (Becton, Dickinson and Company Franklin Lakes, NJ, USA) between 09.00 and 10.00 a.m. on sitting position by the same phlebotomist. Venous blood samples were acquired from all subjects on the same day, every week for 10 weeks from October to December 2018. After centrifugation at 1,500×*g* for 10 min obtained sera was aliquoted into eppendorf tubes and stored at −80 °C until the analysis time.

Serum trace element levels were analyzed by an atomic absorption spectrometer (AAS) (Thermo ICE 3000, Tokyo, Japan). The selenium level was measured with Zeeman background correction by using the electrothermal atomic absorption spectrometer (ETAAS); Cu and Zn levels were measured with deuterium background correction by using the FAAS. Standard Cu, Zn and Se solutions were prepared by diluting certified standard solutions (high purity standards, Charleston, SC, USA). Two levels quality control material (Seronorm, Billingstad; Norway) was used. Samples were diluted with 1% (1.10) nitric oxide (HNO_3). Standards were measured in the ranges of 70–150 ppb, 0.05–1, and 0.1–0.5 ppm for Se Cu and Zn, respectively.

Statistical analysis

Barlett and Cochran tests were used to identify and remove outliers before the statistical analysis. A data graph (scatter plot diagram) was used for outliers' confirmation.

Checking the normality of within- and between-subject data was done with the Anderson–Darling test. Normal values were presented as mean + SD, or in the case of non-normally distributed data, as median (minimum–maximum).To calculate within-subject biological variation (CV_I), between-subject biological variation (CV_G), analytical variation (CV_A), individuality index (II) and RCV nested analysis of variance (nested ANOVA) was used for normally distributed data. CV-ANOVA was used for CV_A and CV_I calculation in non-normally distributed data. Each sample duplicate sample results were used the calculation of analytical variation. The formulas described by Fraser and Harris below was used [23]:

$SDA^2 = (\sum d^2/2n)$,	$CV_A = (SD_A/Mean) \times 100$
$CV_I = (CV_{TI}^2 - CV_A^2)^{1/2}$,	$CV_G = (CV_T^2 - CV_I^2 - CV_A^2)^{1/2}$
$RCV = 2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}$,	$II = CV_I/CV_G$

SD, Standard deviation; CV_I, Within-subject biological variation; RCV, Reference change value; CV_A, Analytical variation; CV_G, Between-subject biological variation; II, Individuality index; CV_{TI}, Total CV; Z=1.96 at 95% confidence interval, CI.

XLSTAT® software (Addinsoft, Paris, France) and Analyse-it for Microsoft Excel 4.0 (Analyse-it Software Ltd., Leeds, UK) were used to analytical performance specifications for analytical bias and analytical imprecision.

Results

Data from 13 volunteers were evaluated after excluding outliers for the copper test. There were no outliers for zinc and selenium. CV_A, CV_I, CV_G values for copper were 8.4, 7.1 and 4.3; 4.2, 9.1, 13.7 for zinc; and 7.6, 2.5, 6.9 for selenium, respectively. CV_A, CV_I, CV_G, II and RCV values for copper, zinc and selenium were given in Table 1. The index of individuality was obtained by dividing the intra-individual coefficient of variation value by the inter-individual coefficient of variation value. The value II was found 1.65, 0.66 and 0.36 for copper, zinc and selenium, respectively. Mean values and absolute range of serum copper, zinc and selenium are given in Figures 1–3, respectively. Quality specifications for trace elements derived from our data on biological variation are given in Table 2.

Discussion

Laboratories should control processes at every stage and minimize variations to create reliable results. For this purpose, biological variation components should be calculated first. Although the number of studies carried out increases day by day, the number of studies about trace elements is very low[21, 24–27].

Trace elements are essential for the body as well as can be toxic at high concentrations. While copper absorption and excretion are under strict control, excess dietary copper intake is not considered a severe health problem, while severe Cu deficiency has a negative effect on various metabolism, especially cholesterol metabolism. There are also two important genetic diseases related to copper metabolism, Wilson and Menkes [6, 28]. Acrodermatitis enteropathica is a rare autosomal recessive disease; there is a problem in zinc absorption Keshan disease, which is an endemic cardiomyopathy disease in China, and Kashin–Beck disease are diseases that occur due to selenium deficiency. Depending on selenium toxicity, symptoms such as diarrhea, fatigue, hair loss, and discoloration of the nails appear [6, 7]. In clinical practice, serum trace element levels are primarily evaluated for the evaluation of symptoms arising due to deficiency or excess in copper, zinc and selenium. Values exceeding the upper and lower limits are associated with clinical conditions and are used in the diagnosis and follow-up of the disease. For this reason, biological variation components should be calculated for serum trace element levels and what should be defined to reveal pathological conditions [29].

To calculate the biological variation components of a test, samples are collected from healthy individuals at regular intervals and the number of replicates determined is studied [30]. Fraser and Harris reported that this calculation could be done with a small number of samples

Table 1: Estimated variance components for copper, zinc and selenium measurements derived from data on biological variation.

Analyte	Mean ± SD (median, min–max)	CV_A% (95% CI)	CV_I% (95% CI)	CV_G% (95% CI)	II	RCV%
Copper, µg/dL	103.34 (80.0–149.76)	8.4 (7.8–9.5)	7.1 (6.4–8.1)	4.3 (2.6–5.6)	1.65	30.46
Zinc, µg/dL	97.74 ± 16.03	4.2 (3.7–4.7)	9.1 (8.3–10.1)	13.7 (9.8–21.8)	0.66	27.56
Selenium, µg/L	78.99 (61.83–110.78)	7.6 (6.8–8.7)	2.5 (2.3–2.9)	6.9 (6.6–7.2)	0.36	22.16

CV_A was calculated using the duplicate results of participants. CV_A, analytical coefficient of variation; CI, coefficient of variation; CV_I, within-subject biological variation; CV_G, between-subject biological variation; II, individuality index; RCV, reference change value.

Table 2: Quality specifications for trace elements derived from our data on biological variation.

Analyte	Quality level	Imprecision %	Bias %
Copper	Optimal	<1.78	<1.18
	Desirable	<3.55	<2.36
	Minimal	<5.33	<3.54
Zinc	Optimal	<2.28	<1.79
	Desirable	<4.55	<3.58
	Minimal	<6.83	<5.37
Selenium	Optimal	<0.63	<1.73
	Desirable	<1.25	<3.47
	Minimal	<1.88	<5.20

Optimal imprecision $CV_A < 0.25\,CV_I$, Bias $< 0.125\,(CV_A^2 + CV_G^2)^{1/2}$

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

Minimal imprecision $CV_A < 0.75\,CV_I$, Bias $< 0.375\,(CV_A^2 + CV_G^2)^{1/2}$

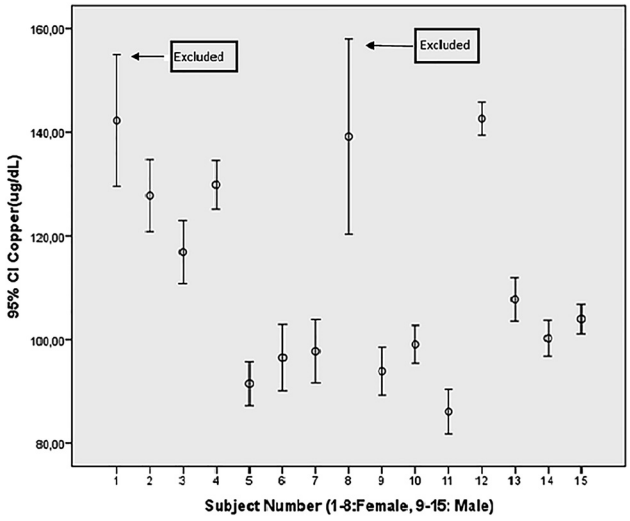

Figure 1: Mean values and absolute range of serum copper.

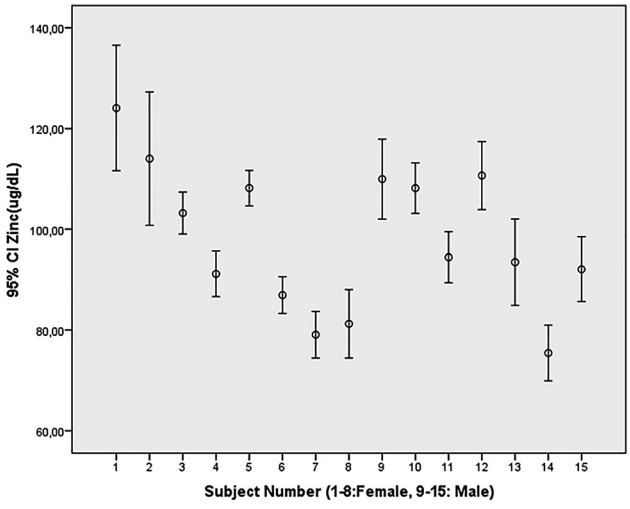

Figure 2: Mean values and absolute range of serum Zinc.

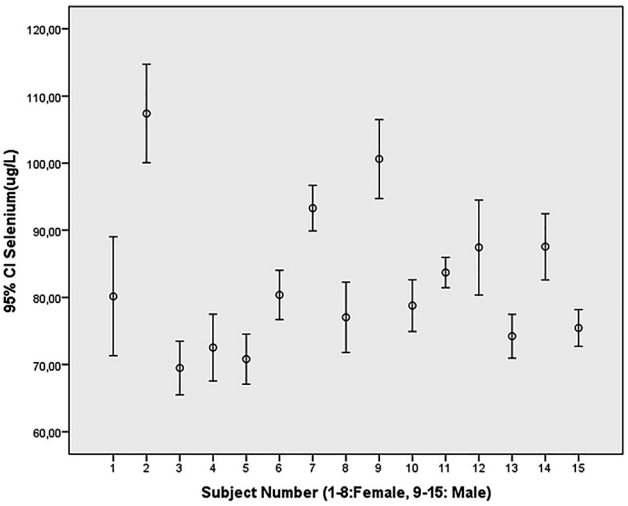

Figure 3: Mean values and absolute range of serum Selenium.

obtained from a small group of people [23].Therefore, in our study, the number of samples collected from 15 people for 10 weeks is sufficient for the number of biological variations.

In our study, we calculated the CV_A, CV_I, CV_G, II and RCV values for copper, zinc and selenium and compared these values with the results obtained from other studies. We found II values for copper, zinc and selenium as 1.65, 0.66 and 0.36, respectively. CV_A, CV_I, CV_G values for copper were 8.4, 7.1 and 4.3; 4.2, 9.1, 13.7 for zinc; and 7.6, 2.5, 6.9 for selenium, respectively. In the literature, in the study of Lux and Naidoo who calculated the BV parameters of copper, zinc and selenium in the same study, CV_A, CV_I, CV_G values for copper were 2, 8 and 19; 2, 11, 14 for zinc; and 4, 12, 14 for selenium, respectively [24]. In the study conducted by Yücel et al., CV_A, CV_I, CV_G values were 4.24, 6.05, 19.64 for copper and 4.59, 6.26, 23.27 for zinc, respectively [21]. We could not compare our results with the other studies because they are not similar to our study design. Gonzalez's study evaluated BV of zinc, copper and magnesium took only four weeks and there were differences in the calculation [25].Giles et al. calculated the plasma zinc variation in individuals over 60 years of age [26]. Gallagher investigated the effect of nutritional status on short and long term variance of copper and other trace elements in two groups of five people [27]. Although all studies were performed with AAS in general, there are differences in the measurement method. In our study, copper and zinc measurements were performed with FAAS, and selenium with ETAAS. In other studies, while FAAS or ETAAS was used for copper and zinc, hydride generation atomic absorption spectroscopy (HGAAS) was used for selenium analysis. Plasma was used as a sample in some of the

studies and serum in some [21, 24–27]. We think that the difference in results may be caused by these factors.

The index of individuality is a value that indicates whether a test result can be evaluated with traditional population-based reference interval values. While the reference interval use is recommended when II >1, the use of the reference interval for tests with II <1 is thought to provide limited use and benefit in tracking changes that would be considered clinically significant. In our study, the value of II was found 1.65, 0.66 and 0.36 for copper, zinc and selenium, respectively. While the II value for selenium and zinc was <1, it was >1 for copper. Except for copper, these values are compatible with the literature. For zinc and selenium, the use of traditional reference intervals provides limited benefits, especially in diagnostic values close to cut-off values. Traditional reference intervals can be used for copper.

In the repeated measurements of a person, falls and rises that fall within the reference interval that will affect the diagnosis or treatment of the patient may not attract clinicians' attention. Therefore, it is recommended to use the RCV value in determining whether there is a clinically significant change in repeat measurements. In our study, we found RCV values for copper, zinc and selenium as 30.46, 27.56 and 22.16%, respectively. Yücel et al. found these values for copper as 20.47 and 21.51% for zinc. We think that the difference between these values is due to the differences in the measurement method used. Since we could not find a calculated RCV value for selenium, we did not compare the calculated RCV value with other results.

In this study, optimal, desirable and minimal for imprecision and bias (Table 1) values were calculated from CV_A, CV_I, CV_G values, biological variation components for copper, zinc and selenium. In addition, this study is the first to calculate RCV values for copper, zinc and selenium at the same time. The calculation of BV parameters for copper, zinc and selenium is very important for the effective use of these tests in clinical laboratories. According to the results of this study, we do not recommend using traditional reference intervals zinc and selenium. We think that it would be more accurate to use RCV value in terms of following significant changes in recurrent results of a patient. Traditional reference intervals can be used for copper.

As a result, in this study, BV data of serum copper, zinc and selenium were studied in an acceptable time, with a sufficient number of participants and samples, and with the correct methodology. We think that this study will pave the way for future studies with different populations, different clinical conditions and different trace elements.

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