Use of Reference Change Value for Dyslipidemia Follow up

Dislipidemi Takibinde Referans Değişim Değerinin Kullanımı

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Başvuru Tarihi: 19 Mart 2019 **Kabul Tarihi:** 17 Mayıs 2019

Abbreviations: ANOVA: Analysis of variance, CV: Coefficient of variation, CVA: Analytic coefficient of variation, CVI: Intra-individual coefficient of variation, CVG: Inter-individual coefficient of variation, II: Index of individuality, HDL-cholesterol: High-density lipoprotein-cholesterol, CVD: Cardiovascular diseases, LDL-cholesterol: Low-density lipoprotein-cholesterol, RCV: Reference change value, SD: Standard deviation

ABSTRACT

Aim: Lipid profile is used to determine the risk of cardiovascular diseases (CVD) and to monitor treatment. Reference change value (RCV) represents the clinical significance of the variation between the results of two consecutive tests and laboratory test results exceeding this value are associated with the individual's disease status. In our study, we aimed to calculate the RCV for lipid profile tests such as total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol, additionally, to assess the clinical use of the RCV in order to determine to the risk of CVD and monitoring their treatment.

Materials and Methods: The analytic coefficient of variation (CVA) was calculated from internal quality control results. The intra-individual coefficient of variation (CVI) and inter-individual coefficient of variation (CVG) were obtained from Westgard's website. For each test, the index of individuality (II) was calculated with the CVI/CVG ratio. To assess tests with index of individuality below 1, percentage of the RCV (RCV%) was used. The RCV% is calculated with the formula z.21/2.[CVA2+CVI2] 1/2.

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Results: The RCV% results were calculated as 15% for total cholesterol, 47% for triglyceride, and 19% for HDL-cholesterol and LDL-cholesterol. The II was calculated as 0.388 for total cholesterol, 0.608 for triglyceride, 0.344 for HDL-cholesterol and 0.382 for LDL-cholesterol.

Conclusion: The results for RCV% values identified for lipid profile in the literature are similar to the results obtained in our study. In clinical practice, when the difference between two consecutive lipid profile tests exceed the RCV%, it should be interpreted as a significant change. The RCV% may be used to assess the CVD risk and the efficacy of dyslipidemia treatment.

Key words: Reference change value; lipid profile; dyslipidemia

ÖZET

Amaç: Kardiyovasküler hastalıklar (KVH) için risk belirlemede ve tedavi takibinde lipit profilinden yararlanılır. Referans değişim değeri (RCV), ardışık iki test sonucu arasındaki değişikliğin klinik önemini ifade eder ve bu değeri aşan laboratuvar test sonuçları bireyin hastalık durumu ile ilişkilidir. Çalışmamızda total kolesterol, trigliserit, HDL-kolesterol ve LDL-kolesterol gibi lipit profili testlerine ait referans değişim değerini (RCV) hesaplamayı ve kardiyovasküler hastalıklar için risk belirleme ve tedavi takibinde RCV'nin klinik kullanımını değerlendirmeyi amaçladık.

Gereç ve Yöntem: İç kalite kontrol sonuçlarından analitik varyasyon katsayısı (CVA) hesaplandı. Birey içi varyasyon katsayısı (CVI) ve bireyler arası varyasyon katsayısı (CVG) değerleri Westgard'ın web sitesinden elde edildi. Herbir test için bireysellik indeksi (BI), CVI/CVG oranı ile hesaplandı. BI değeri 1'in altında olan testlerin değerlendirilmesinde %RCV esas alındı. Elde edilen verilerle %RCV=(z.21/2.[CVA2+CVI2] 1/2) formülü ile hesaplandı.

Bulgular: %RCV sonuçları; total kolesterol için %15, trigliserit için %47, HDL-kolesterol ve LDL-kolesterol için %19 olarak hesaplandı. BI; kolesterol için 0,388, trigliserit için 0,608, HDL-kolesterol için 0,344 ve LDL-kolesterol için 0,382 olarak hesaplandı.

Sonuç: Literatürde lipid profili için belirtilen % RCV değerleri, çalışmamızda elde edilen sonuçlara benzerdir. Klinik pratikte, iki lipit profili test sonucu arasındaki fark %RCV'yi aştığı zaman anlamlı bir değişiklik olarak yorumlanmalıdır. KVH riski ve dislipidemi tedavisi etkinliğini değerlendirmede %RCV kullanışlı olabilir.

Anahtar kelimeler: Referans değişim değeri; lipid profili; dislipidemi

INTRODUCTION

The variation in laboratory test results may be due to many things such as hormonal changes, sunlight (e.g. vitamin D), circadian rhythm, sleep, posture, physical activity, nutrition, stress, etc. (1). The laboratory tests for every individual can be seen to fluctuate randomly around a certain value. This situation is called the intra-individual variation. Random fluctuations between individuals are called the inter-individual variation. Mathematically these fluctuations are represented by the "coefficient of variation (CV)" and the intra-individual and inter-individual variations are shown by CV_I and CV_G, respectively (2). Additionally, changes during analysis of the test in laboratories are called analytic variation (CV_A) and may affect the laboratory test results. In short, just as differences in serial measurements of a test for individuals may be due to progression of disease, amelioration of disease or treatment efficacy, they may also be due to biological and analytic variations.

The numerical value representing the clinical significance of the variation between the results of two consecutive tests is the reference change value (RCV) (3). This value is beneficial to identify clinically significant differences between serial measurements when given along with patient results (4). Laboratory test results exceeding the RCV are associated with the individual's disease status.

Cardiovascular diseases (CVD) are the most important cause of mortality in developed

countries. In America nearly 34% of all deaths under the age of 75 years are linked to CVD (5). Total cholesterol, triglyceride, high-density lipoprotein-cholesterol (HDL-cholesterol) and low-density lipoprotein-cholesterol (LDL-cholesterol) levels are used routinely for risk determination in CVD. Total cholesterol and LDL-cholesterol levels, especially, are primarily recommended for assessment of CVD risk and treatment response. Additionally, it has been shown in many studies that reducing total cholesterol and LDL-cholesterol reduces mortality (6).

In our study, we aimed to calculate the RCV for lipid profile tests such as total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol, additionally, to assess the clinical use of the RCV in order to determine to the risk of CVD and monitoring their treatment.

MATERIALS AND METHODS

In our study, the internal quality control results for total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol tests studied in 2018 using a Beckman Coulter AU5800 model autoanalyzer in the biochemistry laboratory of İstanbul Okmeydanı Training and Research Hospital were obtained from the laboratory information system. For each test, two-level internal quality control studies were performed with Beckman Coulter (Ireland, INV) brand control material and the acceptance or rejection criteria for internal quality control results used the Westgard rules (7). Standard deviation and means was calculated for the two-level controls. Later using the formula (Standard Deviation (SD)/Mean*100) formula, the CV values for the two-level control of each test was calculated. The mean CV for both levels of each test were used to calculate the CV_Avalue.

Tests related to the CV_I and CV_G values were obtained from the current database on the Westgard website (8). For each test the index of individuality (II) was calculated using the CV_I/CV_G formula (9). The RCV was taken as the basis for assessment of lipid profile tests with II value below 1.

The percentage of the RCV (RCV%) values was calculated using the formula $z.2^{1/2}.[CV_A^2+CV_I^2]^{1/2}$. The z constant in this formula was determined as 1.65 for the 95% confidence interval ($p<0.05$) (10).

Lipid profile was studied with colorimetric methods using a Beckman Coulter brand AU5800 model autoanalyzer.

RESULTS

Table 1. Biological variation coefficients and individuality index values for tests
Tablo 1. Testler için biyolojik varyasyon katsayıları ve bireysellik indeksi değerleri

Lipid profile	Biological variation		
	CV_I	CV_G	II
Total cholesterol	5.95	15.3	0.388
Triglyceride	19.9	32.7	0.608
HDL-cholesterol	7.3	21.2	0.344
LDL-cholesterol	7.8	20.4	0.382

CV_I = Intra-individual coefficient of variation
CV_G = Inter-individual coefficient of variation
II = Index of individuality

Table 1 shows the CV_I and CV_G values obtained from the Westgard website (8) and the calculated II value for each analyte.

Table 2. Number, mean, SD, CV and CVa of internal quality control data for the tests
Tablo 2. Testler için iç kalite kontrol verilerinin sayısı, ortalamaları, SD, CV ve CVa değerleri

Internal Quality Controls	Control number (n)	Mean	SD	CV	Mean CV_A
Total cholesterol Control-1	423	150	4.67	3.11	3.10
Total cholesterol Control-2	465	284	8.79	3.09	
Triglyceride Control-1	408	147	6.98	4.74	4.94
Triglyceride Control-2	449	305	15.71	5.15	
HDL-cholesterol Control-1	522	36	1.45	4.02	4.14
HDL-cholesterol Control-2	521	68	2.91	4.27	
LDL-cholesterol Control-1	460	84	3.03	3.60	3.45
LDL-cholesterol Control-2	461	171	5.66	3.30	

Table 2 shows the control number, mean, SD, CV and mean CV_Avalue created from internal quality control data for each analyte.

Table 3. Calculated RCV% for lipid prolife tests
Tablo 3. Lipid prolife testleri için hesaplanan% RCV değerleri

	RCV%
Total cholesterol	15%
Triglyceride	47%
HDL-cholesterol	19%
LDL-cholesterol	19%

The RCV% value was calculated by inserting data from tables 1 and 2 into the $z.2^{1/2}.[CV_A^2+CV_I^2]^{1/2}$ formula.

Table 4. Assessment of virtual patient receiving dyslipidemia treatment with RCV% of consecutive test results
Tablo 4. Dislipidemi tedavisi alan hastanın ardışık test sonuçlarının % RCV'si ile değerlendirilmesi

Values of the virtual patient	Total cholesterol	LDL-cholesterol	HDL-cholesterol	Triglyceride
First visit-basal values (mg/dl)	280	205	48	120
Values after 2 months treatment (mg/dl)	180	130	55	100
Difference (mg/dl)	100	75	7	20
% change in test results	35%	36%	14%	16%
RCV%	**15%**	**19%**	**19%**	**47%**
Interpretation	**Significant**	**Significant**	Insignificant	Insignificant
RCV% exceeded	**Present (35>15)**	**Present (36>19)**	Absent (14<19)	Absent (16<47)

**RCV% data are specific to our laboratory and are not appropriate for use to assess results from other laboratories*

Table 4 shows the consecutive test results for a virtual patient receiving dyslipidemia treatment and assessment according to the calculated RCV%. In the table, when the % change exceeded the RCV% value, the test results for the patient were assessed as changing significantly. In opposite situations, the variation in both test results was insignificant; in other words, due to variation.

DISCUSSION

Factors such as dyslipidemia (high total cholesterol, LDL-cholesterol and triglyceride levels and low HDL-cholesterol levels), smoking, sedentary lifestyle, obesity, family history, high blood pressure and glucose levels lead to increases in the risk of CVD (11). According to World Health Organization data, in European countries high lipid levels are the main factors in 8.7% of heart and vascular diseases (12). High blood pressure and high cholesterol levels are correctable risk factors and are observed in 80% of CVD (13). Additionally, the treatment that those with CVD should start is linked to many factors. These include factors like the patient's present lipid levels, medication use linked to comorbid diseases and side effects of medications (14).

Decision limits and population-based reference intervals are used to interpret lipid profile tests in CVD, as for other tests. However, using the RCV% to interpret the results of two consecutive tests can identify significant differences. As a result, in our study we calculated the RCV% for lipid profile tests. The RCV% we obtained indicates that differences larger than 15% for total cholesterol, 47% for triglyceride, and 19% for HDL-cholesterol and LDL-cholesterol in serial measurements from our laboratory may show a significant change for these tests.

When the literature is investigated, some studies calculated the RCV% values for tests belonging to the lipid profile. A study researching the effects of intake of 300 ml water 1 hour before the phlebotomy procedure calculated RCV% as 17.2% for total cholesterol, 56% for triglyceride, 22.1% for LDL-cholesterol and 21.7% for HDL-cholesterol (15). A study investigating the effects of long-term fasting and mild physical activity on laboratory tests identified 44.6% as RCV% for triglyceride (16). A study calculating RCV% for biochemical tests of subjects with regular aerobic training in the 19-22 year age group found it was 27% for total cholesterol and 66.4% for triglyceride (17). The results for RCV% values identified for lipid profile in the literature are similar to the results obtained in our study. The RCV% for the triglyceride test in our study was identified to have high values, similar to the literature. The high RCV% values for triglyceride may be due to larger effect of daily diet compared to other lipid tests and showing exogenous lipid intake due to high biological variability.

In our study we used the biological variation values calculated by Westgard. For calculation of intra-individual and inter-individual biological variation, a certain number of biological samples could also be taken from a certain number of people (18). In some studies, researchers have used their own serial measurements in biological variation calculations. A study measuring prostate specific antigen levels took four samples from each individual at fourteen-day intervals from 26 healthy males and found the reference change value was 49.4%. Additionally, when assessing the increase in patient's test results, they stated it was necessary to examine whether the RCV% value was exceeded or not and interpretations should be made accordingly (19). Garner et al. in blood samples taken periodically form 22 healthy women monitored for breast cancer found the RCVs% were 32% and 72% for carcinoembryonic antigen and tissue polypeptide antigen tests, respectively (20).

In our study the II for the lipid profile tests were <1. For individuals with II index below 1, two consecutive results may exceed the RCV% but be within the population-based

reference interval. However, analytes with II value above 1 should be compared with the population-based reference interval (18). II is showed to be low in most routine biochemical tests. As a result, differences in repeated measurements of people may remain within the population reference interval and thus rather than comparing the analyte results with the reference interval it appears more appropriate to assess the difference in consecutive measurements; in other words, to use the RCV% value (21). Low II value for lipid profile tests shows that RCV% use may be beneficial for CVD risk and dyslipidemia treatment monitoring.

Comparison of an individual's test results with previous values may provide more valuable information than comparison with a classic population-based reference interval. As a results, reporting of the RCV% along with the population-based reference interval will contribute to interpretation of patient test results. Additionally, the RCV% for each test should be calculated and reported in laboratories. Knowing and reporting the RCV% will reduce patient's concerns related to increasing or reducing test results.

CONCLUSIONS

In conclusion, when the difference in the results of two consecutive lipid profile tests exceeds RCV%, it should be interpreted as a significant change. The RCV% may be used for assessment of CVD risk and efficacy of dyslipidemia treatment. Prospective advanced studies about calculation of biological variations in a certain CVD patient population and related to the use of RCV% after specific treatments should be performed.

Ethics Committee Permission: The study received permission from Health Sciences University Okmeydanı Education and Research Hospital Ethics committee dated 18.12.2018 numbered 1068.

Conflict of Interest: The authors report no conflict of interest.

Financial Support: The authors report they received no financial support.

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