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Research Article / Araştırma

Comparison of different formulas used for LDL calculation

LDL hesaplamasında kullanılan farklı formüllerin karşılaştırılması

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ABSTRACT

Aim: Elevated low-density lipoprotein cholesterol concentration in the blood is a well-defined atherogenic risk factor and it is highly predictive for Coronary Heart Disease. Therefore, the analysis of serum low-density lipoprotein cholesterol levels must be carried out in both accuartely and precisely. This study was designed to compare the results obtained with directly measured low-density lipoprotein cholesterol in our hospital and various formulas used to calculate low-density lipoprotein cholesterol. **Materials and Methods:** 175 patients with directly measured low-density lipoprotein cholesterol results were included in the study. Patient results were divided into four groups based on their triglyceride values. low-density lipoprotein cholesterol values were calculated using 11 different formulas by using the results of total cholesterol, triglyceride, and high-density lipoprotein cholesterol of the patients. For each patient group, directly measured low-density lipoprotein cholesterol and low-density lipoprotein cholesterol values calculated with formulas were evaluated in terms of correlation. **Results**: As a result, a single formula that was valid in all groups could not be determined, but instead, it was obvious that formulas that could be valid at different triglyceride levels came to the fore. **Conclusions**: It seems that the best formula for low-density lipoprotein cholesterol estimation in the Turkish population is not yet available, but we think that different formulas can be preferred according to the triglyceride levels of the patients. In addition, it would be beneficial to develop a new formula by conducting new studies according to the Turkish population.

ÖZ

Amaç: Kandaki yüksek LDL konsantrasyonu, iyi tanımlanmış bir aterojenik risk faktörüdür ve koroner kalp hastalığı için yüksek oranda prediktiftir. Bu nedenle serum LDL düzeylerinin analizi hem doğru hem de kesin olarak yapılmalıdır. Bu çalışma, hastanemizde direkt ölçülen LDL ile indirekt LDL hesaplanmasında kullanılan çeşitli formüllerden elde edilen sonuçları karşılaştırmak için tasarlanmıştır. **Gereç-Yöntem:** Çalışmaya direkt LDL sonuçları ölçülen 175 hasta dahil edildi. Hasta sonuçları trigliserit değerlerine göre dört gruba ayrıldı. Hastaların total kolesterol, trigliserit ve yüksek yoğunluklu lipoprotein kolesterol sonuçları kullanılarak 11 farklı formül kullanıldı ve LDL değerleri hesaplandı. Her hasta grubu için doğrudan ölçülen LDL ve formüllerle hesaplanan LDL değerleri korelasyon açısından değerlendirildi. **Bulgular**: Sonuç olarak, tüm gruplarda geçerli olan tek bir formül belirlenemedi, bunun yerine farklı trigliserit seviyelerinde geçerli olabilecek formüllerin ön plana çıktığı görüldü. **Sonuç**: Türk popülasyonunda LDL tahmini için en iyi formül henüz mevcut değil gibi görünmektedir, ancak hastaların trigliserit düzeylerine göre farklı formüllerin tercih edilebileceğini düşünmekteyiz. Ayrıca Türk nüfusuna göre yeni çalışmalar yapılarak yeni bir formül geliştirilmesi faydalı olacaktır.

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INTRODUCTION

Cardiovascular diseases are among the most common mortality causes worldwide. Low-density lipoprotein cholesterol (LDL) concentration in serum is a predictor of risk of Coronary Heart Disease (CHD) (1). High serum LDL concentration is a commonly accepted atherogenic risk factor which has high predictive value for CHD (2). The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) recommends that serum LDL concentration should be aimed to be kept <100mg/dL at an optimal level. Serum LDL levels are also used for treatment plans and risk classification of the patient (3). Therefore, it is extremely important to detect serum LDL levels precisely and accurately.

β-quantification (separation of lipoproteins by combining ultracentrifugation and precipitation with polyanions) is the reference method for quantitation of LDL in blood. β-quantification needs the use of an ultracentrifuge, needs large sample volume, and is a time-consuming and high-cost technique. For these reasons, the method is not convenient for daily laboratory analyses, and its use is limited to research and specific laboratories (4,5).

Other suggested methods include homogeneous direct measurement (6,7). Homogeneous tests for direct estimation of LDL cholesterol (D-LDL) were developed in 1998 (8). Direct methods require high-cost automated systems and an abundancy of laboratories in developing countries can not afford these systems . Because of these limitations, calculation formula method is used in most laboratories, which is a cheaper and easier approach to LDL estimation.

NCEP ATP III guidelines (3) offer the use of LDL calculated with the Friedewald formula for the prevention of cardiovascular diseases and for determining LDL treatment targets. In routine practice, most clinical laboratories estimate serum LDL concentrations from Total Cholesterol (TC), Triglyceride (TG), and High-Density Lipoprotein Cholesterol (HDL) concentrations using the Friedewald formula. Calculation of LDL with the traditional Friedewald formula is expressed as LDL (mg/dl) = TC-HDL-TG/5 (10). LDL calculated by the Friedewald formula is well correlated with LDL measured by β-quantification, however, it has some shortcomings. The main one is that LDL cholesterol is underestimated at higher triglyceride levels while overestimated at lower triglyceride levels (9). The Friedewald formula is not used to calculate LDL in non-fasting individiauls, when serum TG is >400 mg/dl or <100 mg/dL, or in patients with type III or type I hyperlipoproteinemia (10,11). Obtaining fasting samples is a restriction for Friedewald formula as it assumes the triglyceride/cholesterol ratio to be constant in the Very Low-Density Lipoprotein (VLDL). However, this ratio is modified in post-prandial samples (containing chylomicrons and chylomicron residues). So, if a non-fasting sample is used for LDL calculated by the Friedewald formula, the VLDL level will be overestimated while the LDL level will be underestimated (10). The friedwald formula is also not recommended in patients with Type II diabetes, nephrotic syndrome, and chronic alcohol consumers, as the triglyceride/cholesterol ratio in VLDL changes due to these conditions (12,13,14).

Different formulas for calculating LDL have been proposed over the years, but neither of them has been validated in different populations. This study was designed to compare a variety of formulas used to calculate LDL in our hospital, assuming that the results obtained with directly measured LDL in our laboratory are correct.

MATERIALS AND METHODS

Analytical Methods

The study was carried out in Gülhane Training and Research Hospital Medical Biochemistry Laboratory between 15.01.2022 and 15.04.2022. Serum LDL values measured by the homogeneous method directly with the Beckman Coulter AU680 autoanalyzer in the clinical biochemistry laboratory of our hospital were divided into five groups according to the risk values determined by NCEP (3). According to this classification, LDL results were grouped as follows; Group 1 (optimal): LDL<100 mg/dL, group 2 (near-optimal/over-optimal): LDL=100-129 mg/dL, group 3(at the limit of height): LDL=130-159 mg/dL, group 4 (high): 160-189 mg/dL, group 5 (very high): LDL≥190 mg/dL. A total of 175 patient results, with 35 results in each group, were used in the study. LDL calculations were performed using the 11 formulas given below to compare with directly measured LDL

Table 1: Formulas used for LDL estimation

Formula	Equation
Friedewald (9)	LDL = TC – HDL – (TG/5)
Puavilai (18)	LDL = TC – HDL – (TG/6)
Vujoviç (20)	LDL = TC – HDL – (TG/6,85)
Hattori (19)	LDL = (0,94 × TC) – (0,94 × HDL) – (0,19 × TG)
Anandaraja (17)	LDL = (0,9 × TC) – (0,9 × (TG/5) – 28
Chen (22)	LDL = (0,9 × TC) – (0,9 × HDL) – (0,1 × TG)
Cordova (15)	LDL = 0,7516 × (TC – HDL)
Teerakanchana (23)	LDL = (0,91 × TC) – (0,634 × HDL) – (0,111 × TG) 6,755
Ahmedi (11)	LDL=TC/1,19+TG/1,9-HDL/1,1
DeLong (24)	LDL = TC – HDL – (0,16 × TG)
Rao (21)	LDL = [(4,7 × TC) – (4,364 × HDL) –TG] / 4,487

Table 2: Correlation data of LDL values calculated with formulas in different triglyceride groups according to directly measured LDL

Formula		Group 1 (n=58)	Group 2 (n=49)	Group 3 (n=36)	Group 4 (n=32)
Friedwald	Corr. Coe.	0,691	0,873	0,820	0,754
	p value	<0,001	<0,001	<0,001	<0,001
Puavilia	Corr. Coe.	0,690	0,874	0,824	0,778
	p value	<0,001	<0,001	<0,001	<0,001
Vujoviç	Corr. Coe.	0,688	0,874	0,824	0,858
	p value	<0,001	<0,001	<0,001	<0,001
Hattori	Corr. Coe.	0,691	0,873	0,824	0,754
	p value	<0,001	<0,001	<0,001	<0,001
Anadaraja	Corr. Coe.	0,667	0,854	0,792	0,742
	p value	<0,001	<0,001	<0,001	0,001
Chen	Corr. Coe.	0,686	0,874	0,824	0,859
	p value	<0,001	<0,001	<0,001	<0,001
Cordova	Corr. Coe.	0,680	0,874	0,826	0,662
	p value	<0,001	<0,001	<0,001	0,001
Teerakanchana	Corr. Coe.	0,685	0,871	0,833	0,859
	p value	<0,001	<0,001	<0,001	<0,001
Ahmedi	Corr. Coe.	0,599	0,838	0,811	0,522
	p value	<0,001	<0,001	<0,001	0,018
Delong	Corr. Coe.	0,689	0,874	0,824	0,827
	p value	<0,001	<0,001	<0,001	<0,001
Rao	Corr. Coe.	0,690	0,873	0,824	0,751
	p value	<0,001	<0,001	<0,001	0,001

Corr. Coe.: Correlation Coefficient, Group 1: Triglyceride <150 mg/dL, Group 2: Triglyceride =151-199 mg/dL, Group 3: Triglyceride = 200-399 mg/dL, Group 4: Triglyceride >400 mg/dL. Results with the highest correlation with directly measured LDL are marked in bold.

results. For this, TC, TG, and HDL results of the patients were also driven by Laboratory Information System (LIS) (Table 1). All patient results were sub-grouped to 4 cathegories according to Triglyceride levels (1st group: TG<150 mg/dL, 2nd group: TG=151-199 mg/dL, 3rd group: TG= 200-399 mg/dL, 4th group: TG>400 mg/dL), and within each group; LDL measured directly and LDL values calculated with formulas were evaluated in terms of correlation. The study was approved by the institutional ethics committee (Date: 02.09.2021, Decision no: 2021/14).

Statistical Analysis

All statistical analyzes were performed using the SPSS 22.0 program. Shapiro-Wilk test was used for normality testing. Parametric data were expressed as mean±standard deviation , while nonparametric data were presented as median (min.-max.). The values determined using the formula were evaluated in terms of correlation with the directly measured LDL results. Pearson correlation test was used for parametric data and the Spearman correlation test was used for nonparametric data. Statistical significance was accepted as $p<0.05$ for all analyses.

RESULTS

The correlation results of the formulas used in the calculation for directly measured LDL values among the patient groups classified according to different TG values are presented in Table 2. Accordingly, a single valid formula could not be determined in the entire population, and different formulas came to the fore in each group.

DISCUSSION

It is imperative to analyze serum LDL levels with high precision and accuracy as serum LDL level is an accepted atherogenic risk factor. It is also the basis for CHD risk stratification and the defining factor for treatment settlement. The relationship between LDL levels and CHD risk runs in a wide range of LDL levels from low to high (2,3).

The Friedewald formula is preferred for LDL calculation in majority of clinical laboratories worldwide. A reasonable number of studies have demonstrated the limitations of this formula, while others have proposed that different equations show a superior performance in certain populations. The present study compares different formulas (Friedewald included) with direct LDL detection. Our data show that the Friedewald formula does not show the best performance, except for the group 1 population when TG<150 mg/dL. This shows a contradiction with the results of Sha MFR et al.s results in Bangladesh population which proposed that the Friedewald formula can be chosen until Triglyceride concentrations exceed 700 mg/dL (9).

Cordova et al. proposed an alternative formula which outperformed the Friedewald formula in Brazilian population in a wide range of TG levels (10). Our results showed that the Cordova formula can also be preferred over other formulas, including the Friedewald formula for groups 2 (TG= 151-199 mg/dL) and 3 (TG= 200-399 mg/dL) in the Turkish population.

The Ahmadi formula was validated at TG<300 mg/dL in Iranian subjects (11). In our study, in none of the groups, this formula showed superiority over other formulas. Therefore, it is not suitable for use in the Turkish population. Similarly, the Anandaraja formula was not superior to other formulas in any group. This finding is supported by Gupta S et al., who have shown that the Anandaraja formula did not show any superiority over the Friedewald formula in estimating LDL (12). But these results are in concordant with Anandaraja et al.,'s study, which proposed that their formula had higher accuracy than Friedewald's formula when TG levels were <350 mg/dL (13).

The formulas used showed the best performance in group 2 (TG= 151-199 mg/dL), and Puavilia (14), Vujovic (15), Chen (16), Cordova (10), and Delong (17) formulas gave similar results. In Puivilia et al's work, the modified Friedwald equation performed better than the Friedewald formula in the Indian population within the range of TG>200 mg/dL (14). In our study, on the other hand, the Puavilia formula gave more accurate results than Friedwald in the group with TG>400 mg/dL. The Hattori formula, developed by Hattori et al outperformed the Friedewald formula in the Japanese population, but it did not prooved to be superior than Friedewald formula (18).

In the Serbian population, Vujovic et al. confirmed a modified formula in individuals with triglyceride levels less than 400mg/dL (15). They did not found any significant difference between LDL calculated with the Vujovic formula and direct LDL. Our results showed that the Vujovic formula showed the best performance in

group 2 (TG= 151-199 mg/dL), but did not provide an advantage in any group over the other formulas. The formula developed by Rao et al. didn't show the best correlation in any of the groups, so it is not suitable for use in our population (19). On the other hand, Chen (16) and Teerakanchana formulas showed a better correlation in the group with Triglycerides over 400 mg/dL when compared with other formulas (20).

This study compares the calculated LDL directly with the measured LDL test, and the method compared is not the reference method which is the -quantification method with ultracentrifuge precipitation. Also, only one test for TG, TC, LDL, and HDL parameters was used in the study, and different test methodologies were not taken into account. Low sample numbers in other groups, especially those with TG>400mg/dL can also be listed as a drawback. Finally, besides those used here, there are other equations defined for LDL calculation.

CONCLUSION

We think that the best formula for estimating LDL in the Turkish population is not yet established, but different formulas can be preferred according to the TG levels of the patients. Formulas can be used in different TG ranges and different formulas can be preferred for calculating LDL inappropriate conditions. It will also be beneficial to develop a new formula by conducting new studies according to the Turkish population. However, further studies are needed to be developed in different countries, ethnic and geographic populations, in different settings, and preferably using larger sample sizes compared to the reference method.

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