Evaluation of alternative calculation methods for determining LDL cholesterol

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Summary: *Background:* Due to limitations of the Friedewald formula, alternative methods for calculating low-density lipoprotein cholesterol (LDL-C) were suggested. We evaluated utility of these methods. *Methods:* Ninety three subjects free of coronary heart disease were considered. LDL-C was measured by the homogeneous method, and calculated by the Friedewald formula LDL-C = TC – HDL – (TG/2.2) (LDL1) and alternative formulas LDL-C = 0.41 TC – 0.32 TG + 1.70 apoB – 0.27 (LDL2) and LDL-C = 0.94 TC – 0.94 HDL – 0.435 TG (LDL3). *Results:* All three formulas underestimated the measured LDL-C, both in the whole group and in subgroups according to TG levels (TG < 1.7 and in a range of 1.7–4.5 mmol/l, p < 0.001 for all). We found significantly higher bias for all three formulas in subjects with 1.7 ≤ TG < 4.5 mmol/l levels. The Friedewald formula showed the lowest assay bias in all the groups investigated. The mean absolute bias for LDL1 was 7.6%, 18.3% for LDL2 and 13.6% for LDL3, respectively. Linear regression analysis showed correlation of calculated LDL-C values with the direct method in the range of r = 0.82 – 0.90 (p < 0.0001 for all, except of LDL2 in 1.7 ≤ TG < 4.5 mmol/l group where p = 0.0011). *Conclusions:* The Friedewald formula seems to be a better estimator of LDL-C in our study than the other two alternative formulas; however, it underestimated the LDL-C levels.

Key words: LDL-C – alternative calculation formula – Friedewald formula

Zhodnotenie alternatívnych metód pre výpočet LDL cholesterolu

Súhrn: *Úvod:* Vzhľadom na známe limity Friedewaldovej formuly boli navrhnuté aj alternatívne metódy výpočtu LDL cholesterolu (LDL-C). V tejto práci sme posudzovali užitočnosť týchto metód. *Metódy:* Do súboru sme zaradili deväťdesiat tri osôb bez ischemickej choroby srdca. LDL-C sme priamo stanovili homogénnou metódou, vypočítali Friedewaldovou formulou LDL-C = TC – HDL – (TG/2.2) (LDL1) a alternatívne ako LDL-C = 0,41 TC – 0,32 TG + 1,70 apoB – 0,27 (LDL2) a LDL-C = 0,94 TC – 0,94 HDL – 0,435 TG (LDL3). *Výsledky:* Všetky tri výpočty podhodnotili priamo stanovené hladiny LDL-C, v celom súbore a aj v skupinách podľa hladín TG (TG < 1,7 a v rozsahu 1,7–4,5 mmol/l, p < 0,001 pre všetky porovnania). Zaznamenali sme však významne vyššiu odchýlku vo všetkých troch výpočtoch u osôb s hladinou 1,7 ≤ TG < 4,5 mmol/l. Najnižšiu odchýlku v oboch sledovaných skupinách sme zaznamenali pre Friedewaldovu formulu. Absolútna odchýlka bola 7,6 % pre LDL1, 18,3 % pre LDL2 a 13,6 % pre LDL3. Výpočtami stanovené hladiny LDL-C korelovali s metódou priameho stanovenia v rozmedzí r = 0,82 – 0,90 (p < 0,0001 pre všetky s výnimkou LDL2 pre skupinu 1,7 ≤ TG < 4,5 mmol/l, kde p = 0,0011). *Záver:* Friedewaldova formula sa javí v našej štúdii optimálnejšou metódou výpočtu LDL-C ako ďalšie dve alternatívne metódy, avšak podhodnocuje priamo stanovené LDL-C.

Kľúčové slová: LDL-C – alternatívne metódy výpočtu – Friedewaldova formula

Introduction

Low-density lipoprotein cholesterol (LDL-C) is a well established risk factor of atherosclerosis and cardiovascular disease (CVD), and CVD risk calculation and treatment goals both in primary and secondary preventions are based on LDL-C levels. The reference method for LDL-C measurement is direct measurement by the β-quantification method [1]. Because the direct method is time-consuming and involves ultracentrifugation and a chemical precipitation step that is not available in routine laboratories, the Friedewald formula for LDL-C calculation is used as a standard method in clinical practice [2]. There are, however, several limitations of the formula. It multiplies the errors derived from total cholesterol (TC), triglyceride (TG) and HDL-C measurements, is based on an assumption of fixed mass ratio of plasma TG to very-low density lipoprotein cholesterol (VLDL-C), is not valid if TG > 4.5 mmol/l and in patients with type III hyperlipoproteinemia, and requires fasting specimens. Moreover, the reliability of the Friedewald calculation decreases considerably with increasing TG concentrations, even in specimens with TG concentration of 2.26–4.52 mmol/l [3]. In the last decades, there have been several attempts to suggest alternative ways for LDL-C calculation [4,5]. Recently, a new generation of homogeneous methods for LDL detection, have also been introduced. Homoge-

Table 1. Main characteristics of the study population.

	All	Tg < 1.7 mmol/l	1.7 ≤ TG < 4.5 mmol/l
N	93	77	16
age (years)	43.5 ± 12.3	43.2 ± 12.9	45.2 ± 8.9
male/female (%)	18.3/81.2	16.9/83.1	25/75
TC (mmol/l)	5.97 ± 1.16	5.74 ± 1.07	7.08 ± 0.97
TG (mmol/l)	1.31 ± 0.56	1.10 ± 0.30	2.30 ± 0.47
HDL (mmol/l)	1.43 ± 0.33	1.46 ± 0.33	1.25 ± 0.26
apoB (gl/l)	1.00 ± 0.23	0.95 ± 0.20	1.25 ± 0.17

TC – total cholesterol, TG – triglycerides

neous assays seem to be able to meet current National Cholesterol Education Program (NCEP) requirements for LDL-C testing for precision (CV < 4%) and accuracy (bias < 4%) [3]. The aim of this study was to evaluate the alternative calculation methods for determining LDL cholesterol and its' utility regarding TG levels.

Methods

Ninety three subjects older than 18 years, with TG levels less than 4.5 mmol/l, and free of coronary heart disease (CHD) participating in a cross-sectional screening for cardiovascular risk factors in Slovakia were involved in the analysis. We report results for the whole group and in subgroups with normotriglyceridemia and TG levels in range of 1.7–4.5 mmol/l. The main characteristics of the group are in the table 1. Venous blood samples were collected after overnight fasting without cubital compression. Serum levels of total cholesterol (TC) and triglycerides (TG) were measured enzymatically, apoB levels were measured by an immunoturbidimetric method, HDL cholesterol (HDL-C) was determined directly by commercial kit (Genzyme) on an autoanalyser (Hitachi 911). LDL-C was measured by homogeneous method (LDL), based on the elimination principle (Direct LDL-Cholesterol Randox, Randox Laboratories Ltd, Crumlin, UK). The assay was performed according to the manufacturer's recommendation. Using control sera level I, II and III, the within-run imprecision was 0.69, 0.57, and 0.50 (%) and between-run imprecision was 1.67, 1.21 and 1.69 (%), respectively. LDL-C was calculated by the Friedewald formula LDL-C = TC – HDL – (TG/2.2) (LDL1) [2] and alternative formulas LDL-C = 0.41 TC – 0.32 TG + 1.70 apoB – 0.27 (LDL2) [4] and LDL-C = 0.94 TC – 0.94 HDL – 0.435 TG (LDL3) [5], expressed in mmol/l.

Values are expressed as mean ± SD. Linear regression analysis was used for the determination of correlation between parameters. The H0 hypothesis was that slope = 1 and intercept = 0, and was tested in the regression analysis. Assay bias expressed as delta (Δ) was calculated as the direct measurement of LDL result minus the formulas' calculation result. The differences between the direct measurement and calculated values referred as a delta values were evaluated using a signed rank test. Intergroup differences in assay bias were compared by using a Mann-Whitney two-sample ranksum test. A two-tailed P value of less than 0.05 was considered to indicate statistical significance. All computations were carried out with the SAS statistical package (SAS System for Windows V8) and with STATA (STATA/SE 9.0 for Windows). The study was approved by the local Ethics Committee of the Medical School of Comenius University in Bratislava and all subjects signed informed consent.

Results

Means of directly measured and calculated LDL-C, together with regression models and correlation coeficients are in the table 2. All three formulas significantly underestimated the LDL-C levels compared to directly measured LDL both in the whole group and in two subgroups divided according to their TG levels ($p < 0.001$ for all Δ values). In fact, only 15.1% of values calculated by the Friedewald formula and 2.2% by LDL3 formula were equal or higher than values measured by the direct method. LDL2 formula underestimated the LDL in all the cases. When we compared assay bias according to the TG levels, we found significantly higher bias for all three formulas in subjects with 1.7 ≤ TG < 4.5 mmol/l levels. The Friedewald formula showed the lowest assay bias in all the groups investigated. The mean absolute bias for LDL1 was 7.6%, 18.3% for LDL2 and 13.6% for LDL3, respectively. Linear regression analysis showed significant correlation of calculated LDL-C values with the direct method with a range of $r = 0.82–0.90$ ($p < 0.0001$ for all, except of LDL2 in 1.7 ≤ TG < 4.5 mmol/l group where $p = 0.0011$).

Discussion

A causative relation of LDL-C to CHD has been well established in epidemiological studies and clinical trials. The accurate determination of LDL-C is thus an important component of the assessment of the CHD risk. In our study we evaluated alternative formulas for the LDL-C calculation in comparison with a homogeneous LDL-C assay. LDL2 formula was based on a calculation using TC, TG and apoB levels. ApoB is believed to be a better predictor of CHD risk than LDL-C [6] and several advantages of LDL-C calculation by using the apoB based formula have been suggested. The formula can be used in hypertriglyceridemic patients and HDL-C measurement by precipitation can be avoided. Moreover, the formula has been suggested to be superior to the Friedewald calculation in estimating LDL-C levels [7–9].

Table 2. Description of LDL-C data by different methods of estimation.

	All	Tg < 1.7 mmol/l	1.7 ≤ TG < 4.5 mmol/l
N	93	77	16
LDL (mmol/l)	4.29 ± 1.11	4.08 ± 1.01	5.30 ± 1.02
LDL1 (mmol/l)	3.96 ± 1.05	3.79 ± 0.98	4.79 ± 0.99
LDL2 (mmol/l)	3.46 ± 0.76	3.34 ± 0.72	4.02 ± 0.73
LDL3 (mmol/l)	3.70 ± 0.98	3.54 ± 0.92	4.48 ± 0.93
assay bias, mmol/l			
ΔLDL1 (LDL-LDL1)	0.33 ± 0.35	0.29 ± 0.32	0.50 ± 0.43$^{\$}$
ΔLDL2 (LDL-LDL2)	0.83 ± 0.49	0.74 ± 0.44	1.28 ± 0.50$^{£}$
ΔLDL3 (LDL-LDL3)	0.59 ± 0.36	0.54 ± 0.32	0.82 ± 0.43$^{\#}$
absolute bias (%)			
LDL1	7.6%	7.2%	9.5%
LDL2	18.3%	17.2%	23.7%
LDL3	13.6%	13.2%	15.5%
linear regression model*			
LDL1	0.90x + 0.097 (0.90)	0.92x + 0.024 (0.90)	0.88x + 0.110 (0.82)
LDL2	0.64x + 0.720 (0.86)	0.66x + 0.654 (0.86)	0.64x + 0.631 (0.79)
LDL3	0.84x + 0.095 (0.90)	0.86x + 0.021 (0.90)	0.82x + 0.112 (0.82)

*$y = \beta_1 x + \beta_0$, x = LDL (correlation coeficient), $^{\$}p < 0.05$, $^{£}p < 0.001$, $^{\#}p < 0.01$ for comparisons of different TG groups

LDL – directly measured LDL, LDL1 Friedewald formula: LDL-C = TC – HDL – (TG/2.2), LDL2: LDL-C = 0.41 TC – 0.32 TG + 1.70 apoB – 0.27, LDL3: LDL-C = 0.94 TC – 0.94 HDL – 0.435 TG, expressed in mmol/l

In our study, all of the formulas significantly underestimated the LDL-C levels measured by the homogeneous LDL-C assay. Our finding of underestimation of LDL-C by the Friedewald formula is in agreement with a large Canadian study revealing a tendency of the Friedewald formula to underestimate LDL-C in comparison to β-quantification with a mean percent error of calculated LDL-C less than 5% in subjects with TG < 4.5 mmmol/l [10]. The apoB based LDL2 formula showed the biggest assay bias and worst correlation with the homogeneous LDL-C assay. This is in contrast with results published by Bairaktari et al [7–9], where the apoB based formula was superior to Friedewald when compared with β-quantification method. This can be at least partially explained by different populations included in the studies (hemodialysis patients) and by different reference method of LDL-C measurement used. However, the homogeneous LDL-C assay by Roche has been shown to have a tendency to underestimated LDL-C levels compared to β-quantification method [3], suggesting that the potential imprecision of the homogeneous LDL-C assay used in our study can shift our result towards underestimation of true LDL-C values by the calculation formulas. Although the apoB based LDL2 formula in our study was inferior to other two calculation formulas, apoB itself is a close reflection of the number of LDL particles, can be measured with a high precision, and it has been suggested being a better index of cardiovascular risk than LDL cholesterol [6]. ApoB measurement can be especially useful in patients with hypertriglyceridemia and in disorders associated with hypertriglyceridemia, such as diabetes mellitus or metabolic syndrome. In such disorders measurement of LDL cholesterol may not be an accurate reflection of LDL particles, due to an increased number of small dense LDL particles. We have recently shown increased apoB levels despite of comparable LDL cholesterol levels in subjects with TG in a range of 2.0–4.5 mmol/l compared to normotriglyceridemic subjects [11].

When we compared our data according to TG status, we have found higher assay bias and worse correlations between the calculated LDL-C and measured ones in subjects with 1.7 ≤ TG < 4.5 mmol/l. These findings are in accordance to previously published data for the Friedewald and LDL3 formulas [3,8], however, it has been suggested that apoB based LDL2 formula is less affected by increased TG levels [8,9].

Our study has several limitations. First of all, we used a homogeneous LDL-C assay and not the reference β-quantification method to measure LDL-C levels. However, homogeneous assays seem to be able to meet current NCEP requirements for LDL-C testing for both precision and accuracy. Second, the results of the hypertriglyceridemic group is based on 16 observations only, therefore we can not exclude spurious findings due to small number of observations. Moreover, we did not search for presence of type III hyperlipidemia for which the Friedewald formula is not valid. Even that type III hy-

perlipidemia is an uncommon genetic disorder of lipoprotein metabolism we thus can not exclude a presence of type III patient(s) in our set of subjects.

In conclusion, the Friedewald formula seems to be a better estimator of LDL-C in our study than the other two alternative formulas, both in normo and hypertriglyceridemic subjects (with TG < 4.5 mmol/l), but it underestimated the LDL-C levels measured by direct method for LDL-C measurement (Randox, UK). We should however consider, that all the current clinical guidelines and recommendations for lipid monitoring, treatment and cardiovascular disease prevention are based on data from large epidemiological studies and clinical trials, that have estimated LDL cholesterol by the Friedewald formula. Therefore in a routine clinical practice underestimation of LDL cholesterol levels by the Friedewald formula compared to the direct LDL measurement in fact does not create a significant problem.

Acknowledgements
The study was supported by the VEGA grant of Slovak Ministry of Education (AFAV 1/7236/20) and the Grant Agency of School of Medicine, Comenius University, Bratislava, Slovakia

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Doručeno do redakce: 15. 5. 2008
Přijato po recenzi: 19. 6. 2008