JOURNAL OF CLINICAL MEDICINE OF KAZAKHSTAN (E-ISSN 2313-1519)
Original Article DOI: https://doi.org/10.23950/jcmk/12904

Comparison of direct low density lipoprotein cholesterol measurement with the Friedewald formula and alternative formulas

Nilgun Isiksacan[1], Cennet Yildiz[2], Fatma Nihan Turhan Caglar[2], Murat Koser[3], Pinar Atar[1], Ismail Biyik[4], Dilay Karabulut[1], Mehmet Erturk[5]

1Department of Biochemistry, University of Health Sciences, Bakirköy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey
2Department of Cardiology, University of Health Sciences Bakirkoy Dr. Sadi Konuk Education and Research Hospital, Istanbul, Turkey
3Department of Biochemistry, Goztepe Prof. Dr. Suleyman Yalcin City Hospital, Istanbul, Turkey
4Department of Cardiology, Usak University, School of Medicine, Education and Research Hospital, Usak, Turkey
5Cardiology Department, University of Health Sciences, Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, Istanbul, Turkey

Received: 2022-10-21.
Accepted: 2022-12-24

J Clin Med Kaz 2023; 20(1):24-30

Corresponding author:
Cennet Yildiz.
E-mail: cennet_yildiz@live.com;
ORCID: 0000-0003-2456-3206

Abstract

Aim: Our aim was to compare the direct enzymatic measurement with four formulas which are used in determining the value of low density lipoprotein cholesterol (LDL-C) levels.

Material and methods: A total of 33842 patients' files were retrospectively reviewed and data was collected. Triglyceride (TG) group 1, 2, 3, 4 and 5 were consisted of TG levels ≤99 mg/dl, 100-199 mg/dl, 200-299 mg/dl, 300-399 mg/dl and ≥ 400 mg/dl, respectively. LDL-Group 1, 2, 3, 4 and 5 were composed of LDL-C≤100 mg/dl, 101-130 mg/dl, 131-160 mg/dl, 160-190 mg/dl and >190 mg/dl, respectively.

Results: All formulas tended to undervalue LDL-C concentrations compared to direct method (p<0.001 for all). The Chen formula had higher degree of correlation compared to other formulas. Acceptable result of Friedewald formula was 53.77%, Chen formula was 62.72%, Hattori formula was 24.72, and Anandaraja formula was 45.98%. Bland-Altman plot results showed disagreement of four formulas with significant proportional and systematic bias compared to direct method. There was no agreement of calculated LDL-C with direct LDL-C when the data was subgrouped according to TG levels. No agreement between direct LDL-C and calculated LDL-C was found. Correlation analysis showed moderate to high level of correlation for Friedewald, Chen, and Hattori calculations, whereas Anandaraja formula showed low to moderate correlation. The Friedewald and Anandaraja formulas mostly misclassified LDL-Group 3 subjects, whereas the Chen and Hattori formulas mostly misclassified LDL-Group 4 subjects.

Conclusion: The Chen formula might be an acceptable alternative of the Friedewald formula and other formulas.

Key words: lipids, laboratory methods, cardiology

Introduction

Low density lipoprotein cholesterol (LDL-C) is complicit in the pathophysiology of atherosclerotic coronary artery disease (CAD). LDL-C lowering therapy has been a major target both in the treatment and follow-up of patients with hyperlipidemia and CAD. Current cardiac guidelines highlight the importance of achieving and maintaining recommended LDL concentrations based on cardiovascular risk exposure. National Cholesterol Education Programme (NCEP) Working Group advocates that the total analytical error of LDL-C measurement should be within ±12% [1]. Hence accurate estimation of LDL-C levels is crucial.

Ultracentrifugation followed by beta-quantification

is the best method for measuring LDL-C levels. However, it is expensive, laborious and requires skilled staff which makes it difficult for them to use in most clinical laboratories [2]. In addition homogenous direct methods are used to measure cholesterol from LDL fraction [3]. But these methods are also expensive and not readily available in most laboratories. Therefore indirect calculation of LDL-C levels from other lipid parameters is more practical approach in daily practice. The Friedewald formula continues to be the most frequently used method in clinical settings. This formula assumes a fixed factor of 5 for triglyceride (TG) to very low density lipoprotein cholesterol (VLDL-C) ratio and its use is limited in patients who had TG > 400 mg/dl, diabetes mellitus, nephrotic syndrome, and alcoholism [4,5]. This formula does not take in account inter-individual variability in TG to VLDL-C ratio. Lipid Research Clinics Prevalence study demonstrated that TG to VLDL-C ratio range from 5.2 to 8.9 [6]. It has been shown that Friedwald formula underestimates LDL-C levels by 8% in diabetic patients. Another drawback of Friedwald formula is that it needs fasting in order to calculate LDL-C levels since nonfasting status leads to underestimation of LDL-C levels. If TG levels are greater than 150 mg/dL, the formula commonly calculates LDL-C levels less than 70 mg/dL, despite directly measured levels of greater than 70 mg/dL [2]. In the era when the Friedewald equation was proposed, an LDL-C ≤70 mg/dL was not yet established as an ideal secondary prevention target for the treatment of high-risk patients. As such, Friedewald formula could lead to misclassification of the patients [7]. In order to circumvent the limitations of the Friedewald formula, several other formulas have been proposed with different results in different populations. In this study direct enzymatic measurement of LDL-C levels was compared with measurements using four different formulas that are used in determining the value of LDL-C levels.

Material and methods

This was a retrospective comparative study which compares direct method of LDL-C measurement with 4 different formulas. It was conducted in a tertiary hospital and the data regarding patient's biochemical variables was obtained from hospital database system which was screened between January 2016 and January 2018. Ethical approval was obtained from Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Education Hospital ethical committee and it was carried out in conformity with the Declaration of Helsinki. A total of 33842 patients were included in the study. Mean age of the study population was 53.23±14.35 years, 18816 (55.6%) of them were female, 15026 (44.4%) of them were male. Patients with hepatic/renal failure, ischemic heart disease, stroke, heart failure, diabetes mellitus, malignancy, thyroid function abnormalities, high density lipoprotein cholesterol (HDL-C) < 20 mg/dl were excluded. After overnight fast venous blood samples were drawn from the patients. All measurements were done by using Roche/Hitachi Cobas c 501 auto analyzer system (Roche Diagnostics catalog number: 07005717 system ID 07 7565 7.). A homogeneous enzymatic colorimetric assay was used to measure direct LDL-C. This automated direct estimation of LDL-C based on micellar solubility of LDL-C with nonionic detergent and interaction of a sugar compound and lipoproteins. For the assessment of cholesterol in lipoprotein subgroups, cholesterol esterase/cholesterol oxidase linking reaction was conducted. This analysis met the NCEP criteria in terms of precision, accuracy. And total analytical error was less than 12 %. TG level evaluated by lipoprotein lipase.

LDL-C levels were measured by four different calculations including Friedewald, Chen, Hattori, and Anandaraja formulas. Calculation of LDL-C for each formula were as follows:

Friedewald formula: LDL-C (mg/dL) = Total cholesterol (TC) -HDL-C -TG/5

Chen formula: LDL-C (mg/dL) = (TC-HDL) x 90 TG×%10

Hattori formula: LDL-C(mg/dL) = 0.94x TC-0.94 ×HDL -0.91 × TG

Anandaraja formula: LDL-C (mg/dl) = 0.9 x TC - 0.9xTG/5 – 28

Since Friedewald formula has acceptable accuracy when LDL-C is average and TG levels are not elevated, we divided patients according to their TG and LDL-C levels [8]. Data were splitted into 5 groups according to TG levels. TG group 1, 2,3,4 and 5 were consisted of TG levels ≤99 mg/dl, 100-199 mg/dl, 200-299 mg/dl, 300-399 mg/dl and ≥ 400 mg/dl, respectively. Subjects were also split into five groups according to their LDL-C levels: LDL-Group 1, 2, 3, 4 and 5 were composed of LDL-C≤100 mg/dl, 101-130 mg/dl, 131-160 mg/dl, 160-190 mg/dl and >190 mg/dl, respectively. For each group, calculated LDL-C was compared with direct method. In addition, misclassification percentages of LDL-C levels were also calculated. Each LDL-Group was further analysed according to TG concentrations: TG concentrations less than 200 mg/dl (n=25357), TG concentrations between 200-400 (n=7715), and TG concentrations higher than 400 mg/dl (n=770). If the difference between calculated and direct LDL-C concentration fell into range of ±10 mg/dl, that was described as an acceptable result. Acceptable result of each formula was also calculated. Flowchart of the study is shown in Figure 1.

Statistics

Normality of the data was assessed by Kolmogorow-Smirnow test. Normally and non-normally distributed data were expressed as mean±SD and median-IQR, respectively. For the comparison of two groups Mann-Whitney U test was used. Correlation analysis was done by Spearman Correlation analysis. In order to assess agreement of two methods, Bland Altman plot analysis was done. Analyses were done by using MedCalc Statistical Software version 12.7.7 programme.

Results

Average age of the study population was 54.27+13.09 years, 15183 (44.9%) of them were male and 18659 (55.1%) were female. The mean LDL-C concentration value via direct measurement was 128.43±31.46 mg/dl, TC levels were 199.86±37.68 mg/dl, TG levels were 170.82±83 mg/dl. Most of the subjects had LDL-C values between 100-130 mg/dl (32.8%). Males had significantly higher levels of LDL-C levels in contrast to females (129.92±31.01 mg/dl vs 126.59±31.90 mg/, p<0.001). A comparison analysis showed that all formulas tended to undervalue LDL-C concentration compared to direct method (p<0.001 for all). Mean differences between direct method and the Friedewald, Chen, Hattori, and Anandaraja formulas were 9.22±16.19 mg/dl, 7.47±13.42 mg/dl, 16.71±15.65 mg/dl and 7.31±18.15 mg/dl, respectively. Mean percentage change of LDL-C levels between calculated and direct methods were -7.22±14.17%, -5.37±11.73%, -13.06±13.38%, -5.12±15.69% for the Friedewald, Chen, Hattori, and Anandaraja formulas, respectively. Correlation analysis showed that the Chen formula had higher degree of correlation compared to other formulas. Biochemical results and correlation analysis of the calculated and direct LDL-C are shown in Table 1. Bland-Altman plot results showed disagreement of four formulas with significant proportional and systematic bias compared to direct method (Table 2, Figure 2). There were no agreement of calculated LDL-C with direct LDL-C when the data was subgrouped

Table 1 Biochemical parameters of the patients.

		TG Group 1 TG≤99 mg/dl (n=3888)	TG Group 2 100-199 mg/dl (n=21469)	TG Group 3 200-299 mg/dl (n=6146)	TG Group 4 300-399 mg/dl (n=1565)	TG Group 5 ≥400 mg/dl (n=774)
Age (years)	55 (18-97)	54 (18-93)	55 (18-97)	54 (18-92	53 (19-92)	53(19-86)
TC (mg/dl)	199 (81-379.6)	181(81-289)	196(84-304)	211(101-319)	223(120-305)	240(151-379.6)
TG (mg/dl)	148 (87-985)	93(87-99.9)	138(100-99,9)	233(200-299.8)	335(300-399)	474(400.1-985)
HDL-C (mg/dl)	45 (30-108)	43(30-105)	44.4(30-108)	46(30-104)	46(30-92.30)	47(30-93)
LDL-C (mg/dl)	128 (35-200)	84(35-100.3)	122.5(71-160.3)	162(130.9-200)	174(118.11-200)	175(160.6-200)
F-LDL-C (mg/dl)	(16.6-216.12)	75.6(16.6-211.8)	113.6(17-215)	150.6(17.6-216.1)	162.4(21.4-214)	165.3(36.8-214.6)
Direct-Friedewald						
difference	9.22±16.19	6.17±15.77	8.82±15.48	10.33±15.85	16.91±23.15	11.24±17.56
r**	0.886	0.767	0.817	0.815	0.553	0.593
C-LDL-C (mg/dl)	(24.4-210.06)	80.2(24.4-199.9)	115.4(25.7-207.9)	150.8(26.7-210.0)	161(38.7-200.6)	163(61.9-208)
Direct- Chen						
difference	7.47±13.42	1.8±13.25	6.79±12.49	10.40±12.54	17.19±19.49	12.15±13.94
r**	0.913	0.819	0.861	0.868	0.657	0.688
H-LDL-C (mg/dl)	111.34 (14.9-202.9)	70.75(15.17-198.86)	106.42(14.89-201.85)	141.13(15.89-202.89)	152.34(19.42-200.96)	154.93(33.81-201.39)
Direct-Hattori						
difference	16.71±15.65	10.95±14.98	15.97±14.77	19.76±15.03	26.85±21.87	21.49±16.64
r**	0.885	0.765	0.815	0.813	0.550	0.590
A-HDL-C (mg/dl)	120.68(22.94-215.36)	79.64(23.66-213.56)	116(22.94-215)	150.15(25.64-215)	161(36.8-214.28)	164(36.6-215.36)
Direct-Anandaraja						
Difference	7.31±18.15	1.09±17.42	6.45±17.43	10.90±17.50	17.90±23.20	12.24±19.16
r**	0.842	0.658	0.736	0.739	0.472	0.509

** All correlations are significant at the 0.01 level (2-tailed). TC: Total Cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, F-LDL-C: Friedewald low density lipoprotein cholesterol, C-LDL-C: Chen low density lipoprotein cholesterol, H-LDL-C: Hattori low density lipoprotein cholesterol, A-LDL-C: Anandaraja low density lipoprotein cholesterol

Table 2 Bland-Altman plot results of the four formulas.

	Mean	Upper limit	Lower limit	p
Friedewald	9.22	40.97	-22.52	<0.0001
Chen	7.47	33.79	-18.83	<0.0001
Hattori	16.71	47.39	-13.95	<0.0001
Anandaraja	7.31	42.89	-28.28	<0.0001

Table 4 Misclassification of subjects according to calculated LDL-C values.

	Friedewald	Chen	Hattori	Anandaraja
LDL Group 1 (%)	6.3	6.73	3.89	15.90
LDL Group 2 (%)	33.67	24.05	49.28	38.65
Underclassified	28.61	19.97	47.01	26.66
Overclassified	5.06	4.08	2.27	11.99
LDL Group 3 (%)	39.60	34.74	60.98	45.20
Underclassified	35.64	32.41	59.84	37.54
Overclassified	3.96	2.33	1.14	7.66
LDL Group 4 (%)	46.1	45.4	69.6	53.3
Underclassified	42.5	44.3	69.1	47.7
Overclassified	3.6	1.1	0.5	5.6
LDL Group 5 (%)	64.6	77.4	91.0	69.8

LDL-C: low density lipoprotein cholesterol.

according to TG levels (Bland-Altman p value <0.0001, for all). All formulas underestimated LDL-C concentration in all TG groups. Acceptable result of the Friedewald formula was 53.77%, the Chen formula was 62.72%, the Hattori formula was 24.72, and the Anandaraja formula was 45.98%.

We analyzed data according to LDL-C levels in order to evaluate whether there were an agreement between calculated and directly measured LDL-C levels. There were no agreement between direct LDL-C and calculated LDL-C by four formulas.

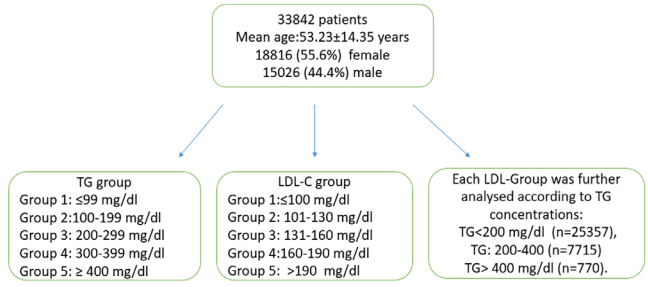

Figure 1 - Flowchart of the study.

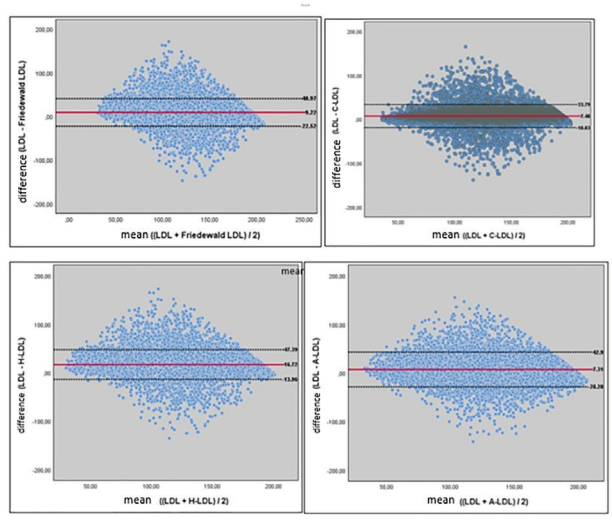

Figure 2 - Bland-Altman plots showing proportional and systemic bias between two sets of measurements. The solid line shows the mean difference between the values while the dotted lines are the upper and the lower limits of agreement (95% observed differences).

Table 3 Correlation analysis and Bland-Altman plot results of LDL-C groups.

LDL GROUP 1 (<100 mg/dl, n=6701)	**Bland-Altman plot results**				**Correlation analysis**		**Correlation analysis (TG <200)**		**Correlation analysis (TG 200-400)**		**Correlation analysis (TG >400)**	
	Mean	Upper limit	Lower limit	p	r	p	r	p	r	p	r	p
Friedewald LDL-C	6.31	39.01	-26.38	**<0.0001**	0.706	**<0.001**	0.766	**<0.001**	0.666	**<0.001**	0.643	**<0.001**
Chen LDL-C	2.09	29.58	-25.39	**<0.0001**	0.770	**<0.001**	0.784	**<0.001**	0.707	**<0.001**	0.595	**<0.001**
Hattori LDL-C	11.27	42.27	-19.28	**<0.0001**	0.703	**<0.001**	0.765	**<0.001**	0.664	**<0.001**	0.645	**<0.001**
Anandaraja LDL-C	1.25	36.57	-34.04	**<0.0001**	0.589	**<0.001**	0.652	**<0.001**	0.628	**<0.001**	0.638	**<0.001**
LDL GROUP 2 (100-130 mg/dl, n=11115)	**Bland-Altman plot results**				**Correlation analysis**		**Correlation analysis (TG <200)**		**Correlation analysis (TG 200-400)**		**Correlation analysis (TG >400)**	
	Mean	Upper limit	Lower limit	p	r	p	r	p	r	p	r	p
Friedewald LDL-C	8.65	36.72	-19.41	**<0.0001**	0.616	**<0.001**	0.699	**<0.001**	0.648	**<0.001**	0.484	**<0.001**
Chen LDL-C	6.06	28.04	-15.51	**<0.0001**	0.713	**<0.001**	0.732	**<0.001**	0.680	**<0.001**	0.420	**<0.001**
Hattori LDL-C	15.18	42.04	-11.16	**<0.0001**	0.612	**<0.001**	0.697	**<0.001**	0.647	**<0.001**	0.483	**<0.001**
Anandaraja LDL-C	-6.14	26.71	-38.38	**<0.0001**	0.459	**<0.001**	0.532	**<0.001**	0.581	**<0.001**	0.492	**<0.001**
LDL GROUP 3 (130-160 mg/dl n=10360)	**Bland-Altman plot results**				**Correlation analysis**		**Correlation analysis (TG <200)**		**Correlation analysis (TG 200-400)**		**Correlation analysis (TG >400)**	
	Mean	Upper limit	Lower limit	p	r	p	r	p	r	p	r	p
Friedewald LDL-C	10.08	40.33	-20.15	**<0.0001**	0.571	**<0.001**	0.660	**<0.001**	0.625	**<0.001**	0.510	**<0.001**
Chen LDL-C	9.32	33.37	-14.72	**<0.0001**	0.671	**<0.001**	0.684	**<0.001**	0.661	**<0.001**	0.452	**<0.001**
Hattori LDL-C	18.49	47.14	-10.62	**<0.0001**	0.568	**<0.001**	0.658	**<0.001**	0.624	**<0.001**	0.510	**<0.001**
Anandaraja LDL-C	9.37	43.06	-24.32	**<0.0001**	0.447	**<0.001**	0.530	**<0.001**	0.578	**<0.001**	0.476	**<0.001**
LDL GROUP 4 (160-190 mg/dl, n=954)	**Bland-Altman plot results**				**Correlation analysis**		**Correlation analysis (TG <200)**		**Correlation analysis (200-400)**		**Correlation analysis (TG >400)**	
	Mean	Upper limit	Lower limit	p	r	p	r	p	r	p	r	p
Friedewald LDL-C	11.95	49.05	-25.14	**<0.0001**	0.389	**<0.001**	0.609	**<0.001**	0.584	**<0.001**	0.514	**<0.001**
Chen LDL-C	12.53	42.66	-17.59	**<0.0001**	0.442	**<0.001**	0.637	**<0.001**	0.630	**<0.001**	0.547	**<0.001**
Hattori LDL-C	22.01	57.07	-13.90	**<0.0001**	0.387	**<0.001**	0.608	**<0.001**	0.582	**<0.001**	0.511	**<0.001**
Anandaraja LDL-C	13.15	52.27	-12,59	**<0.0001**	0.383	**<0.001**	0.522	**<0.001**	0.534	**<0.001**	0.561	**<0.001**
LDL GROUP 5 (>190 mg/dl, n=712)	**Bland-Altman plot results**				**Correlation analysis**		**Correlation analysis (TG <200)**		**Correlation analysis (TG 200-400)**		**Correlation analysis (TG >400)**	
	Mean	Upper limit	Lower limit	p	r	p	r	p	r	p	r	p
Friedewald LDL-C	13.75	58.67	-31.15	**<0.0001**	0.176	**<0.001**	0.226	**<0.001**	0.265	**<0.001**	0.353	0.044
Chen LDL-C	15.01	52.84	-22.81	**<0.0001**	0.260	**<0.001**	0.270	**<0.001**	0.316	**<0.001**	0.434	0.012
Hattori LDL-C	23.46	67.41	-17.30	**<0.0001**	0.174	**<0.001**	0.226	**<0.001**	0.264	**<0.001**	0.346	0.049
Anandaraja LDL-C	16.47	59.47	-26.51	**<0.0001**	0.121	**<0.001**	0.159	**0.001**	0.241	**0.001**	0.352	0.044

LDL-C: low density lipoprotein cholesterol.

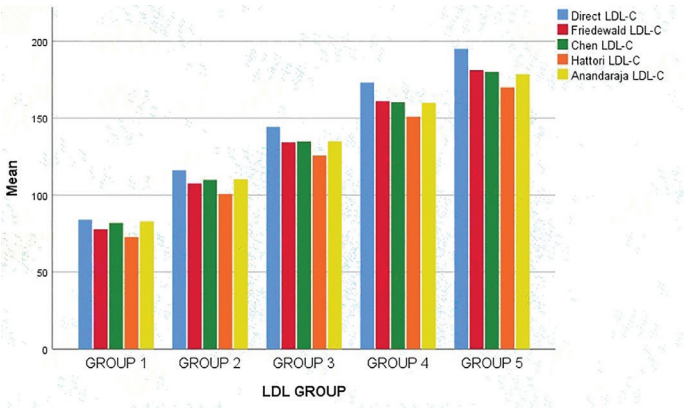

Figure 3 - Comparison of direct LDL-C measurement with Friedewald, Chen, Hattori and Anandaraja formulas in LDL-C groups.

In addition, all of the four formulas were in disagreement with respect to TG level analysis (TG<200 mg/dl, TG 200-400 mg/dl, and TG>400 mg/dl. Bland-Altman plot analysis showed p values less than 0.0001 for all calculations. Figure 3 shows the mean level of LDL-C of LDL-C groups. Correlation analysis showed moderate to high level of correlation for the Friedewald, Chen, and Hattori calculations, whereas the Anandaraja formula showed low to moderate correlation. Bland-Altman plot results and correlation analysis of the four groups are shown in Table 3. The Friedewald and the Anandaraja formulas mostly misclassified LDL-Group 3 subjects, whereas the Chen and the Hattori formulas mostly misclassified LDL-Group 4 subjects. The percentages of misclassification of the subjects with respect to calculated formulas are shown in Table 4.

Discussion

Our analysis indicates that all of the formulas tested underestimated the LDL-C concentration levels compared to direct enzymatic method and were in disagreement with it. The Bland-Altman plot results reveal a systematic and proportional bias in the four formulas. Further analysis of the data as a function of TG fractions indicates that there is no agreement between the calculated LDL-C values and the direct measurement values. Although Anandaraja formula had a slightly lower mean value than that obtained using the formula, the Chen formula had the narrowest range of limits of agreement (between 33.79 and to 18.83 mg/dl). Furthermore, the acceptable result of the Chen formula was 62.72%, higher than the results yielded by the other formulas. Of all the formulas, only the Chen formula had very high correlation with the direct LDL-C measurement.

Despite its limitations, the Friedewald formula remains the most widely used LDL-C calculation method in laboratories. Several studies have shown that the Friedewald formula yields erroneous estimations of LDL-C levels in clinical situations where the TC, TG, and LDL-C concentration levels are low. In addition, the Friedewald formula loses its accuracy when the values for HDL-C levesl are considerably low [9]. This accuracy is dependent on the accurate measurement of TG, TC, and HDL-C levels and a mathematical formula that estimates VLDL-C level. Our findings on the Friedewald formula are consistent with most of the other studies, which found that the Friedewald formual yields lower LDL-C concentration values than the direct enzymatic method [10-13]. According to a Korean study, the Friedewald formula tends to underestimate LDL-C concentration values when TG >150 mg/dl, and then overestimates these values when <150 mg/dl [14]. We found a mean difference of 9.22±16.19 mg/dl between the direct method and the Friedewald calculation. The Friedewald formula underestimated LDL-C concentration values for all TG subgroups in our study, and its correlation with the direct measurement was highest at TG levels between 100 mg/dl-299 mg/dl, with only a moderate correlation at TG levels higher than 300 mg/dl. When the data was analyzed as a function of LDL-C concentrations levels, the correlation between the Friedewald formula and the direct enzymatic method decreased with increasing LDL-C concentration values, with a significantly weak correlation at LDL-C levels greater than 190 mg/dl.

It has been proposed that the Anandaraja formula, which requires two parameters for LDL-C estimation, has a lower analytical error than other formulas [15]. Anandaraja et al. found a strong correlation between their formula and direct measurements, with a correlation coefficient of 0.97. Other studies have reported correlation coefficient values of between 0.658 to 0.930 [16-19]. In most previous reports, the Anandaraja formula underestimated LDL-C levels compared to the direct enzymatic method. Gupta et al. showed that the Friedewald and Anandaraja formulas underestimated LDL-C concentration values, with reported values of 10.8 and 14 mg/dl, respectively [18]. Yet another study also reported that these two formulas underestimated LDL-C concentration leves, with reported values of 17 and 22 mg/dl, respectively [19]. In a study by Gasko et al., the mean difference between the direct method and the Anandaraja formula was only -1 mg/dl [20]. Krishnavemi et al. discovered that the Friedewald calculation had a stronger correlation with the direct enzymatic method than the Anandaraja calculation [21]. In our study, the Anandaraja formula showed a moderate correlation with the direct method ($r=0.842$, $p<0.001$), with an average underestimation of 7.31±18.15 mg/dl. The correlation decreased with increasing LDL-C concentration levels and approximately half of the subjects in LDL-Group 3 and LDL-Group 4 and two thirds of LDL-Group 5 subjects were underclassified.

Martin et al. compared the Friedewald, Chen, Cordova, and Hattori formulas using a sample of hospitalized South African patients and found that the Chen formula overestimated LDL-C concentration values, while the Hattori formula had outperformed other formulas, with an underestimation value of only 1.55 mg/dl [22]. In an Iranian study, eight different formulas were evaluated using a sample of healthy subjects, and values from the Hattori and Cordova formulas were the least different from the estimation values. The Hattori formula over- and underestimated LDL-C levels at TG levels below 150 mg/dl and above 150 mg/dl, respectively. Although the Chen formula overvalue LDL-C levels at all TG concentrations, the Anandaraja formula overestimated and underestimated LDL-C levels at TG levels below 60 mg/dl and above 60 mg/dl, respectively [23]. In the present study, the Hattori formula had the highest mean of difference, which increased with increasing LDL-C concentration values. Ninety one percent of the patients in LDL-group 5 were underclassified. LDL-C is the paramount target for cardiovascular risk stratification, preventive strategies and medical treatment of patients. In this context, difference between the direct and calculated methods of deriving LDL-C values is critical for the classification of patients. Our results favored the Chen formula because of all the formulas, it had the highest correlation with the direct enzymatic method, had a mean difference with a narrowest limits of agreement, and lower misclassification rate than the Friedewald, Hattori, and Anandaraja formulas.

Because beta quantification via ultracentrifugation is costly and time-consuming, direct homogenous measurement of LDL-C is the preferred alternative method in most biochemistry laboratories [24]. Research showed that most of the homogenous methods meet the requirements prescribed by the NCEP [25,26]. This present study used the Roche direct LDL-C method, which is a precise and justifiable alternative of beta quantification. Miller et al. compared direct method, which was performed according to Roche/Hitachi analyzer manufacturer instructions, with reference measurement procedures. Their results showed that direct method met the NCEP goals for measuring HDL-C and LDL-C concentration levels in healthy individuals [27]. Our total analytical error was less than 12 %, which is within the total error goal stipulated by the NCEP. Major factor behind the incorrect of LDL-C concentration calculations of various formulas is that they typically need three terms. Hence, any measurement error in the TC, TG, and HDL-C values affect LDL-C estimation. It has been shown that direct measurements of TC and TG levels are in agreement with our reference method; however, it is not the case for HDL-C measurement. Oliveira et al. compared eight different direct HDL-C methods. They found that the accuracy of calculated formula was depend on the specific HDL-C measurement [28]. Measurement errors of HDL-C might be one of the reasons for underestimation of LDL-C.

Limitations

Our study was not generalizable to patients with various comorbidities since we enrolled only healthy subjects in this study. In addition, we did not evaluate outcomes of the subjects. Beta-quantification procedure is international standard method for determining the values obtained from LDL-C direct method by homogeneous assay. In our study, calculated LDL-C levels were not compared with reference method. Lastly, we did not measure lipoprotein(a) concentrations which would have impact on LDL-C measurement.

Conclusion

Our study aimed to find an important research question for countries where homogenous direct measurement methods are not in general distribution. According to our results, the Chen formula might be an acceptable alternative of the Friedewald formula. All the formulas analyzed in the present study had the best correlation at TG levels between 100 mg/dl-299 mg/dl and LDL-C concentrations less than 130 mg/dl. Neverthless, it should be remembered that direct enzymatic LDL-C measurement does not need for fasting and allows us to get results from single analysis.

Disclosures: There is no conflict of interest for all authors.

Acknowledgements: None.

Funding: None.

References

1. Bachorik PS. Ross JW. National Education Program recommendations for measurements of low density lipoprotein cholesterol: executive summary. National Cholesterol Education Program Working Group on Lipoprotein Measurements. *Clin Chem.* 1995;41:1414-20. https://doi.org/10.1093/clinchem/41.10.1414
2. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, et al. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA*. 2013;310(19):2061-8. https://doi.org/10.1001/jama.2013.280532
3. Anwar M, Khan DA, Khan FA. Comparison of Friedewald formula and modified Friedewald formula with direct homogeneous assay for low density lipoprotein cholesterol estimation. *J Coll Physicians Surg Pak.* 2014;24:8-12.
4. Marniemi J. Maki J. Maatela J. Jarvisalo J. Impivaara O. Poor applicability of the Friedewald formula in the assessment of serum LDL cholesterol for clinical purposes. *Clin Biochem*. 1995; 28:285-289. https://doi.org/10.1016/0009-9120(94)00095-D
5. Martin SS. Blaha MJ. Elshazly MB. Brinton EA, Toth PP, McEvoy JW, et al. Friedewald-estimated versus directly measured low-density lipoprotein cholesterol and treatment implications. *J Am Coll Cardiol.* 2013a;62:732-739. https://doi.org/10.1016/j.jacc.2013.01.079
6. DeLong DM, DeLong ER, Wood PD, Lippel K, Rifkind BM. A comparison of methods for the estimation of plasma low- and very low-density lipoprotein cholesterol. The Lipid Research Clinics Prevalence Study. *JAMA*. 1986;256:2372-2377. https://doi.org/10.1001/jama.1986.03380170088024
7. Authors/Task Force Members; ESC Committee for Practice Guidelines (CPG); ESC National Cardiac Societies.2019 ESC/EAS guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. Societies. *Atherosclerosis*. 2019;290:140-205. https://doi.org/10.1016/j.atherosclerosis.2019.08.014
8. Cordova CM, Schneider CR, Juttel ID, Cordova MM. Comparison of LDL-cholesterol direct measurement with the estimate using the Friedewald formula in a sample of 10,664 patients. *Arq Bras Cardiol.* 2004; 83: 482-7. https://doi.org/10.1590/S0066-782X2004001800006
9. Timón-Zapata J, Laserna-Mendieta EJ, Pineda- Tenor D, Agudo-Macazaga M, Narros-Cecilia C, Rocha-Bogas MJ, et al. Extreme concentrations of high density lipoprotein cholesterol affect the calculation of low density lipoprotein cholesterol in the Friedewald formula and other proposed formulas. *Clin Biochem.* 2011; 44: 1451-6. https://doi.org/10.1016/j.clinbiochem.2011.09.009
10. Jun KR, Park H, Chun S, Park H, Min WK. Effects of total cholesterol and triglyceride on the percentage difference between the low-density lipoprotein cholesterol concentration measured directly and calculated using the Friedewald formula. *Clin Chem Lab Med.* 2008;46(Supp 3):371-5. https://doi.org/10.1515/CCLM.2008.064
11. Marniemi J, Maki J, Maatela J, Jarvisalo J, Impivaara O. Poor applicability of the Friedewald formula in the assessment of serum LDL cholesterol for clinical purposes. *Clin Biochem.* 1995;28:285-9. https://doi.org/10.1016/0009-9120(94)00095-D
12. Tighe DA, Ockene IS, Reed G, Nicolosi R. Calculated low density lipoprotein cholesterol levels frequently underestimate directly measured low density lipoprotein cholesterol determinations in patients with serum triglyceride levels or B4.52 mmol/l: an analysis comparing the LipiDirect_ magnetic LDL assay with the Friedewald calculation. *Clin Chim Acta.* 2006;365:236-42. https://doi.org/10.1016/j.cca.2005.08.026
13. Bansal E, Kaur N. Does Friedewald Formula Underestimate the Risk of Ischemic Heart Disease? *Ind J Clin Biochem.* 2014; 29(4):496-500. https://doi.org/10.1007/s12291-013-0392-2
14. Hwang YC, Ahn HY, Jeong IK. Optimal range of triglyceride values to estimate serum low density lipoprotein cholesterol concentration in Korean adults: the Korea National Health and Nutrition Examination Survey, 2009. *J Korean Med Sci.* 2012;27:1530-1535. PMID: 23255853. https://doi.org/10.3346/jkms.2012.27.12.1530
15. Anandaraja S, Narang Ahn KJ, Chung HY. R, Godeswar R, Laksmy R, Talwar KK. Low density lipoprotein cholesterol estimation by a new formula in Indian population. *Int J Cardiol.* 2005;102:117-20. https://doi.org/10.1016/j.ijcard.2004.05.009
16. Ephraim RKD, Acheampong E, Swaray SM, Anto EO, Agbodzaykey H, Adoba P, et al. Developing a Modified Low-Density Lipoprotein (M-LDL C) Friedewald's Equation as a Substitute for Direct LDL-C Measure in a Ghanaian Population: A Comparative Study. *Lipids*. 2018;2018:7078409. https://doi.org/10.1155/2018/7078409
17. Vujovic A, Stevulijevic JK, Spasic S, Bujisic N, Martinovic J, Vujovic M, et al. Evaluation of different formulas for LDL-C calculation. *Lipids Health Dis.* 2010;9:27. https://doi.org/10.1186/1476-511X-9-27
18. Gupta S, Verma M, Singh K. Does LDL-C Estimation Using Anandaraja's Formula Give a Better Agreement with Direct LDL-C Estimation than the Friedewald's Formula? *Ind J Clin Biochem.* 2012; 27(2):127-133. https://doi.org/10.1007/s12291-011-0186-3
19. Kamal AHM, Hossain M, Chowdhury S, Mahmud NU. A comparison of calculated with direct measurement of low density lipoprotein cholesterol level. JCMCTA. 2009;20:19-23. https://doi.org/10.3329/jcmcta.v20i2.5621
20. Gasko R. Low density lipoprotein cholesterol estimation by the Anandaraja's formula confirmation. *Lipids Health Dis.* 2006;5: 18. https://doi.org/10.1186/1476-511X-5-18

21. Krishnaveni P, Gowda VM. Assessing the validity of Friedewald's formula and Anandaraja's formula for serum LDL-cholesterol calculation. *J Clin Diagn Res.* 2015;9:BC01-4. https://doi.org/10.7860/JCDR/2015/16850.6870
22. Martins J, Olorunju SA, Murray LM, Pillay TS. Comparison of equations for the calculation of LDL-cholesterol in hospitalized patients. *Clin Chim Acta.* 2015;444:137-42. https://doi.org/10.1016/j.cca.2015.01.037
23. Karkhaneh A, Bagherieh M, Sadeghi S, Kheirollahi A. Evaluation of eight formulas for LDL-C estimation in Iranian subjects with different metabolic health statuses. *Lipids Health Dis.* 2019;18(1):231. https://doi.org/10.1186/s12944-019-1178-1
24. Mora S, Rifai N, Buring JE, Ridker PM. Comparison of LDL cholesterol concentrations by Friedewald calculation and direct measurement in relation to cardiovascular events in 27,331 women. *Clin Chem.* 2009;55:888-94. https://doi.org/10.1373/clinchem.2008.117929
25. Nauck M, Graziani MS, Bruton D, Cobbaert C, Cole TG, Lefevre F, et al. Analytical and clinical performance of a detergent-based homogeneous LDL-cholesterol assay: a multicenter evaluation. *Clin Chem.* 2010;46:506-14. https://doi.org/10.1093/clinchem/46.4.506
26. Esteban-Salan M, Aguilar-Doreste JA, Arranz-Pena ML, Juve- Cuxart S, Gich-Salarich I, Zapico-Muniz E, et al. Multicentric evaluation of the homogeneous LDL-cholesterol Plus assay: comparison with beta quantification and Friedewald formula. *Clin Biochem.* 2008;41:1402-9. https://doi.org/10.1016/j.clinbiochem.2008.07.014
27. Miller WG, Myers GL, Sakurabayashi I, Bachmann LM, Caudill SP, Dziekonski A, et al. Seven Direct Methods for Measuring HDL and LDL Cholesterol Compared with Ultracentrifugation Reference Measurement Procedures. *Clinical Chemistry.* 2010; 56(6):977-986. https://doi.org/10.1373/clinchem.2009.142810
28. Oliveira MJA, van Deventr HE, Bachmann LM, Warnick GR, Nakajime K, Nakamura M, et al. Evaluation of Four Different Equations for Calculating LDL-C with Eight Different Direct HDL-C Assays. *Clin Chim Acta.* 2013;423:135-40. https://doi.org/10.1016/j.cca.2013.04.009