Original Article :

Does Fredrickson Friedewald's formula and direct assay of LDL concentration in serum give varying results? - A pilot retrospective study in a tertiary care hospital.

Aditi Singh[1], Mukesh Kumar Meena[2], Shailata Prisi[3], Preeti Chauhan[2], Ritu Singh[2], Ankur[2]

1. AGILUS Diagnostics Ltd., FMRI, Gurugram
2. Department of Biochemistry, Lady Hardinge Medical College, New Delhi
3. Department of Biochemistry, Maulana Azad Medical College, New Delhi

Received on Jan 16, 2024, accepted for publication on May 05, 2024, dx.doi.org/10.5455/mjhs.2024.03.004

Corresponding author:

Dr. Shailata Prisi

E-mail: drshailataprisi@gmail.com - Contact No: +91-7903593242

OPEN ACCESS Distributed under Creative Commons CC BY-NC-ND 4.0

Abstract

Background and Aims:
The LDL-C levels guide the cardiovascular risk assessment under the National Cholesterol Education Program Adult Treatment Panel (NCEP) as well as used for the management of dyslipidemia. Traditionally, LDL-C level is calculated using the Friedewald formula i.e., cLDL, however, recently many of the laboratories have shifted to newer methods of direct LDL-C estimation, i.e., dLDL. With this transition, there is increased demand to compare and establish updated LDL-C level cutoffs for the risk assessments of cardiovascular events.

Methods:
A retrospective observational study, comprising 216 study participants, was conducted in the Department of Biochemistry, Lady Hardinge Medical College, and associated hospitals, in New Delhi for 12 months. Data was collected from 216 adult patients (> 18 years) whose routine fasting lipid profile was done and documented in our clinical biochemistry lab. LDL-C levels done by direct method and calculated methods were compared using the Intraclass correlation coefficient (ICC). Appropriate statistical tests were used with a p-value <0.05 taken as statistically significant.

Results:
When compared between dLDL-C and cLDL-C significant difference was seen with mean values as 104 ±38.8 and 85.5 ±35.6 mg/dl respectively. However, a strong positive correlation was seen between dLDL-C and cLDL-C, r=0.9, p<0.01. The ICC showed good reliability for overall comparison between dLDL-C and cLDL-C, however, reliability was found to be poor for group 2.

Conclusions:
Friedewald and modified Friedewald equations can be used as a substitute for direct homogenous LDL estimation when resources are scarce.

Keywords:
Direct homogeneous method, Friedewald equation, Lipid profile, LDL, National Cholesterol Education Program Adult Treatment Panel.

Introduction

The link between various cardiovascular diseases (CVD) and lipoprotein has been found in various studies, which led to the formulation of the current national guidelines for cardiovascular risk assessment. These guidelines have used LDL-C for assessing the risk of CVD.[1, 2] For estimating LDL-C, previously the methods used frequently were either Friedewald calculation or β-quantification. The commonly used Friedewald calculation method used triglycerides(TG), total cholesterol(TC), and high-density lipoprotein (HDL) cholesterol levels[3]. Despite being a routinely used method, it has certain limitations i.e., it cannot be used in conditions of hypertriglyceridemia (>400 mg/dL), type III hyperlipoproteinemia, and non-fasting patient's sample. Moreover, there is reduced precision and accuracy as it is a derived equation used to measure LDL-C. The newer methods like the direct measurement method, also recommended by the National Cholesterol Education Program (NCEP) expert laboratory panel have supplanted the older procedures for LDL-C estimation which depends on the physical separation of the different lipoprotein classes[4,5]. The major drawback of the β-quantification method is being labour intensive involving manual pre-treatment.[5] Hence the direct measurement methods overcome this disadvantage as automation has been incorporated into it leading to improved imprecision over the older methods.

A few retrospective and cross-sectional studies have been conducted to explore the analytical equivalence between the older and newer methods. Therefore, we conducted this retrospective study to evaluate the comparison of baseline LDL-C concentrations as determined by a direct assay compared with Friedewald calculation.

Material and Methods

The retrospective observational study was conducted in the Department of Biochemistry, Lady Hardinge Medical College and associated hospitals, New Delhi for a duration of 12 months from October 2022 to September 2023 with IEC registration no. LHMC/IEC/2023/PG Thesis/18. Serum samples of 216 adult patients (> 18 years) were collected, after 12-14 hours of overnight fasting for analysing the lipid profile levels and processed in our clinical biochemistry lab. Hence, we extracted the data from the report entry register of our clinical biochemistry lab. The subjects of this study included individuals whose LDL-C by direct homogeneous method and HDL-C, TC, and TG which are variables of the Friedewald LDL-C estimation

formula were measured. All the cases with TG >500 mg/dl were excluded from the study sample. A total of 216 study participants were placed into three groups based on their TG levels according to NCEP with TG <150 mg/dl, 150-199 mg/dl, and > 199 mg/dl as group 1(142 subjects), group 2 (36 subjects) and group 3 (38 subjects) respectively.

TC, TG, HDL-C, and LDL-C levels were estimated by enzymatic methods on Beckman Coulter AU5800, and absorbance was measured spectrophotometrically. Serum HDL-C and LDL-C levels were estimated by direct homogenous method (two reagent assay), serum TC by enzymatic direct cholesterol oxidase peroxidase method and serum TG by enzymatic triglyceride glycerol phosphate oxidase method. For calculated LDL, the Friedwald formula was used, i.e., LDL-C = TC-HDL-(TG/5). The other methods of cLDL are Modified Friedewald equation, LDL = TC-(TG/6 =HDL), Anandraja equation: LDL = (0.9TC)-(0.9TG/5)-28, Chen equation: LDL = (TC-HDL) x 0.9- (TG x 0.1), Hattori equation: LDL = (0.94 x TC)- (0.94 x HDL)- (0.19 x TG) and Vujovic equation: LDL = TC-HDL-(TG/6.58)6.

Statistical analysis:

The data retrieved from the laboratory system was analysed using SPSS v23. Appropriate statistical tests were used, and parametric data was expressed as mean and standard deviations. The Pearson test was used to find out the correlation between the methods. Receiver operator curve analysis (ROC) was done to assess the diagnostic accuracy of dLDL and cLDL Intraclass correlation coefficient (ICC) was used to compare different methods of LDL estimation. p-value <0.05 was taken as statistically significant.

Results

The mean serum total cholesterol, triglycerides, dHDL, dLDL, cLDL of the study population was 157 ± 41, 144 ± 84, 40 ±13.8, 104 ± 38.8, and 85.5 ± 35.6 mg/dl respectively. The mean values were comparable between male and female study participants. The mean serum total cholesterol, triglycerides, dHDL, dLDL, cLDL of the study male and female population was 157 ± 43, 142 ± 79, 41.9 ±12.9, 108 ± 41.9 and 91.6 ± 35, and 156 ± 40.1, 154.9 ± 90, 39.3 ±14.5, 100 ± 35.5 and 82.3 ± 29 mg/dl respectively. When a comparison between dLDL-C and cLDL-C was done, the mean dLDL-C was found to be higher than the mean cLDL-C. A strong positive correlation was seen between dLDL-C and cLDL-C as shown in Figure 1.

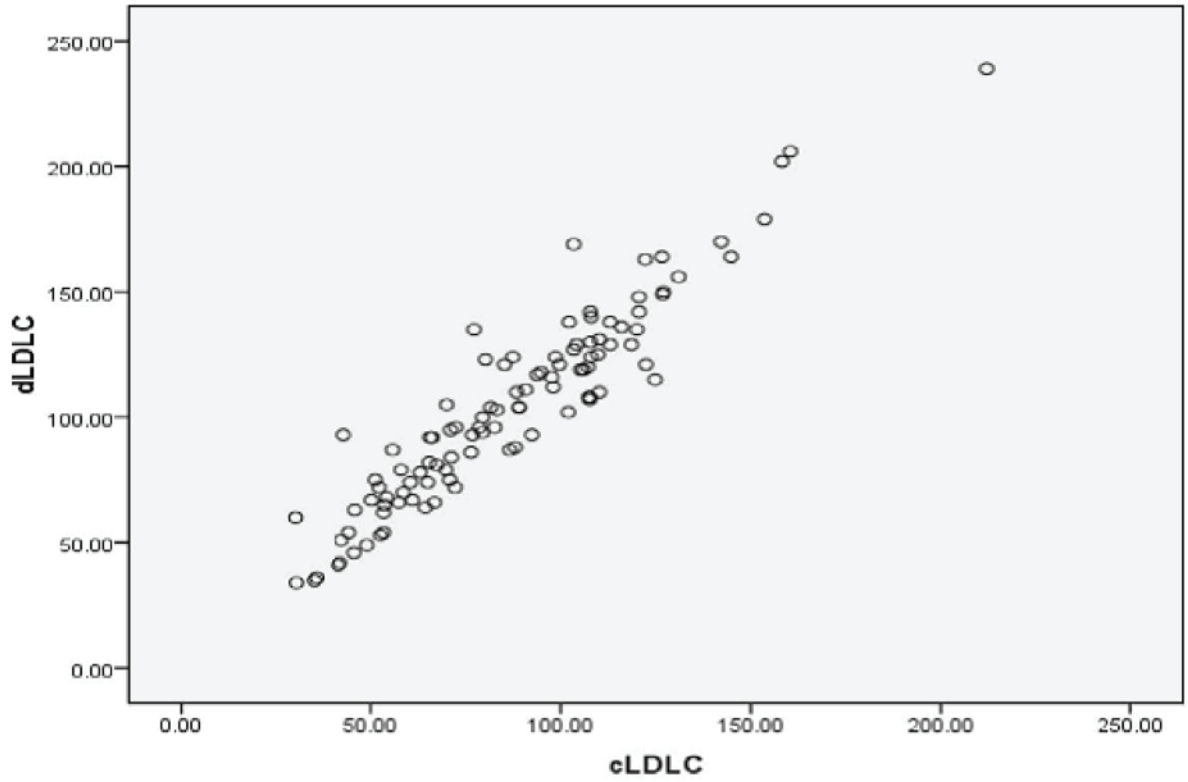


Figure 1. Shows Scattered Plot of dLDL-C by Friedewald equation. r=0.9, p<0.01

The ICC was used to compare different levels of LDL among the three groups as methods of LDL estimation with varying shown in Table 1.

Table 1: Intraclass correlation coefficient of different methods of LDL estimation among groups

	LDL-C measurement	Mean ± SD	Mean difference	ICC(CI)
OVERALL	Direct	100 ±38.5	NA	
	Friedewald	89.9 ±37.5	-10.1	**0.88**(0.85-0.90)
	Modified Friedewald	94.6 ±37.5	-5.4	**0.87**(0.83-0.89)
	Anandraja	73.7 ±56.9	-26.3	0.62(0.50-0.71)
	Chen	92.2 ±34.01	-7.8	0.65(0.54-0.73)
	Hattori	84.2 ±35.3	-15.8	0.31(0.10-0.45)
	Vujovic	96.7 ±37.5	-3.3	0.66(0.55-0.74)
GROUP 1(n=142) cLDL <150mg/dl	Direct	94.5 ±34.6	NA	
	Friedewald	88.6 ±35.2	-5.9	0.63(0.49-0.73)
	Modified Friedewald	92.01 ±35.3	-2.5	0.65(0.52-0.75)
	Anandraja	45.4 ±33.4	-49.1	0.32(0.1-0.6)
	Chen	87.9 ±31.8	-6.6	0.67(0.54-0.76)
	Hattori	83.1 ±33.2	-11.4	0.61(0.45-0.73)
	Vujovic	93.5 ±35.3	-1	0.66(0.52-0.76)

	LDL-C measurement	Mean ± SD	Mean difference	ICC(CI)
GROUP 2(n=36) cLDL= 150-199 mg/dl	Direct	110.3 ±40.4	NA	
	Friedewald	95.9 ±42.4	-14.4	0.46(0.02-0.72)
	Modified Friedewald	101.4±42.4	-8.9	0.47(0.03-0.73)
	Anandraja	90.9 ±21.2	-19.4	0.10(0.01-0.4)
	Chen	99.5 ±38.2	-10.8	0.46(0.03-0.72)
	Hattori	89.8±39.9	-20.5	0.45(0.04-0.7)
	Vujovic	103.9±42.4	-6.4	0.47(0.03-0.73)
GROUP 3(n=38) cLDL > 199mg/dl	Direct	111.2±46.5	NA	
	Friedewald	89.7±41.3	-21.5	0.73(0.39-0.86)
	Modified friedewald	98.3±40.8	-12.9	0.75(0.52-0.87)
	Anandraja	156.9±48.7	45.7	0.50(0.01-0.17)
	Chen	101.3±36.1	-9.9	0.73(0.49-0.86)
	Hattori	83.8±38.9	-27.4	0.69(0.21-0.86)
	Vujovic	102.0±40.5	-9.2	0.76(0.53-0.87)

While all the methods of cLDL-C equations showed a negative mean difference with dLDL-C, a positive mean difference of 45.7 was seen with Anandraja for group 3. The ICC showed excellent reliability for the overall comparison between dLDL-C and cLDL-C by the Friedewald equation, however, reliability was found to be poor for group 2. Overall, the Friedewald equation and Modified Friedewald equation showed near excellent reliability with ICC of 0.88(0.85-0.90) and 0.87(0.83-0.89) respectively, whereas the ICC of the Hattori equation lied in the poor reliability range in comparison to dLDL.

Discussion

For formulating the diagnostic criteria for patients of hypercholesterolemia and further treating them the main target is LDL-C according to the latest NCEP guidelines[7]. The data available for assessing the predictive performance of various direct methods in relation to cardiovascular events is limited[8]. In our study, it was found that the mean dLDL-C was higher than the mean cLDL-C. In a similar study conducted by Miler WG et al, two direct LDL assays viz., LDL-C (GZ, Genzyme) and LDL-C (RD, Roche Diagnostics) were compared with the Friedwald calculation method[9]. It was observed that both the direct assay methods were better in taking medical decision cut point range in comparison to the Friedewald equation.

The possible explanation for this observation may be due to the dependency of the Friedewald equation on other parameters (triglyceride, total cholesterol, and HDL-C). The dLDL-C method has certain advantages over calculated methods as it is relatively unaffected by the fasting status of patients and the increased triglyceride(>400mg/dl) levels don't affect the result[10]. Various studies have found comparative results between direct and calculated methods of LDL-C estimation for the diagnostic and treatment assessment of hyperlipidaemic patients. [9-13]

On the contrary, there are studies that have questioned the credibility of direct assay methods in clinical decision-making[14]. Moreover, certain clinical trials have also proposed the role of estimating LDL-C by the Friedewald calculation method over the direct method in treating hyperlipidaemic patients [15, 16]. Faas et al conducted a study on 464 patients to compare a direct LDL-C with the cLDL-C methods and found out that dLDL-C was 18% higher than the C-LDL-C which is consistent with our findings also. However, treatment guidelines by NCEP-ATP III use calculated LDL-C, whereas the most common method nowadays is the direct method which can lead to overtreatment of hyperlipidaemic patients[15]. Hence, more studies are required to establish direct LDL-C in the treatment guideline goals before modifying them according to non-fasting sample values.

Limitations

Limitations associated with this work could be that the gold standard method i.e., beta quantification could not be used to compare LDL-C levels. Another shortcoming could not retrieve adequate patient history including drug history like statins.

Conclusion

A number of conventional calculated methods as well as newer direct methods are available to measure the LDL-C, however, there is still a paucity of data to establish their performance and use in clinical setup. In the study, it was observed that the Friedewald equation and Modified Friedewald equation showed an excellent performance among the calculated methods for estimating LDL-C. Hence, both Friedewald and Modified Friedewald equations can be used as a substitute for direct homogenous LDL estimation when resources are scarce.

References:

1. Misra S, Lyngdoh T, Mulchandani R. Guidelines for dyslipidemia management in India: A review of the current scenario and gaps in research. Indian

Heart Journal. 2022;74(5):341-50.

2. Daniels SR, Greer FR Committee on Nutrition. Lipid screening and cardiovascular health in childhood. Pediatrics. 2008;122:198–208.
3. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Cslin Chem. 1972;18:499–502.
4. National Cholesterol Education Program. Recommendations on lipoprotein measurement: from the Working Group on Lipoprotein Measurement. Bethesda (MD): NIH, National Heart, Lung, and Blood Institute; 1995. [Accessed April 2010]. p. 186. Available at: www.lassa.org.za/guidelines/lipoprot.pdf. NIH publication no. 95-3044.
5. Nauck M, Warnick GR, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. Clin Chem. 2002;48:236–254.
6. Thapa S, Gyawali P, Bhatta RD, Risal P. Comparison between direct assay and popular equations for Low-Density Lipoprotein-cholesterol estimation in Nepalese population. Journal of Pathology of Nepal. 2020 May 12;10(1):1618-24.
7. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) JAMA. 2001;285:2486–2497.
8. Charlton-Menys V, Liu Y, Moorhouse A, Durrington PN. The robustness of the Roche 2nd generation homogenous HDL cholesterol (PEGME) method: assessment of the effect of serum sample storage for up to 8 years at −80 degrees C. Clin Chim Acta. 2007;382:142–144.
9. Miller WG, Waymack PP, Anderson FP, Ethridge SF, Jayne EC. Performance of four homogeneous direct methods for LDL-cholesterol. Clin Chem. 2002;48:489–498.
10. Warnick GR, Nauck M, Rifai N. Evolution of methods for measurement of HDL-cholesterol: from ultracentrifugation to homogeneous assays. Clin Chem. 2001;47:1579–1596.
11. Jeong YW, Koo JH, Huh JH, Kim YJ, Jeong H, Kim EY, Kang DR. Comparison of Newly Proposed LDL-Cholesterol Estimation Equations. Journal of Korean Medical Science. 2023;38(19).

12. Alpdemir MF, Alpdemir M. Comparison of different equations for estimation of low-density lipoprotein (LDL)–cholesterol. Turkish Journal of Biochemistry. 2020;45(5):601-11.

13. Esteban-Salán M, Guimón-Bardesi A, de la Viuda-Unzueta JM, Azcarate-Ania MN, Pascual-Usandizaga P, Amoroto-Del-Río E. Analytical and clinical evaluation of two homogeneous assays for LDL-cholesterol in hyperlipidemic patients. Clin Chem. 2000;46:1121–1131.

14. Usui S, Kakuuchi H, Okamoto M, Mizukami Y, Okazaki M. Differential reactivity of two homogeneous LDL-Cholesterol methods to LDL and VLDL subfractions, as demonstrated by ultracentrifugation and HPLC. Clin Chem. 2002;48:1946–1954.

15. CLSI. CLSI document C37-A. Wayne (PA): CLSI; 1999. Preparation and validation of commutable frozen human serum pools as secondary reference materials for cholesterol measurement procedures; approved guideline.

16. Nauck M, Wiebe D, Warnick GR. Measurement of high-density-lipoprotein cholesterol. In: Rifai N, Warnick GR, Dominiczak MH, editors. Handbook of lipoprotein testing. 2nd ed. Washington (DC): AACC Press; s2000. pp. 227–230