|| Online ISSN: 2589-9139 || Print ISSN: 2589-9120 ||
International Journal of Medical Science and Applied Research (IJMSAR)
Available Online at www.ijmsar.com
NLM - National Library of Medicine ID: 101775327
Volume - 8 , Issue - 3; June : 2025; Page No. 62-69

Article Type: Original Research Article

A Comparative Study of Various Formulae for LDL-C Estimation in Patients with Serum Triglycerides > 400mg/dl

[1]Dr V Naga Guhan, Associate Professor, Department of Biochemistry, All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh, India

[2]Dr Desai Vidya Sripad, Professor & Head, Department of Biochemistry, All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh, India

[3]Dr Montosh Chakraborty, Additional Professor, Department of Biochemistry, All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh, India

[4]Dr Bari Siddiqui, Associate Professor, Department of Biochemistry, All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh, India

[5]Dr T Sibi Mandela, Assistant Professor, Department of Biochemistry, All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh, India

[6]Dr Nichenametla Gautam, Assistant Professor, Department of Biochemistry, All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh, India

[7]Mr Saidaiah Ikkurthi, Tutor/Demonstrator, Department of Biochemistry, All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh, India

Corresponding Author: Dr V Naga Guhan, Associate Professor, Department of Biochemistry, All India Institute of Medical Sciences, Mangalagiri, Andhra Pradesh, India

Conflict of interest: Nil

Abstract

Background & Rationale: Cardiovascular diseases (CVD) resulting from atherosclerosis remain the leading cause of morbidity and mortality worldwide. Among the various risk factors, elevated low-density lipoprotein cholesterol (LDL-C) is strongly associated with an increased risk of CVD-related mortality. While the Friedewald formula is widely used for estimating LDL-C, its accuracy diminishes significantly in cases of severe hypertriglyceridemia (TG > 400 mg/dL). This limitation necessitates the evaluation of alternative estimation methods. The present study aimed to identify the most accurate LDL-C estimation formula in patients with TG levels exceeding 400 mg/dL, by comparing LDL-C values derived from nine different formulas with directly measured LDL-C.

Methodology: This hospital-based retrospective comparative study was conducted using data from 108 patients with TG > 400 mg/dL, following approval from the Institutional Ethics Committee, AIIMS Mangalagiri, and in accordance with ICMR National Ethical Guidelines. LDL-C was estimated using nine formulas—Friedewald, Chen, de Cordova, Vujovic, Anandaraja, Hattori, Ahmadi, Puavilai, and the Martin-Hopkins equation—and compared with directly measured LDL-C values.

Results: Due to non-normal data distribution, results were expressed as median (interquartile range, IQR) and analyzed using the Wilcoxon signed-rank test. Among all the equations tested, the Martin-Hopkins LDL calculator provided LDL-C estimates (median 113.17 mg/dL; IQR 83.39–132.84) that were statistically not significantly different from the directly measured LDL-C (median 118.05 mg/dL; IQR 80.38–152.55), with a p-value of 0.44. In contrast, all other formulas significantly underestimated LDL-C values compared to the direct method. The intraclass correlation coefficient (ICC) for the Martin-Hopkins formula was 0.72 with a 95% confidence interval of 0.616 to 0.800 ($p < 0.001$), indicating good agreement with direct LDL-C values.

Conclusion: The Martin-Hopkins LDL calculator, which uses an adjustable factor for very-low-density lipoprotein cholesterol (VLDL-C) based on TG and non-HDL-C values, was found to be the most reliable and accurate alternative in this study. Given its statistical equivalence to directly measured LDL-C and its non-invasiveness and cost-effectiveness, the Martin-Hopkins equation can be recommended as a practical and trustworthy method for LDL-C estimation in patients with severe hypertriglyceridemia, particularly in resource-limited settings where direct assays may not always be feasible.

Keywords: Fasting Lipid profile, LDL cholesterol, Direct LDL, Friedewald formula, Martin Hopkin equations, hypertriglyceridemia.

Introduction

Cardiovascular diseases (CVD) continue to represent the foremost cause of mortality and morbidity globally, exerting a significant burden on healthcare systems and economies in both high-income and low- to middle-income countries.[1] According to the Global Burden of Disease (GBD) study, India exhibits an alarming mortality rate of 272 per 100,000 population attributable to CVD, which notably surpasses the global average.[2] This reflects not only a growing public health concern but also highlights the urgency of early identification and effective management of CVD risk factors in the Indian population.

Among the various modifiable risk factors implicated in the pathogenesis of atherosclerosis, dyslipidemia—particularly elevated low-density lipoprotein cholesterol (LDL-C)—has emerged as a pivotal player.[3] LDL-C is known to contribute directly to the initiation and progression of atherosclerotic lesions. Mechanistically, LDL particles infiltrate the arterial intima and undergo oxidative modifications. These modified lipoproteins are then internalized by macrophages through scavenger receptors, culminating in the formation of foam cells—an early and critical event in plaque development.[4] Numerous large-scale epidemiological and interventional studies have reinforced the causal role of LDL-C in atherogenesis and its utility as a therapeutic target in cardiovascular risk reduction.[5]

Given its clinical significance, accurate quantification of LDL-C is essential for both risk stratification and treatment monitoring in patients with or at risk for CVD. The gold standard for LDL-C measurement involves ultracentrifugation-based methods; however, these are labor-intensive, expensive, and not routinely available in most clinical laboratories. As a practical alternative, LDL-C is commonly estimated using mathematical equations, the most widely used being the Friedewald formula: LDL-C = Total Cholesterol – (HDL-C + Triglycerides/5)[6]

While convenient and cost-effective, the Friedewald formula has important limitations. It assumes a fixed ratio of triglycerides to very-low-density lipoprotein cholesterol (VLDL-C), which becomes inaccurate in certain metabolic conditions. Its reliability significantly diminishes in patients with hypertriglyceridemia, particularly when triglyceride levels exceed 400 mg/dL.[7] Under such conditions, the misestimation of LDL-C can lead to erroneous risk categorization and inappropriate clinical decision-making. To address these limitations, a number of alternative equations have been developed and proposed in recent years. These include formulas by Martin-Hopkins, Chen, Vujovic, de Cordova, Anandaraja, Ahmadi, Hattori, and Puavillai, each with unique approaches to adjusting for triglyceride variability and VLDL-C estimation.

Several studies have attempted to validate the accuracy of these formulas across different populations and lipid profiles. For example, research conducted by Azam Karkhaneh and colleagues in the Iranian population found the Puavillai formula to be particularly effective in estimating LDL-C in individuals with elevated triglycerides.[8] However, contrasting findings were reported in a study by Nishtha et al. in an Indian cohort, where the Puavillai formula showed poor correlation with directly measured LDL-C values in individuals with triglyceride levels above 400 mg/dL.[9]

Given these inconsistencies and the need for population-specific validation, the present study aims to evaluate and compare LDL-C values estimated by Friedewald and eight alternative formulas—Martin-Hopkins, Chen, de Cordova, Vujovic, Anandaraja, Hattori, Ahmadi, and Puavillai—with direct LDL-C measurements in individuals from South India with severe hypertriglyceridemia (TG > 400 mg/dL). By evaluating the accuracy and reliability of these formulas in a high-risk population, the study aims to inform laboratory physicians on the most appropriate method for LDL-C estimation when direct measurement is not feasible, thereby improving cardiovascular risk assessment and patient care in routine clinical practice.

Materials & Methods

Study Design and Setting

This was a retrospective comparative study conducted at the All India Institute of Medical Sciences (AIIMS), Mangalagiri, Andhra Pradesh, India. The study was carried out over a period of two months following approval from the Institutional Ethics Committee (IEC). As this was a retrospective analysis of anonymized laboratory data, informed consent was waived. Data were extracted from the Laboratory Information System (LIS).

Participants

We included data from 108 patients who had undergone lipid profile analysis and direct LDL-C measurement and had serum triglyceride (TG) levels > 400 mg/dL. The inclusion criterion was availability of all four lipid parameters: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and directly measured LDL-C. Patients with incomplete data or known secondary causes of hyperlipidemia (e.g., nephrotic syndrome, liver disease, hypothyroidism) were excluded.

Sample Size Calculation

Sample size was determined using nMaster software. Based on a previous study that reported a correlation coefficient of 0.7 between directly measured LDL-C and calculated LDL-C values, and assuming a baseline correlation of 0.5, with a type I error (α) of 0.05 and power (1–β) of 90%, the minimum required sample size was calculated to be 108.[9]

Variables and Data Collection

After obtaining ethical clearance, retrospective data were extracted from the institutional Laboratory Information System (LIS). The following variables were collected: Serum Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein Cholesterol (HDL-C) and Directly measured Low-Density Lipoprotein Cholesterol (LDL-C).Using the above parameters, LDL-C was also estimated using the following nine formulae:

- Friedewald: LDL-C = TC – HDL-C – (TG/5)[10]
- Chen: LDL-C = (TC – HDL-C) × 0.9 – (TG × 0.1)[11]
- de Cordova: LDL-C = 0.7516 × (TC – HDL-C)[12]
- Vujovic: LDL-C = TC – HDL-C – (TG/6.85)[13]
- Anandaraja: LDL-C = (0.9 × TC) – (0.9 × TG/5) – 28[14]
- Hattori: LDL-C = (0.94 × TC) – (0.94 × HDL-C) – (0.19 × TG)[15]
- Ahmadi: LDL-C = TC/1.19 + TG/1.9 – HDL-C/1.1[16]
- Puavillai: LDL-C = TC – HDL-C – (TG/6)[17]
- Martin-Hopkins: LDL-C derived using Martin-Hopkins LDL calculator application[18]

Statistical Analysis

All data were entered into Microsoft Excel and analyzed using SPSS version 20.0 (IBM Corp., Armonk, NY). The Kolmogorov-Smirnov test was used to assess the normality of data distribution. Variables were reported as median with interquartile range (IQR). The Wilcoxon signed-rank test was applied to compare directly measured LDL-C and estimated LDL-C. The agreement between directly measured LDL-C and estimated LDL-C values from each formula was evaluated using the Bland-Altman method. Bland-Altman plots were constructed to visualize the mean difference (bias) and the 95% limits of agreement (mean difference ± 1.96 × SD). These plots provided insight into whether the calculated LDL-C values systematically overestimated or underestimated the directly measured LDL-C across the range of values. A two-sided p-value < 0.05 was considered statistically significant.

Results

A total of 108 patient samples with serum triglyceride levels >400 mg/dL were included in the study. The LDL-C values estimated by nine different formulas were compared with directly measured LDL-C values. The data were not normally distributed; hence, results are expressed as median (interquartile range, IQR) and analyzed using the Wilcoxon signed-rank test. The median (IQR) of directly measured LDL-C was 118.05 mg/dL (72.2). Among the calculated methods, Martin-Hopkins formula yielded the closest estimate to the direct measurement, with a median LDL-C of 113.17 mg/dL (49.4) and a p-value of 0.44, indicating no statistically significant difference from the direct LDL-C value. All other formulas showed statistically significant differences from the direct method ($p < 0.05$), indicating a lack of agreement. (Table 1). Bland-Altman analysis for the Martin-Hopkins formula (Figure 1) further supported its reliability, demonstrating a narrow bias and acceptable limits of agreement when compared to the direct method. The intraclass correlation coefficient (ICC) for the Martin-Hopkins formula was 0.72 with a 95% confidence interval of 0.616 to 0.800 ($p < 0.001$), indicating good agreement with direct LDL-C values.

Table 1: Comparison of calculated LDL by various formula with direct LDL estimation using Wilcoxon signed rank test

Method	**Median (IQR)**	**P value (comparison with direct LDL)**
Direct LDL	118.05(72.2)	-
Friedewald	72.15(65.8)	<0.001
Chen	109.13(54.3)	0.034
de Cordova	137.73(45.9)	<0.001
Vujovic	102.49(58.7)	<0.001
Anandaraja	74.42(69.1)	<0.001
Hattori	66.47(61.6)	<0.001
Puavillai	90.07(61.2)	<0.001
Martin – Hopkin	113.17(49.4)	0.44

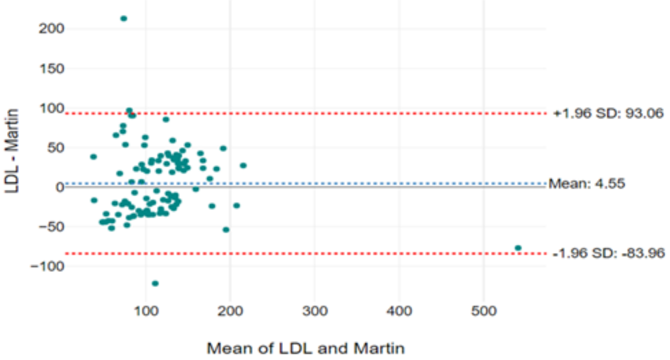

Figure 1: Bland Altman plot analysis – Agreement between Direct LDL and LDL calculated by Martin-Hopkins formula

Discussion

Accurate estimation of LDL-C is critical in assessing cardiovascular risk, especially in patients with hypertriglyceridemia, where standard formulas often become unreliable. In this retrospective comparative study, we assessed the performance of nine different LDL-C estimation formulas against directly measured LDL-C values in a South Indian population with serum triglycerides >400 mg/dL. Among all the formulas evaluated, the Martin-Hopkins formula showed the best agreement with directly measured LDL-C, as demonstrated by a non-significant p-value (0.44), a strong intraclass correlation coefficient (ICC = 0.72), and narrow limits of agreement on Bland-Altman analysis.

Our findings are in agreement with Martin et al. (2013), who introduced this formula and demonstrated its superior accuracy across a wide range of lipid profiles, especially in individuals with high triglyceride levels.[18] The strength of the Martin-Hopkins equation lies in its use of an adaptive factor for VLDL-C estimation, as opposed to the fixed divisor (TG/5) used in the Friedewald formula. This dynamic approach allows the formula to better account for individual variations in lipid metabolism, which is particularly beneficial in cases of hypertriglyceridemia. In contrast, the Friedewald formula, despite being widely used in routine clinical practice, significantly underestimated LDL-C levels in our study cohort. This is consistent with findings by Nishtha et al. (2021), who reported that Friedewald's equation is unreliable when triglyceride levels exceed 400 mg/dL, particularly in Indian patients.[19] Similarly, Vujovic et al. (2010) noted considerable underestimation by Friedewald in hypertriglyceridemic individuals, supporting our observation of its limited clinical utility in such settings.[13]

Interestingly, the Puavillai formula, which showed good agreement with direct LDL-C in an Iranian population as reported by Azam Karkhaneh et al. (2018), did not perform well in our South Indian cohort.[20] This discrepancy may be attributed to population-specific differences in lipid metabolism influenced by genetic, dietary, and environmental factors. Our results are consistent with Nishtha et al.'s conclusion that the Puavillai formula does not correlate well with direct LDL-C measurements in Indian patients with TG >400 mg/dL.[19] Other formulas such as Chen, de Cordova, Anandaraja, Hattori, and Ahmadi also showed statistically significant differences from direct LDL-C and displayed broader limits of agreement on Bland-Altman plots. The de Cordova formula, for instance, significantly overestimated LDL-C values in our study, which was also observed by Akolkar et al. (2014) in a Western Indian population.[21] This overestimation may be problematic in clinical settings, potentially leading to overtreatment or misclassification of cardiovascular risk.

The Anandaraja formula, which was developed based on an Indian population and simplifies LDL-C estimation by excluding HDL-C from the equation, surprisingly did not perform well in our hypertriglyceridemic cohort. This is consistent with previous studies (e.g., Ramalingam et al., 2020) that suggest its reduced applicability in extreme lipid profiles, despite its utility in resource-limited settings.[22] The Bland-Altman analysis further validated the superiority of the Martin-Hopkins formula, which showed minimal bias and tight limits of agreement with direct LDL-C, suggesting clinical interchangeability. In contrast, formulas like Hattori and Friedewald demonstrated wider bias and poor agreement, making them less suitable in patients with elevated triglycerides.

One of the strengths of our study is the inclusion of multiple formulas and use of robust statistical tools such as intraclass correlation and Bland-Altman plots, which offer a more nuanced understanding of agreement than correlation alone. Moreover, our study adds to the limited body of evidence from South Indian populations, where lipid profiles and cardiovascular disease patterns may differ from other regions. However, certain limitations should be acknowledged. The study was retrospective and conducted at a single center, which may limit generalizability. Additionally, the direct LDL-C measurements were based on enzymatic methods rather than ultracentrifugation, which is considered the gold standard. Nonetheless, enzymatic direct LDL-C assays are widely accepted in clinical practice and provide a valid reference for comparative analysis.

References

1. Benjamin EJ, Muntner P, Alonso A, et al. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. *Circulation* 2019;Jan 31
2. Dorairaj Prabhakaran, Panniyammakal Jeemon, Ambuj Roy. Cardiovascular Diseases in India Current Epidemiology and Future Directions. Circulation. 2016;133:1605–1620.
3. Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. Int J Prev Med. 2014; 5 (8):927–946.
4. Singh RB, Mengi SA, Xu YJ, Arneja AS, Dhalla NS. Pathogenesis of atherosclerosis: A multifactorial process. Exp Clin Cardiol. 2002;7(1):40–53.
5. Tarchalski J, Guzik P, Wysocki H. Correlation

between the extent of coronary atherosclerosis and lipid profile. Mol Cell Biochem. 2003 Apr;246(1-2):25-30.

6. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low- density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499–502.
7. A. J. Tremblay, H. Morrissette, J.-M. Gagné, J. Bergeron, C. Gagné, and P. Couture, “Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol compared with β-quantification in a large population,” Clinical Biochemistry, vol. 37, no. 9, pp. 785–790, 2004.
8. Azam Karkhaneh, Molood Bagherieh, Solmaz Sadeghi and Asma Kheirollahi. Evaluation of eight formulas for LDL-C estimation in Iranian subjects with different metabolic health statuses. Lipids in Health and Disease (2019) 18:231
9. Wadhwa N, Krishnaswamy R. Comparison of LDL-Cholesterol Estimate using Various Formulae with Directly Measured LDL-Cholesterol in Indian Population. J Clin Diagn Res. 2016;10(12): BC11-BC13.
10. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low- density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972 ;18(6):499–502.
11. Chen Y, Zhang X, Pan B, Jin X, Yao H, Chen B, et al. A modified formula for calculating low-density lipoprotein cholesterol values. Lipids Health Dis.2010;9(1):52
12. de Cordova CMM, de Cordova MM. A new accurate, simple formula for LDL- cholesterol estimation based on directly measured blood lipids from a large cohort.Ann Clin Biochem. 2013;50(1):13–9.
13. Vujovic A, Kotur-Stevuljevic J, Spasic S, Bujisic N, Martinovic J, Vujovic M et al.Evaluation of different formulas for LDL-C calculation. Lipids Health Dis. 2010; 9(1):27.
14. Anandaraja S, Narang R, Godeswar R, Laksmy R, Talwar KK. Low-density lipoprotein cholesterol estimation by a new formula in Indian population. Int JCardiol. 2005;102(1):117–20
15. Hattori Y, Suzuki M, Tsushima M, Yoshida M, Tokunaga Y, Wang Y, et al. Development of approximate formula for LDL-chol, LDL-apo B and LDLchol/ LDL-apo B as indices of hyperapobetalipoproteinemia and small dense LDL. Atherosclerosis. 1998;138(2):289–99
16. Ahmadi SA, Boroumand M-A, Gouhari MK, Tajik P, Dibaj S-M. The impact of low serum triglyceride on LDL-cholesterol estimation. Arch Iran Med. 2008; 11(3):318–21.
17. Puavilai W, Laoragpongse D. Is calculated LDL-C by using the new modified Friedewald equation better than the standard Friedewald equation? J Med AssocThail. 2004;87(6):589–93
18. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, Jones SR. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. JAMA. 2013 Nov 20;310(19):2061-8. doi: 10.1001/ jama.2013.280532. PMID: 24240933; PMCID: PMC4226221
19. Nishtha, Bajaj RK, Arora R, Kapil M, Varun. Comparative Evaluation of Various LDL Cholesterol Calculation Formulae with Direct Estimation of

LDL-C in Hypertriglyceridemic Subjects. Int J Adv Res. 2021;9(1):420–8.

20. Karkhaneh A, Kargar Z, Ebrahimian S. Evaluation of different formulas for LDL-C estimation in hypertriglyceridemia: a study in Iranian subjects. Iran J Pathol. 2018;13(2):191–8.
21. Akolkar PN, Barik PR, Kulkarni SR. Evaluation of various LDL-C calculation formulas against direct LDL-C estimation in Indian population. Indian J Clin Biochem. 2014 Jan;29(1):89–95.
22. Ramalingam S, Vinayagam B, Das S. A comparative study of LDL-C estimation formulas in various triglyceride ranges and the applicability of Anandaraja's formula in an Indian population. J Clin Diagn Res. 2020;14(3):BC01–BC04.