Section: Biochemistry

Original Research Article

COMPARISON OF DIRECT AND CALCULATED LDL CHOLESTEROL USING MULTIPLE FORMULAE IN A TERTIARY CARE HOSPITAL IN NORTH KERALA

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ABSTRACT

Background: Accurate estimation of low-density lipoprotein cholesterol (LDL-C) is essential for cardiovascular risk assessment and dyslipidaemia management. Although direct LDL-C measurement is reliable, it is costly and may be infeasible in resource-limited settings. Several calculation formulas have been proposed as cost-effective alternatives; however, their agreement with direct measurement requires validation. **Materials and Methods:** This analytical cross-sectional study included 52 participants. LDL-C was measured directly (by laboratory testing) and calculated using 19 formulas. Agreement between direct and calculated LDL-C was assessed by correlation analysis (statistical association), relative error (per cent difference), Bland–Altman analysis (measurement comparison), and intraclass correlation coefficient (ICC; reliability). **Results:** The study population had a mean age of 49.0 ± 11.9 years, with 80.8% males. The mean direct LDL-C was 96.9 ± 45.3 mg/dL. Anandaraja, Hattori, Martin/Hopkins, Rao, and Friedewald formulas produced values closest to direct LDL-C. Ahmadi significantly overestimated LDL-C, whereas Ephraim, Ghasemi, and Hatta underestimated it. Most formulas showed relative errors between 4–6%, with the lowest for deCordova (3.93%). Strong correlations were observed for Martin (r = 0.900) and for Dansethakul, Friedewald, and Rao (r = 0.899), while Ahmadi showed a weak correlation (r = 0.348). Bland–Altman and ICC analyses demonstrated the best agreement for Friedewald, Martin, Rao, and Anandaraja formulas. **Conclusion:** Calculated LDL-C closely correlates with direct measurement, though agreement varies. The Rao, Martin, Friedewald, and Anandaraja formulas provide reliable, cost-effective alternatives in resource-limited settings.

INTRODUCTION

The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) guidelines emphasise the importance of low-density lipoprotein cholesterol (LDL-C) in the classification and management of dyslipidaemia.[1] The gold standard method for measuring LDL-C is β-quantification; however, this technique is expensive, time-consuming, and not suitable for routine clinical use.[2] In current clinical practice, LDL-C is commonly estimated using Friedewald's equation or measured by direct LDL-C assays.[3-5]Multiple formulas provide cost-effective LDL-C estimates. The Friedewald equation is common, but it has limitations at higher triglyceride levels. Newer formulas, such as the Martin–Hopkins and Sampson formulas, expand applicability.

A recent meta-analysis reported that only a limited number of formulas demonstrated good correlation with directly measured LDL-C values, with the Martin equation performing better than the traditionally used Friedewald equation.[6] In the present study, 19 different LDL-C calculation equations (Table 1) were evaluated.Given this context, this study specifically aimed to assess how

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closely LDL-C values calculated using 19 equations align with directly measured LDL-C levels in the local population, thereby determining the reliability and clinical applicability of each calculation method.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Clinical Biochemistry section of the Central Laboratory, Government Medical College, Thrissur, over one month (October 2023). Adult patients (>18 years) who underwent complete lipid profile testing during this period were included. Only samples with triglyceride (TAG) levels of 250–400 mg/dL were analysed. Samples with incomplete lipid profiles or TAG <250 mg/dL were excluded.

Fasting lipid profiles were analysed on a Beckman Coulter AU 680 analyser. Total cholesterol was measured by CHOD–PAP, triglycerides by GPO–PAP, and HDL-C by a direct enzymatic assay. LDL-C was measured directly by a homogeneous enzymatic method and used as the reference standard. LDL-C was also calculated with 19 established formulas for comparison. Internal and external quality controls remained within acceptable limits throughout the study period.

Data were analysed using MedCalc (version 23.4.9). Descriptive statistics were used to summarise the data. The relationship between directly measured and calculated LDL-C values was assessed using correlation analysis. The agreement was evaluated using appropriate statistical methods. These included Bland–Altman analysis and intraclass correlation coefficient (ICC).

RESULTS

A total of 52 participants were included in the study, with a male predominance (80.8%). The mean age was 49.0 ± 11.9 years. The mean directly measured LDL-C level was 96.9 ± 45.3 mg/dL, with values ranging from 9 to 237 mg/dL. Comparison of LDL-C values calculated using 19 different equations revealed considerable variability across formulas. The Rao equation produced mean LDL-C values closest to direct measurement, followed by Dansethakul, while Anandaraja, Hattori, Martin/Hopkins, and Friedewald equations also showed comparable estimates. In contrast, the Ahmadi equation markedly overestimated LDL-C, whereas the Ephraim, Ghasemi, and Hatta equations underestimated LDL-C values.

Most formulas demonstrated relative errors of 4–6%, indicating acceptable precision, with the lowest error observed for the deCordova equation (3.93%). Higher errors were noted with the Ghasemi, Hatta, and Ephraim equations. Correlation analysis showed that all formulas had statistically significant correlation with directly measured LDL-C ($p < 0.05$). Strong correlations were observed for Martin ($r = 0.900$), Dansethakul, Friedewald, and Rao ($r = 0.899$), and Anandaraja ($r = 0.898$), whereas Ahmadi showed a weak correlation ($r = 0.348$).

Agreement analysis using Bland–Altman plots and intraclass correlation coefficient (ICC) demonstrated variable performance among formulas. Specifically, the Friedewald, Martin/Hopkins, and Anandaraja equations showed minimal bias, with narrow limits of agreement and high ICC values (≈0.90), indicating excellent agreement with direct LDL-C. In contrast, Dansethakul and deCordova showed low bias but wider limits of agreement, while several other formulas demonstrated moderate agreement. Notably, the Ahmadi equation showed poor agreement with a large bias and a very low ICC. Overall, the Rao, Martin, Friedewald, and Anandaraja equations demonstrated the best agreement with directly measured LDL-C in this study population.

Table1:19 different equations used for calculating LDL

S.NO:	LDL-C CALCULATING EQUATIONS	FORMULA
1	Ahmadi's equation	LDL = (TC/1.19) − (HDL/1.1) + (TG/1.9) − 38
2	Anandaraja's equation	LDL = (0.9 × TC) − (0.9 × TG/5) − 28
3	Chen's equations	LDL = (TC − HDL) × 0.9 − (TG × 0.1)
4	Choi's equations	LDL = TC − 0.87 × HDL − 0.13 × TG
5	Dansethakul's equations	LDL = 0.9955*TC − 0.9853*HDL-C − 0.1998*TG + 7.1449
6	DeCordova's equation	LDL = 0.7516 × (TC-HDL)
7	DeLong's equation	LDL = TC − (HDL + 0.16 × TG)
8	Ephraim's equation	LDL (mmol/L) = TC-HDL-TG/4
9	Friedewald's equation	LDL = TC-HDL-(TG/5)
10	Ghasemi's equation	LDL = TC-HDL-TG/4
11	Hatta's equation	LDL = TC-HDL-TG/4
12	Hattori's equation	LDL = (0.94 × TC) − (0.94 × HDL) − (0.19 × TG)
13	Martin-Hopkin's equation	LDL-C = TC-HDL-C-TG/adjustable factor By online calculator[13,14]
14	Molavi's equation	LDL = (0.97 × TC) − (0.93 × HDL) − (0.19 × TG)
15	Puavilai's equation	LDL = TC-HDL-(TG/6)
16	Rao's equation	LDL = [(4.7 × TC) − (4.364 × HDL-C) − TG]/4.487
17	Sampson's equation	LDL-C = TC/0.948 − HDL-C/0.971 − (TG/8.56) + (TG × (Non-HDLC/2140) − TG2 /16100) − 9.44
18	Teerakanchana's equation	LDL-C = (0.91 × TC) − (0.634 × HDL-C) − (0.111 × TG) − 6.755
19	Vujovic's equation	LDL = TC-HDL-(TG/6.58)

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Table 2: Direct LDL- C and calculated LDL -C values using various equations with corresponding relative errors and Pearsons coefficient with p- value

S.NO	LDL - C	Mean mg/dl	SD	SEof Mean	Relative Error (%)	Pearsons coefficient	p-value
1	Direct LDL	96.9	45.3	-	-	-	-
2	Ahmadi's Equation	332.9	96.3	13.4	4.02	0.348	0.011
3	Anandaraja's Equation	88.7	42.0	5.83	6.57	0.898	<0.001
4	Chen's Equation	119.2	40.7	5.65	4.74	0.869	<0.001
5	Choi's Equation	129.0	45.0	6.23	4.83	0.882	<0.001
6	Dansethakul's Equation	101.7	43.5	6.03	5.93	0.899	<0.001
7	deCordova's Equation	134.9	38.2	5.30	3.93	0.775	<0.001
8	DeLong's Equation	111.8	44.0	6.10	5.46	0.893	<0.001
9	Ephraim's Equation	73.8	44.0	6.10	8.27	0.891	<0.001
10	Friedewald's Equation	94.9	43.6	6.05	6.37	0.899	<0.001
11	Ghasemi's Equation	73.7	44.0	6.10	8.28	0.891	<0.001
12	Hatta's Equation	73.7	44.0	6.10	8.28	0.891	<0.001
13	Hattori's Equation	88.4	41.0	5.69	6.43	0.899	<0.001
14	Martin's-Hopkin's Equation	95.1	42.4	5.99	6.02	0.900	<0.001
15	Molavi's Equation	156	60.0	5.89	6.19	0.830	<0.001
16	Puavilai's Equation	109.0	43.9	6.09	5.59	0.894	<0.001
17	Rao's Equation	96.3	45.9	6.37	6.61	0.899	<0.001
18	Sampson's Equation	156.2	60.0	8.33	5.33	0.830	<0.001
19	Teerakanchana's Equation	119.2	41.5	5.75	4.82	0.881	<0.001
20	Vujovic's Equation	115.2	44.2	6.13	5.32	0.890	<0.001

Table 3: Bias and 95% LOA between direct LDL-C measurements and calculated LDL-C values

Equation	Bias (mg/dL)	Lower Limit of Agreement (mg/dL)	Upper Limit of Agreement (mg/dL)	Sample Size (n)
Friedewald	2.05	−37.21	41.31	52
Martin	−12.0	−52.3	28.2	52
Rao	0.60	−39.48	40.68	52

Table 4: Intra-Class Correlation coefficient (ICC)

	ICC Agreement	95% CI Lower Limit	95% CI Upper Limit
Martin's /Hopkin's Equation	0.901	0.796	1.02
Friedewald's Equation	0.899	0.797	1.01
Rao's Equation	0.899	0.793	1.02
Dansethakul's Equation	0.895	0.789	1.01
Anandaraja's Equation	0.881	0.788	0.988
Hattori's Equation	0.879	0.777	0.992
Puavilai's Equation	0.864	0.760	0.981
Molavi's Equation	0.862	0.695	0.901
DeLong's Equation	0.847	0.743	0.970
Vujovic's Equation	0.823	0.717	0.943
Ephraim's Equation	0.786	0.675	0.913
Ghasemi's Equation	0.786	0.669	0.917
Hatta's Equation	0.786	0.670	0.916
Teerakanchana's Equation	0.777	0.667	0.911
Chen's Equation	0.764	0.641	0.905
Choi's Equation	0.706	0.595	0.840
deCordova's Equation	0.544	0.396	0.713
Sampson's Equation	0.493	0.383	0.623
Ahmadi's Equation	0.0454	0.00733	0.0847

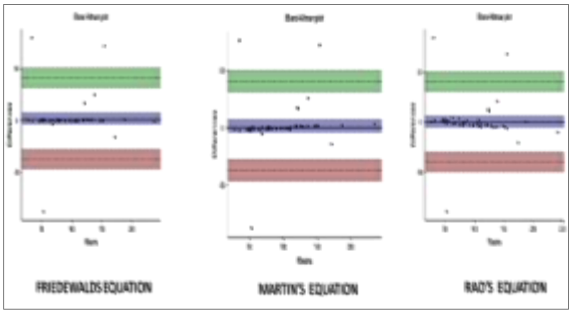

Figure 1: Bland–Altman analysis of agreement between Direct LDL-C and calculated LDL-C values in Friedewald's, Martin's and Rao's Equation

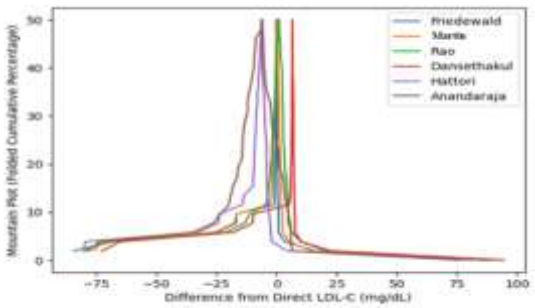

Figure 2: Mountain plot of Calculated LDL-C (best 6) with Direct LDL-C

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DISCUSSION

Clinical significance of LDL-C estimation

Low-density lipoprotein cholesterol (LDL-C) is widely called "bad cholesterol" because it deposits cholesterol in tissues and arteries, contributing to atherosclerosis. High LDL-C levels greatly increase the risk of coronary artery disease. Accurate LDL-C estimation is essential for cardiovascular risk assessment and treatment decisions. Direct measurement is accurate but costly and not always practical in routine care. Therefore, the National Cholesterol Education Program (NCEP) recommends Friedewald's equation as a practical way to estimate LDL-C.

Performance of LDL-C estimation formulas

This study found considerable variation in the accuracy of LDL-C estimation formulas compared with directly measured LDL-C. Discrepancies, from overestimation to underestimation, expose the limitations of indirect calculation methods, especially with dyslipidaemia or in non-fasting states. Overestimation with Ahmadi's and Sampson's equations can lead to unnecessary intensification of lipid-lowering therapy, while underestimation with the Ephraim, Ghasemi, and Hatta equations can lead to undertreatment. Precise LDL-C estimation matters, especially since current AHA/ACC guidelines call for ≥50% LDL-C reduction in high-risk individuals.

In this study, Friedewald, Martin–Hopkins, and Rao equations showed the highest agreement with directly measured LDL-C, indicated by minimal bias and excellent reliability. This aligns with previous evidence from a meta-analysis of 23 LDL-C estimation equations, which found that the Martin/Hopkins method was the most accurate, correctly classifying LDL-C in up to 89.6% of cases. In comparison, Friedewald correctly classified 83.2% of cases, while Sampson, Puavilai, and DeLong equations also showed relatively good performance.

Performance of specific formulas and population relevance

Among the evaluated formulas, the Ahmadi equation performed poorly in this study population. It significantly overestimated LDL-C levels, showed a weak correlation with direct measurement ($r = 0.348$), poor Bland–Altman agreement, and very low reliability (ICC = 0.045). These results show the Ahmadi equation is unsuitable for clinical use here, likely due to population-specific differences in lipid profiles. The equation was originally validated in an Iranian population with triglyceride levels <300 mg/dL, while many in our cohort had higher levels, including >400 mg/dL. Even when excluding high triglyceride samples, it performed worse than the Friedewald equation, highlighting limited generalizability. This emphasises the need to validate LDL-C estimation formulas in specific populations before clinical use.

Limitations

This study has limitations. First, we used directly measured LDL-C by homogeneous assay as the reference standard, not β-quantification, the gold standard. However, β-quantification is less clinically applicable and direct assays are widely used.[12] Second, the study did not cover the full range of triglyceride levels, which may limit generalizability. Third, the sample size was small. Finally, although we evaluated several formulas, we did not include all LDL-C estimation equations from the literature.[10]

CONCLUSION

Significant variability exists in LDL-C values depending on the estimation equation used. Although most formulas demonstrate strong correlation with directly measured LDL-C, the degree of agreement varies considerably. Ahmadi and Sampson equations showed poor agreement and may not be reliable for clinical use. In contrast, the Rao, Martin–Hopkins, Friedewald, Anandaraja, and Dansethakul equations showed better agreement with direct LDL-C measurements and may be more suitable, clinically acceptable alternatives in this population.

REFERENCES

1. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. 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) final report. Circulation. 2002;106(25):3143–4210.
2. Ginsberg HN, Rosenson RS, Hovingh GK, et al. LDL-C calculated by Friedewald, Martin-Hopkins, or NIH equation 2 versus beta-quantification: pooled alirocumab trials. J Lipid Res. 2022;63:100148.
3. 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;18:499–502.
4. Maki KC, Grant JA, Orringer CE. LDL-C estimation: the perils of living with imperfection. J Am CollCardiol. 2022;79(6):542–544.
5. Dansethakul P, Thapanathamchai L, Saichanma S, Worachartcheewan A, Pidetcha P. Determining a new formula for calculating low-density lipoprotein cholesterol: data mining approach. EXCLI J. 2015;14:478.
6. Ephraim RKD, Ativi E, Ashie SA, Abaka-Yawson A, OseiDarkwah K. Assessment of estimated low-density lipoprotein-cholesterol (LDL-C) equations: a systematic review and meta-analysis. Bull Natl Res Cent. 2023;47:71. doi:10.1186/s42269-023-01046-5.
7. Wolska A, Remaley AT. Measuring LDL-cholesterol: what is the best way to do it? CurrOpinCardiol. 2020;35:405–411.
8. Burtis CA, Ashwood ER, Bruns DE. Tietz fundamentals of clinical chemistry. 7th ed. St. Louis: Elsevier; 2008.
9. Martins J, Steyn NP, Rossouw HM, Pillay TS. J ClinPathol. 2023;76:145–151.
10. Samuel C, Park J, Sajja A, Michos ED, Blumenthal RS, Jones SR, Martin SS. Accuracy of 23 equations for estimating LDL cholesterol in a clinical laboratory database of 5,051,467 patients. Glob Heart. 2023;18(1):36. doi:10.5334/gh.1214.

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11. Sampson M, Ling C, Sun Q, et al. A new equation for calculation of low-density lipoprotein cholesterol in patients with normolipidemia and/or hypertriglyceridemia. JAMA Cardiol. 2020;5(5):540–548. doi:10.1001/jamacardio.2020.0013.
12. Sahu S, Chawla R, Uppal B. Comparison of two methods of estimation of low density lipoprotein cholesterol, the direct versus Friedewald estimation. Indian J ClinBiochem. 2005;20(2):54–61.
13. Farheen F, Ambiger S, Jaalam K, Javali S. Martin's formula as the most suitable method for estimation of low-density lipoprotein cholesterol in Indian population. J Lab Physicians. 2023;15(4):545–551. doi:10.1055/s-0043-1768951.
14. Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, et al. LDL calculator [Internet]. Baltimore: Johns Hopkins Medicine; 2013 [cited 2020 Aug 15]. Available from: https://www.ldl-calculator.com/
15. Sonsok S, Sarakarn P, Sanchaisuriya P. Comparison of three equations for estimating low-density lipoprotein-cholesterol in the rural northeastern region of Thailand. J Clin Lab Anal. 2020;34:e23429. doi:10.1002/jcla.23429.
16. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med. 2016;15:155–163.
17. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159–174.
18. Vujovic A, Kotur-Stevuljevic J, Spasic S, Bujisic N, Martinovic J, Vujovic N. Evaluation of different formulae for LDL-C calculation. Lipids Health Dis. 2010;9:27–35.
19. Ahmadi SA, Boroumand MA, Gouhari MK, Tajik P, Dibaj SM. The impact of low serum triglyceride on LDL-cholesterol estimation. Arch Iran Med. 2008;11(3):318–321.

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