Martin's Formula As the Most Suitable Method for Estimation of Low-Density Lipoprotein Cholesterol in Indian Population

Fatima Farheen[1] Sudha Ambiger[2] Kamarudin Jaalam[3] Shivalingappa Javali[4]

[1] Department of Chemical Pathology, USM KLE International Medical Programme, Belgaum, Karnataka, India
[2] Department of Biochemistry, KLE Academy of Higher Education and Research (KAHER), J.N. Medical College, Belagavi, Karnataka
[3] Department of Anaesthesia and Critical Care Medicine, School of Medical Science, PPSP USM Kubang Kerian, Malaysia, and Deputy Dean, USM KLE International Medical Programme, Belgaum, India
[4] Department of Community Medicine, USM KLE International Medical Programme, Belgaum, Karnataka, India

Address for correspondence Sudha Ambiger, MD, Department of Biochemistry, KLE Academy of Higher Education and Research (KAHER), J.N. Medical College, Belagavi 590010, Karnataka, India (e-mail: dr.sudha.ambi@gmail.com).

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Abstract

Background Because of cost effectiveness, most of the laboratories in India estimate low-density lipoprotein cholesterol (LDL-C) levels with the Friedewald's formula. There were many shortcomings of the Friedewald's formula. Recently, Martin and colleagues have derived a new formula for calculating LDL-C. The present study was undertaken to calculate LDL-C using various formulae (Friedewald's formula, Anandaraja's formula, and Martin's formula) and to compare directly measured LDL-C (D-LDL-C) with calculated LDL-C at various ranges of triglyceride (TG) concentration.

Materials and Methods The present study compared LDL-C measured by Martin's formula, Friedewald's formula, and Anandaraja's formula with D-LDL-C in 280 outpatient fasting samples between the age groups of 18 and 50 years. Depending on the TG values, study samples were divided into four groups. Group 1: less than 200 mg/dL; Group 2: 200 to 300 mg/dL; Group 3: 300 to 400 mg/dL; and Group 4: more than 400 mg/dL.

Results Martin's formula shows highest correlation with *r*-value of 0.9979 compared with Friedewald's (0.9857) and Anandaraja's (0.9683) *r*-values. The mean difference was least for Martin's formula (0.31 ± 3.53) compared with other formulae. Among all the groups, percentage of error was least for Martin's formula (0.23%). Martin's LDL-C shows highest concordance (90.90%) compared with Friedewald's (79.60%) and Anandaraja's formulae (82.90%).

Conclusion Among all the groups, Martin's formula shows highest correlation, least percentage of error, highest concordance, and least mean differences. At all TG levels, Martin's formula is the best formula compared with the Friedewald's formula and Anandaraja's formula.

Keywords
- LDL-C
- Martin's formula
- Anandaraja's formula
- Friedewald's formula

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Introduction

The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines suggest to start the drug therapy if low-density lipoprotein cholesterol (LDL-C) levels are more than 130 mg/dL. This makes accurate reporting of LDL-C crucial in the management of dyslipidemia patients.[1] Ultracentrifugation and β-quantitation are the gold standard methods for LDL-C measurement. Other methods include direct measurement of LDL-C using a homogenous assay. These methods are expensive, inconvenient, and not readily available in most of the routine laboratories.[2] Because of these limitation, many clinical laboratories throughout the world use a less expensive and easy approach for the estimation of LDL-C, that is, Friedewald's formula.[3] However, there are several shortcomings of this formula, mainly the underestimation of LDL-C at high triglyceride (TG) levels and overestimation at low TG levels.[4] Many attempts have been made to evaluate and refine Friedewald's formula. The different modified formulae like Anandaraja's formula[5] and Martin's formula[6] have been developed. Compared with Friedewald's formula, Aanandaraja's formula[5] uses only two analytes, TG and total cholesterol (TC), for calculation, which may decrease the total error when compared with the Friedewald's formula.

Friedewald's equation uses a fixed value equal to 5 as a divisor for TG; it does not account for interindividual variability, often resulting in underestimation of risk and potential under treatment.[7] In contrast, Martin et al[7] provided a new formula by introducing adjustable factor in the formula. Martin's formula is: (TC–high-density lipoprotein cholesterol [HDL-C]) – (TGs/adjustable factor).[7] Adjustable factor, defined by levels of TG and non-HDL-C, is divisor for TG. This adjustable factor ranges from 3.1 to 11.9 and was derived from an analysis of TG-to-very-low-density lipoprotein (VLDL)-C ratios of more than 1.3 million people.[7] There are few studies reporting use of this formula in India.

Accurately determining LDL-C values is important in clinical laboratory practice because LDL-C is employed to manage patients having a high risk of coronary heart disease. Therefore, most alternative formulae have been developed to estimate LDL-C to be appropriate for ethnic, specific, as well as other populations. The present study was undertaken with the aim to determine which of these calculated formulas (Friedewald's, Anandaraja's, and Martin's formulas) shows maximum correlation with directly measured LDL-C (D-LDL-C) at different serum TG levels.

Materials and Methods

Study Design

This study is an observational study. Study samples were collected from KLE Centenary Charitable Hospital and Medical Research Center, Belgaum. Total 280 outpatient fasting complete lipid profile patients of 18 to 50 years of age were included in the study. Ethical clearance was obtained from institution ethics committee USM KLE International Medical Program Belgavi: Ethical approval number USM-KLE/IEC/04–2020.Written informed consent was taken from all participants.

Inclusion criteria: 280 outpatient fasting samples coming to laboratory for lipid profile; age group, 18 to 50 years.

Exclusion criteria: Patients with diabetes mellitus, hypothyroidism, cirrhosis, chronic hepatitis, chronic kidney disease, pancreatitis, and patients on active medication including steroids, statins, and omega-3 fatty acids were excluded from the study

Calculation of Sample Size

Direct method LDL-C mean = 118.02[8]
Friedewald method mean = 107.22[8]
Standard deviation in direct method = 35.45
Standard deviation in Friedewald method = 24.35
Effect size: 0.261538461538461
Power = 95%
Alpha error = 1%
Required sample size = 266 should be taken

$$n_{pairs} = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2}{\Delta^2} + \frac{Z^2_{1-\alpha/2}}{2}$$

$$Where\ \Delta = \frac{\bar{x}_2 - \bar{x}_1}{SD}, \qquad SD = \frac{S_1 + S_2}{2}$$

Sample Collection and Lipoprotein Analysis

As a routine procedure, the samples were collected after 10 to 12 hours of overnight fasting by withdrawing 3 mL of venous blood in plain vial. The samples were centrifuged at 3,000 rpm for 15 minutes to obtain serum and were analyzed for lipid profile on the same day. The serum lipid profile parameters were total cholesterol, TG, HDL-C, and LDL-C, which were analyzed on EM 360 clinical chemistry analyzer (TransAsia Bio-Medicals Ltd, Mumbai, Maharashtra, India). All the lipid parameters were estimated using kits purchased from Erba Mannheim XL system packs. The linearity (intra-assay) coefficients of TC, TG, HDL-C, and LDL-C assays were 4.2 to 695 mg/dL (0.98–1.21%), 9.74 to 1,062 mg/dL (0.48–0.86%), 1.90 to 193 mg/dL (1.32–1.95%), and 2.60 to 263 mg/dL (1.74–2.16%), respectively. The intra-assay coefficients observed in our analysis were in concurrence with manufacturer's measurements. All quality controls were performed to ensure the accuracy of the analytical testing (internal and external controls). The internal control is routinely processed every 24 hours on two levels (normal and pathological) by Liquichek Lipids Control from Bio-Rad laboratories, Inc. The results are analyzed daily and periodically for the evaluation of the Levey Jennings graph. The laboratory's external quality control is performed every 3 months. All the lipid parameters' assays meet the National Institutes of Health-NCEP goals for acceptable performance (LDL-CV <4%, Bias <4%and Total Error of <12%, for HDL-CV<4%,Bias $\leq \pm 5\%$ and total error $\leq$13%, for TC-CV<3%,Bias $\leq \pm 3\%$ and total error $\leq$8.9%, for TG-CV<5%,Bias $\leq \pm 5\%$ and total error $\leq$15%,).

Table 1 Comparison of four groups by age and gender

	Group 1	Group 2	Group 3	Group 4	Total	*p*-Value
Gender						
Male	64 (52)	37 (41)	20 (55)	9 (31)	130	0.089
Female	60 (48)	54 (59)	16 (44)	20 (69)	150	
Age						
Mean ± SD	40.9 ± 8.0	38.8 ± 9.2	39.1 ± 10.0	39.8 ± 8.2	39.9 ± 8.7	0.337
Total	124	91	36	29	280	

Abbreviation: SD, standard deviation.
Note: $p < 0.05$ is statistically significant.

LDL Cholesterol Was Calculated by following Formulae

- Friedewald's formula[4] (F-LDL-C) = TC − (TG/5 + HDL-C)
- Anandaraja's formula[5] (A-LDL-C) = (0.9 × TC) − (0.9 × TG/5) – 28
- Martin's formula[6] (M-LDL-C) = (TC-HDL-C) – (TG/adjustable factor*)

*Adjustable factor: The adjustable factor is based on TG and non-HDL-C concentrations. Martin's method matches each person with 1 of 180 different factors to estimate VLDL-C cholesterol from TGs. Martin's LDL-C was calculated using an LDL-C calculator (htttp://www.ldlcalculator.com). Copy the values for total cholesterol, HDL-C, and TGs from research database into the Excel file: non-HDL-C, the adjustable factor, and LDL-C by Martin's formula will be automatically calculated. Depending on the TG values, study samples were divided into four groups:

Group 1: less than 200 mg/dL
Group 2: 200 to 300 mg/dL
Group 3: 300 to 400 mg/dL
Group 4: more than 400 mg/dL

Statistical Analysis

The data obtained were entered into Microsoft Excel sheet and statistical analysis was performed with the SPSS version 16.0. Paired *t*-test and Pearson's correlation were performed to find the significant difference and correlation between D-LDL-C and calculated LDL by different formulas. Scatter plot was used to represent the correlation between the two methods. The mean percentage of error was calculated using the formula: (calculated LDL-C − D-LDL-C)/D-LDL-C × 100. *p*-Value less than 0.05 is considered as significant.

Results

The study consists of total 280 samples. Depending on the TG values (66–533 mg/dL), study population was divided into four groups. There were 124 participants in Group 1, 91 participants in Group 2, 36 participants in Group 3, and 29 participants in Group 4.

►**Table 1** shows the baseline characteristics of the study population, like gender and sex. Comparison of gender and age between groups is statistically not significant. There was no significant difference in age and gender in study population between groups (►**Table1**).

LDL-C was calculated according to three different formulae and compared with D-LDL-C (►**Table 2**). Correlation coefficient *r* was calculated with each formula by correlation analysis of the data. The best formula was chosen in terms of the highest correlation and the lowest mean difference and standard deviation. LDL-C by Martin's formula showed a highest correlation of *r*-value (0.9979), compared with Friedewald's (0.9857) and Anandaraja's (0.9683) formulas (►**Table 2**; ►**Fig. 1**).

Comparison of mean of D-LDL-C with calculated LDL-C (►**Table 3**) by Friedewald's formula and Anandaraja's formula shows that it is underestimated at all levels of TG, and it is statistically significant. Among total sample, mean difference of direct and calculated formulas was least for Martin's formula (0.31 ± 3.53) compared with other formulae. In Group 1, mean difference was least for Anandaraja's formula

Table 2 Correlation between direct LDL-C and calculated LDL-C by different formulas and by Karl Pearson's correlation method

Samples	Variables	*r*-Value	*p*-Value
Total	Friedewald's formula	0.9857	<0.001
	Anandaraja's formula	0.9683	<0.001
	Martin's formula	0.9979	<0.001
Group 1	Friedewald's formula	0.9983	<0.001
	Anandaraja's formula	0.9864	<0.001
	Martin's formula	0.9998	<0.001
Group 2	Friedewald's formula	0.9944	<0.001
	Anandaraja's formula	0.9884	<0.001
	Martin's formula	0.9953	<0.001
Group 3	Friedewald's formula	0.9976	<0.001
	Anandaraja's formula	0.9908	<0.001
	Martin's formula	0.9991	<0.001
Group 4	Friedewald's formula	0.9958	<0.001
	Anandaraja's formula	0.9967	<0.001
	Martin's formula	0.9967	<0.001

Abbreviation: LDL-C, low-density lipoprotein cholesterol.
Note: *r* = correlation coefficient; $p < 0.05$ is statistically significant.

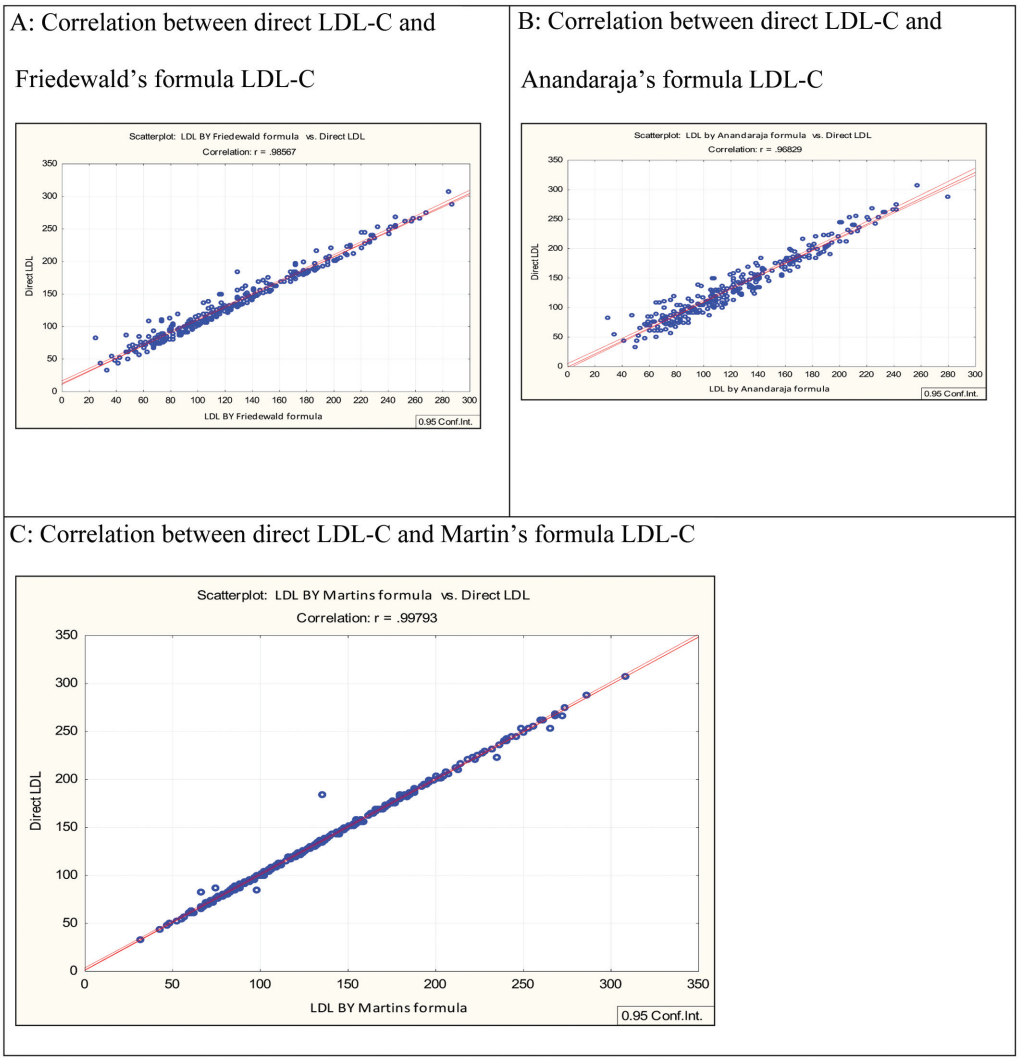

Fig. 1 (**A–C**) Correlation between direct low-density lipoprotein-cholesterol (LDL-C) and calculated LDL-C.

(1.08 ± 8.35) compared with other formulae. In Groups 2, 3, and 4, mean difference was least for Martin's formula with values 0.65 ± 5.17, 0.00 ± 2.47, and 0.77 ± 5.13, respectively, compared with other formulae.

Percentage of error from D-LDL-C and calculated LDL-C was least for Martin's formula (►**Table 4**; ►**Fig. 1**) in total study sample and in all groups compared with other formulae.

The present study compared the concordance of the D-LDL-C with the estimated LDL-C when classifying LDL-C values by NCEP-ATP III. We labeled the result as being "concordant" if the two values were in the same classification, as an "overestimation" if the estimated value was greater than the direct measurement, or as an "underestimation" if the estimated value was less than the direct measurement.

Martin's formula (90.90%) resulted in the best concordance with the direct measurement compared with Friedewald's formula (79.60%) and Anandaraja's formula (82.90%). Overestimation and underestimation rates produced by Martin's formula are less than those produced by Friedewald's and Anandaraja's formulas.

Discussion

The underestimation of LDL-C will lead to delay in initiation of treatment to patients who are at high risk of dyslipidemia. Meanwhile, overestimation can also lead to exposure of patients to unnecessary drug therapy. So there is a need to find an accurate equation for estimation of LDL-C with the best performance comparable to the D-LDL-C. Since Friedewald's formula has limitations, many attempts have been made to derive more accurate formula for LDL-C calculation. The present study was undertaken with the aim to determine which of these calculated formulae (Friedewald's, Anandaraja's and Martin's formula) shows maximum correlation with D-LDL-C at different serum TG levels.

Previous studies like Sahu et al[9] and Molavi et al[10] have shown that the Friedewald's equation performs better for certain groups of populations. But in the study we found calculated LDL-C is underestimated in all the groups. Among all the formulas, mean difference and percentage of error produced by Friedewald's equation are high in total sample and in Groups 2, 3, and 4. The results are consistent with the

Table 3 Comparison of mean value of direct LDL-C and calculated LDL-C by different formulas

Total sample			
Method	**Mean ± SD**	**Mean difference (mg/dL)**	***p*-Value**
Direct	137.42 ± 54.51		
Friedewald's formula	128.06 ± 55.18	9.37	<0.001
Anandaraja's formula	125.56 ± 47.96	11.86	<0.001
Martin's formula	137.11 ± 54.82	0.31	0.1443
Group 1			
Direct	118.61 ± 44.55		
Friedewald's formula	115.60 ± 45.27	3.01	<0.001
Anandaraja's formula	117.53 ± 39.93	1.08	0.1536
Martin's formula	118.21 ± 44.47	0.40	<0.001
Group 2			
Direct	134.85 ± 53.09		
Friedewald's formula	124.33 ± 56.93	10.52	<0.001
Anandaraja's formula	121.54 ± 50.16	13.31	<0.001
Martin's formula	134.20 ± 52.76	0.65	0.2343
Group 3			
Direct	177.48 ± 50.13		
Friedewald's formula	163.74 ± 55.03	13.74	<0.001
Anandaraja's formula	153.87 ± 48.31	23.61	<0.001
Martin's formula	177.47 ± 51.33	0.00	0.9941
Group 4			
Direct	176.21 ± 58.99		
Friedewald's formula	148.70 ± 65.78	27.52	<0.001
Anandaraja's formula	137.39 ± 58.33	38.83	<0.001
Martin's formula	176.98 ± 60.58	−0.77	0.4288

Abbreviations: LDL-C, low-density lipoprotein cholesterol; SD, standard deviation.
Note: Mean difference = direct LDL cholesterol − formula-calculated LDL cholesterol; $p < 0.05$ is statistically significant.

Table 4 Comparison of percentage of error from direct LDL-C and calculated LDL-C by different formulas

	LDL-C by Friedewald	**LDL-C by Anandaraja**	**LDL-C by Martin**
Total	6.82	8.63	0.23
Group 1	2.54	0.91	0.34
Group 2	7.80	9.87	0.48
Group 3	7.74	13.30	0.00
Group 4	15.62	22.04	0.44

Abbreviation: LDL-C, low-density lipoprotein cholesterol.
Note: Percentage of error = (Calculated LDL cholesterol − Direct LDL cholesterol)/Direct LDL-C × 100.

results previously reported by Kamal et al,[11] Agrawal et al,[12] and Tremblay et al,[13]which shows that Friedewald's formula underestimates LDL-C at higher TG ranges. It may be because the performance of Friedewald's equation steadily decreases with increasing TG and is not recommended for hypertriglyceride (<400 mg/dL) ranges. In contradictory studies, Mora et al[14] and Gazi and Elisaf[15] have reported overestimation of LDL-C by Friedewald's formula as compared with D-LDL-C.

The present study shows underestimation by Anandaraja's formula compared with the D-LDL-C. Previous studies conducted by Kapoor et al,[8] Kamal et al,[11] Gupta et al,[16] Kamezaki et al,[17] and Sudha et al[18] also reported underestimation by Anandaraja's formula. In Group 1, mean difference between Anandaraja's formula and D-LDL-C is least compared with other formulas. The results are consistent with Krishnaveni and Gowda.[19] Krishnaveni and Gowda[19] showed that for subjects with serum TG levels less than 100 mg/dL, Anandaraja's formula was the most accurate.

Kamal et al,[11] Miller et al,[20] and Nakanishi et al[21] have showed that as TG levels increase, there is an increase in mean difference between direct and formula-calculated LDL-C. The present study results support this finding: with

an increase in TG concentrations, the difference between D-LDL-C and LDL-C calculated by Friedewald's and Anandaraja's formulas increased. Gupta et al.[16] and Lee et al[22] observed that LDL-C concentrations had no relation with TG concentrations. Martin-LDL-C values were closer to D-LDL-C in all the groups.

Martin's formula (90.90%) resulted in the best concordance with the direct measurement compared with Friedewald's formula (79.60%) and Anandaraja's formula (82.90%). The results are consistent with studies done by Martin et al,[7] Kang et al,[6] and Lee et al.[22] Overestimation and underestimation rates produced by Martin's formula are less than those produced by Friedewald's and Anandaraja's formulas; the difference is particularly pronounced in the underestimation rate. This is of particular importance because underestimation is generally considered riskier than overestimation, especially when screening the general population, as underestimation can cause delays in initiation of treatment.

The present study shows tendency of the Friedewald's formula to underestimate LDL-C. It is in these clinical conditions that Martin's formula may be more useful. In all the groups, Pearson's correlation coefficient *r*-value was high for Martin's formula compared with Friedewald's formula. It was suggested that Martin's formula may prevent undertreatment due to the underestimation of LDL-C using Friedewald's formula. Our results confirmed those of Martin et al,[7] Kang et al,[6] and Lee et al,[22] who stated that Martin's formula offers a significant improvement in LDL-C estimation when compared with Friedewald's formula. Martin's formula can be used instead of routine Friedewald's formula as Martin's formula is more accurate.

In a developing country like India with a burdening population with high TG, there is a need to adopt the novel equation. Martin's 180-cell approach could be coded into an online calculator, smartphone application, or automated laboratory reporting system.

Conclusion

In the present study, Martin's formula showed high correlation, lower mean difference, highest concordance, and low percentage of errors in all the groups compared with Friedewald's formula and Anandaraja's formula. At all TG levels, Martin's formula is best compared with Friedewald's formula and Anandaraja's formula.

Limitation of Study

This present study has a few limitations. First, the results may not be generalizable to the overall population, as there may be differences in baseline characteristics between our subjects and the general population. We had only access to the lipid profiles of the subjects and clinical characteristics or clinical outcomes of patients in our sample were unknown. Second, instead of calculating the adjustable factor for Martin's formula, we used the calculator that was suggested by the authors, and hence there is a possibility that the adjustable factor for the Indian population may be different from what Martin et al reported.

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Authors' Contributions
S.A. developed the concept, designed the study, and prepared the manuscript. F.F. collected the samples, analyzed the samples, and helped in manuscript editing. K.J. prepared and edited the manuscript. S.J. helped in statistical analysis of data and manuscript editing.

Conflict of Interest
None declared.

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