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Biochemistry Section

Correlation of LDL Cholesterol Calculated by Friedewald's, Puavilai's, Vujovic's, de Cordova's and Martin's Formulae with Directly Measured LDL Cholesterol: A Cross-sectional Study

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ABSTRACT

Introduction: Measurement of Low Density Lipoprotein Cholesterol (LDL-C) carries high importance in the management of Cardiovascular Disease (CVD). Direct LDL-C measurement is preferred method but this is expensive and inconvenient for the routine laboratories. To date, various types of formulae have been introduced. However, accurate estimation of LDL-C by formula is a challenge.

Aim: To determine that which of these calculated formulae (Friedewald's, Puavilai's, Vujovic's, de Cordova's and Martin's formulae) show maximum correlation with directly measured LDL-C at different serum triglyceride levels.

Materials and Methods: The present cross-sectional study was conducted in the Department of Biochemistry, KLE Centenary Charitable Hospital and Medical Research Centre, Belgaum, Karnataka, India, from December 2020 to December 2021. A total of 280 outpatient fasting complete lipid profiles of patients, aged between 18-50 years were included in the study. LDL-C measured by Friedewald's formula, Puavilai's formula, Vujovic's formula, de Cordova's formula and Martin's formula were compared with directly measured LDL-C. Comparison of calculated LDL-C with directly measured LDL-C was done at following Triglyceride (TG) ranges as group 1: <200 mg/dL, group 2: 200-300 mg/dL, group 3: >300-400 mg/dL and group 4: >400 mg/dL. Data analysis was done using Pearson's correlation coefficient and two paired t-test.

Results: Of total 280 samples, 124 participants were in group 1, 91 participants in group 2, 36 participants in group 3 and 29 participants in group 4, and there were 130 males and 150 females. The mean age in group 1, 2, 3 and 4 was 40.9±8.0 years, 38.8±9.2 years, 39.1±10.0 years and 39.8±8.2 years, respectively. Martin's formula showed maximum correlation with r-value of 0.9979 compared to Friedewald's formula, Puavilai's formula, Vujovic's formula and de Cordova's formula. The mean difference was least for Martin's formula 0.31±3.53 compared to other formulas. Percentage of error was least for Martin's formula (0.23%) in total study sample and in all groups. Martin's LDL-C shows highest concordance (90.90%) compared to Frielwald's (79.60%), Puavilai's (86.00%), Vujovic's (83.88%) and de Cordova's formula (82.76%).

Conclusion: In the present study, Martin's formula showed highest correlation, least mean difference, highest concordance and low percentage of errors in all the groups compared to Frieldwald's formula, Puavilai's formula, Vujovic's formula and de Cordova's formula.

Keywords: Cardiovascular disease, Cholesterol calculation, Direct assay, Dyslipidaemia, Low density lipoprotein, Triglyceride

INTRODUCTION

High serum Low Density Lipoprotein Cholesterol (LDL-C) concentration is the strongest marker of atherosclerosis and an important risk factor for CVD [1]. The US National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) has recommended that serum LDL-C level should be the primary target in dyslipidaemia treatment [2]. As treatment depends on LDL-C levels, it is very crucial to estimate LDL-C accurately. Due to cost-effectiveness or unavailability of direct LDL measurement, LDL-C is measured by Friedewald's formula [3]. Friedewald's formula uses the assumptions that very LDL-C (VLDL-C) greatly influences TG levels and that the ratio between TG and VLDL-C is 5 [4]. However, the actual ratio varies. Thus, many studies have stated that Friedewald's equation tends to either overestimate or underestimate LDL-C in individuals [5-7]. Many attempts have been made to evaluate and refine Friedewald's formula. The different modified formulas like Puavilai's formula, De Cordova's formula, Vujovic's formula and Martin's formula have been developed. Different formulas are been validated in different populations [8-11]. In the Friedewald's formula, VLDL-C is calculated as TG/5. In order to have a better estimation of LDL-C in Vujovic's and Puavilai's formulas, five is replaced by six and 6.82, respectively. Puavilai W et al., found Puavilai's formula is more accurate than the original Friedewald's formula in estimation of LDL-C [8]. Puavilai's formula can be used for non fasting sample, diabetes mellitus, obese patients and familial hypertriglyceridaemia patients. Puavilai's formula was validated in 1079 samples and the values of LDL-C were compared with direct LDL-C and Friedewald's LDL-C [8]. de Cordova CM and de Cordova MM had a study on Brazilian population and introduced a new formula for estimation of LDL-C in which TG concentration was omitted [9]. de Cordova's formula reported to outperform several of the earlier LDL-C formulae, including Friedewald's formula.

In order to correct Friedewald's formula limitations and improve the LDL-C estimation, Martin SS et al., proposed a new equation derived from Friedewald's formula for the estimation of LDL-C [11]. Martin's formula uses an adjustable factor for the calculation of the VLDL-C fraction based on TG (instead of the fixed divisor of five in

Friedewald's formula). This adjustable factor, which can range from 3.1 to 11.9, was derived from an analysis of triglyceride to VLDL-C ratios in more than 1.3 million people. This method matches each person with one of 180 different factors to estimate VLDL cholesterol from triglycerides [11]. But, there are very few Indian studies on Martin's formula [12,13].

Recently, there have been studies showing the efficiency of different formulae of several researchers in specific populations [1,3,13-20]. As can be seen, there are differences in performance of the formulae, due to the metabolic differences in different regions across varied populations.

Considering that the determination of the lipid profile is of fundamental importance to identify risk factors and to establish adequate therapeutic plans, it is necessary to have high safety regarding the diagnostic methods. Since, LDL-C value obtained by direct assay are more accurate, the present study was designed to compare the LDL calculated by several formulae with directly measured LDL over a wide range of TG levels. Hence, the present study was undertaken with the aim to determine that which of these calculated formulae (Friedewald's, Puavilai's, Vujovic's, de Cordova's and Martin's formula) show maximum correlation with directly measured LDL-C at different serum triglyceride levels in Indian population.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Biochemistry, KLE Centenary Charitable Hospital and Medical Research Centre, Belgaum, Karnataka, India, from December 2020 to December 2021. Ethical clearance was obtained from Institutional Ethics Committee of USM KLE IMP Belgaum (USM-KLE/IEC/04-2020). Written informed consent was taken from all participants.

Inclusion criteria: A total of 280 outpatient fasting complete lipid profiles of patients, aged between 18-50 years were included in the study.

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

Sample size calculation: The calculation was based on the assumption of an α error of 1% and a power of 90% [21,22]. The estimated sample size was 266.

Direct method LDL mean=118.02 [23]

Friedewald's method mean=107.22 [23]

Standard deviation in direct method=35.45

Standard deviation in Friedewald's method=24.35

Effect size: -0.26

Power=90%

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

Study Procedure

The demographic data such as age and sex was collected from all the study subjects. As a routine procedure, the samples were collected after 10-12 hours of overnight fasting by withdrawing 3 mL of venous blood in plain vial. The samples were centrifuged at 3000 rpm for 15 min to obtain serum and were analysed for lipid profile on the same day. Serum cholesterol, Triglyceride, High-density Lipoproteins (HDL) and LDL was estimated by commercial kit by autoanalyser [Table/Fig-1] [24-27].

In homogenous method of LDL-C estimation, LDL-C reacts with cholesterol esterase and oxidase to produce coloured complex

Analyte	Principle of method
Total cholesterol	Cholesterol Oxidase Peroxidase (CHOD-POD) method [24]
Triglyceride	Glycerol Phosphate Oxidase Peroxidase (GPO-PAP) method [25]
HDL-C	Cholesterol Esterase-Cholesterol Oxidase Peroxidase method) [26]
LDL-C	Homogeneous Enzymatic Colorimetric Assay method [27]

[Table/Fig-1]: Analytes and principal of method [24-27].

[27]. Apart from direct assay LDL-C was calculated by following formulae:

- ✓ Friedewald's formula [4]=TC- (TG/5+HDL-C)
- ✓ Puavilai's formula [8]=TC- (TG/6+HDL-C)
- ✓ Martin's formula [11]=(TC-HDL-C)-(Triglycerides/adjustable factor)
- ✓ Vujovic's formula [10]=TC-HDL-(TG/6.82)
- ✓ de Cordova's formula [9]=(TC-HDL)*0.7516

According to NCEP-ATP III criteria, TG >200 mg/dL is high triglyceride levels [28]. Triglyceride levels affect the accuracy of calculated LDL-C. As the triglyceride concentrations increases above 200 mg/dL, there is an increased chances of errors in calculated LDL-C [18]. So in the present study, to improve the comparison between methods, samples were stratified according to triglyceride levels.

- Group 1: <200 mg/dL
- Group 2: 200-300 mg/dL
- Group 3: >300-400 mg/dL
- Group 4: >400 mg/dL

The present study compared the concordance of the directly measured LDL-C with the estimated LDL-C when classifying LDL-C values by NCEP-ATP III. Results were labelled 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. The mean percentage difference/percentage of error was calculated as was done by a previous study by Kapoor R et al., using the formula:

PD=(calculated LDL-C-Direct LDL-C)/Direct LD-C×100 [23]

STATISTICAL ANALYSIS

Data analysis was done by using Statistical Package for the Social Sciences (SPSS) Software version 16.0. The distribution of continuous variables were described as means and standard deviations (mean±SD) and compared using Student t-test. Correlation between various methods of LDL-C was assessed by Pearson's correlation. The level of statistical significance was established at p-value <0.05.

RESULTS

The study consists of total 280 samples. There were 124 participants in group 1, 91 participants in group 2, 36 participants in group 3 and 29 participants in group 4. Mean age of group 1, 2, 3 and 4 is 40.9±8.0, 38.8±9.2, 39.1±10.0 and 39.8±8.2, respectively. There was no significant difference in age and gender in study population between the groups [Table/Fig-2].

Variables	Group 1	Group 2	Group 3	Group 4	Total	p-value
Gender						
Male, n (%)	64 (51.6)	37 (40.7)	20 (55.6)	9 (31)	130	0.089
Female, n (%)	60 (48.4)	54 (59.3)	16 (44.4)	20 (69)	150	
Age (years)						
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, n	124	91	36	29	280	

[Table/Fig-2]: Comparison of four groups by age and gender. Independent t-test

Among total sample mean difference of direct and calculated formula was least for Martin's formula 0.31±3.53 as compared to

other formulas. In group 1, 2, 3 and 4 mean difference was least for Martin's formula with values 0.40±1.2, 0.65±5.17, 0.00±2.47 and -0.77±5.13, respectively compared to other formulas. In group 3, de Cordova's formulas showed statically insignificant mean difference. In group 4 Vujovic's formulas showed stastically insignificant mean difference [Table/Fig-3].

Total sample			
Method	**Mean±SD**	**Mean difference (mg/dL)**	**p-value**
Direct	137.42±54.51	-	-
Frieldwald's formula	128.06±55.18	9.37±9.31	**<0.001**
Puavilai's formula	135.75±56.40	1.67±6.78	**<0.001**
Vujovic's formula	140.37±57.24	-2.95±5.88	**<0.001**
de Cordova's formula	130.94±49.11	6.48±11.27	**<0.001**
Martin's formula	137.11±54.82	0.31±3.53	0.1443
Group 1			
Direct	118.61±44.55	-	-
Frieldwald's formula	115.60±45.27	3.01±2.74	**<0.001**
Puavilai's formula	120.25±45.81	-1.64±2.19	**<0.001**
Vujovic's formula	123.04±46.16	-4.43±2.13	**<0.001**
de Cordova's formula	107.85±36.76	10.76±8.50	**<0.001**
Martin's formula	118.21±44.47	0.40±0.79	**<0.001**
Group 2			
Direct	134.85±53.09	-	-
Frieldwald's formula	124.33±56.93	10.52±6.96	**<0.001**
Puavilai's formula	132.20±56.93	2.65±6.79	**<0.001**
Vujovic's formula	136.94±56.93	-2.09±8.39	**<0.001**
de Cordova's formula	128.94±42.95	5.91±11.61	**<0.001**
Martin's formula	134.20±52.76	0.65±5.17	0.2343
Group 3			
Direct	177.48±50.13	-	-
Frieldwald's formula	163.74±55.03	13.74±6.11	**<0.001**
Puavilai's formula	175.17±55.09	2.31±6.14	**0.0304**
Vujovic's formula	182.04±55.14	-4.56±6.23	**<0.001**
de Cordova's formula	174.62±41.83	2.86±9.83	0.0899
Martin's formula	177.47±51.33	0.00±2.47	0.9941
Group 4			
Direct	176.21±58.99	-	-
Frieldwald's formula	148.70±65.78	27.52±8.87	**<0.001**
Puavilai's formula	164.22±65.79	11.99±8.90	**<0.001**
Vujovic's formula	173.55±65.81	2.66±9.0	0.1224
de Cordova's formula	181.76±49.75	-5.55±12.01	**0.0191**
Martin's formula	176.98±60.58	-0.77±5.13	0.4288

[Table/Fig-3]: Comparison of mean value of direct LDL-C and calculated LDL-C by different formulas.
SD: Standard deviation; Mean Difference=Direct LDL-C- Formula calculated LDL-C; p-value in bold font indicates statistically significant values

Percentage of error from direct LDL to calculated LDL was least for Martin's formula, in total study sample and in all groups compared to other formulas [Table/Fig-4].

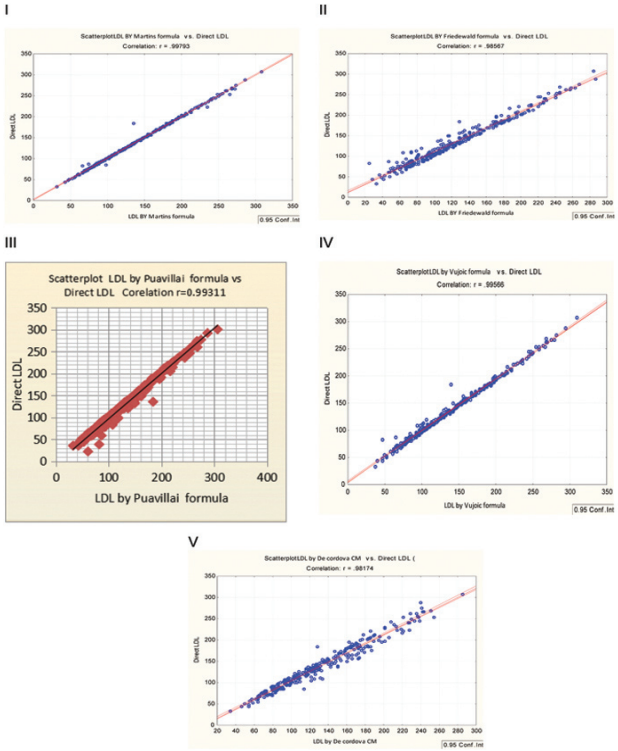
Groups	LDL by Frieldwald's	LDL by Puavilai's	LDL by Vujovic's	LDL by de Cordova CM's	LDL by Martin's	p-value
Total	6.82	1.22	-2.15	4.72	0.23	**<0.001**
Group 1	2.54	-1.38	-3.73	9.07	0.34	**<0.001**
Group 2	7.80	1.97	-1.55	4.38	0.48	**<0.001**
Group 3	7.74	1.30	-2.57	1.61	0.00	**<0.001**
Group 4	15.62	6.80	1.51	-3.15	0.44	**<0.001**

[Table/Fig-4]: Percentage of error between direct LDL with LDL estimated by different formulas.
The p-value in bold font indicates statistically significant values

Among total study sample, a strong correlation was found between direct LDL and calculated LDL by all different formulas in all the groups and it was statistically significant. Martin's formula shows highest correlation with r-value 0.9979, compared to other formulas r-value Friedewald's 0.9857, Puavilai's formula 0.9931, Vujovic's formula 0.9957 and de Cordova's formula 0.9817 [Table/Fig-5,6].

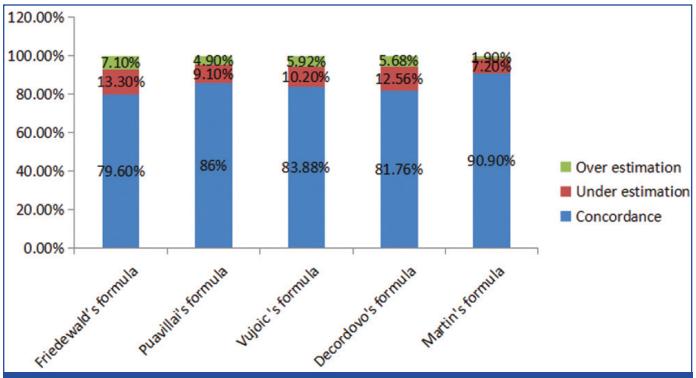
Samples	Variables	r-value	p-value
Total	Frieldwald's formula	0.9857	**<0.001**
	Puavilai's formula	0.9931	**<0.001**
	Vujovic's formula	0.9957	**<0.001**
	de Cordova's formula	0.9817	**<0.001**
	Martin's formula	0.9979	**<0.001**
Group 1	Frieldwald's formula	0.9983	**<0.001**
	Puavilai's formula	0.9992	**<0.001**
	Vujovic's formula	0.9995	**<0.001**
	de Cordova's formula	0.9964	**<0.001**
	Martin's formula	0.9998	**<0.001**
Group 2	Frieldwald's formula	0.9944	**<0.001**
	Puavilai's formula	0.9948	**<0.001**
	Vujovic's formula	0.9949	**<0.001**
	de Cordova's formula	0.9930	**<0.001**
	Martin's formula	0.9953	**<0.001**
Group 3	Frieldwald's formula	0.9976	**<0.001**
	Puavilai's formula	0.9976	**<0.001**
	Vujovic's formula	0.9975	**<0.001**
	de Cordova's formula	0.9934	**<0.001**
	Martin's formula	0.9991	**<0.001**
Group 4	Frieldwald's formula	0.9958	**<0.001**
	Puavilai's formula	0.9958	**<0.001**
	Vujovic's formula	0.9956	**<0.001**
	de Cordova's formula	0.9900	**<0.001**
	Martin's formula	0.9967	**<0.001**

[Table/Fig-5]: Correlation between direct LDL-C with calculated LDL-C by different formula by Karl Pearson's correlation method.
r=Correlation coefficient; The p-value in bold font indicates statistically significant values



[Table/Fig-6]: Correlation between direct LDL-C and calculated LDL-C by different formulae.

Martin's formula (90.90%) resulted in the best concordance with the direct measurement compared to Friedewald's formula (79.60%), Puavilai's formula (86%), Vujovic's formula (83.88%) and de Cordova's formula (81.76%). Overestimation and underestimation rates produced by Martin's formula are less than those produced by other formulas [Table/Fig-7].



[Table/Fig-7]: Comparision of the concordance of the directly measured LDL-C with the calculated LDL-C by different formulas.

DISCUSSION

The present study is undertaken to determine that which of these calculated formulae (Friedewald's, Puavilai's, Vujovic's, de Cordo's and Martin's formula) show maximum correlation with directly measured LDL-C at different serum triglyceride levels. In the present study, Martin's formula showed highest correlation, least mean difference, highest concordance and low percentage of errors in all the groups compared to other formulas. From past decades numerous studies have been conducted to derive more precise formulas for LDL-C calculation in different populations compared to the globally used Friedewald's formula [1,6,23,29-34]. However, some of these modifications were not found to be suitable replacements of the Friedewald's formula [35].

Among all the formulas, mean difference and percentage of error produced by Friedewald's formula is high in total sample and in group 2, 3 and 4. The present study results are consistent with the results previously reported by Kamal AHM et al., Agrawal M et al., Mora S et al., [36-38]. Study conducted by Tremblay AJ et al., shows that Frieldwald's formula underestimates LDL at higher triglyceride ranges [39]. It may be because the performance of Friedewald's formula steadily decreases with increasing TG and is not recommended for hypertriglyceride (<400 mg/dL) ranges.

After Martin's formula, Puvillai's formula performed best in group 1, 2 and 3. The present study results are consistent with studies reported by Kang M et al., Karkhaneh A et al., Garule MD et al., and, Wadhwa N and Krishnaswamy R [1,15,40,41]. Garule MD et al., showed that the Puavilai's formula is the most accurate formula and correlates with the direct method at all triglyceride levels [40]. Wadhwa N and Krishnaswamy R showed in Indian population, Puavilai's formula correlated well with direct measurement and performed better than Friedewald's formula at TG range <150 mg/dL. Puavilai's equation using a TG: VLDL-C ratio of six seems to be superior to Friedewald's equation. It shows less difference and good correlation than Friedewald's equation [41].

The present study showed Vujovic's formula overestimates LDL in total sample and in group 1, 2 and 3. This is contradictory to the study done by Vujovic A et al., and, Wadhwa N and Krishnaswamy R [10,41]. In group 4 at triglyceride >400 mg/dL, Vujovic's formula performed best with mean difference 2.66 and r-value of 0.9956 and low percentage of error 1.51%. Results of the present study are consistent with studies reported by Choi H et al., [42].

de Cordova's formula performed best in group 3 with mean difference 2.86 and r-value of 0.9934. The present study results are consistant with studies done by Karkhaneh A et al., [15]. Karkhaneh A et al., showed that de Cordova's formula could be the best alternatives for LDL-C direct measurement in Iranian population, especially for healthy subjects [15]. Next to Friedewald's equation de Cordova's formula does not performed well in all the groups. Results are consistent with studies reported by Wadhwa N and Krishnaswamy R, who showed that de Cordova's formula it is not suitable to be used in Indian population [41]. This is contradictory to the study done by Karkhaneh A et al., which concluded that de Cordova's formulas can be considered as the best alternatives for LDL-C direct measurement in the Iranian population [15]. May be due to diversity in terms of study populations compared to Brazilian/German population in which de Cordova's formula was validated.

Among five formulas, Martin's formula shows best concordance 90.90%. The present study results are same as that of Martin SS et al., and Chaen H et al., [11,43]. In a study done by Chaen H et al., at TG ≥150 mg/dL Martin's formula demonstrated a better concordance compared with Frieldwald's formula [43]. Martin SS et al., reported overall concordance of 85.4% for Frieldward's formula versus 91.7% for Martin's formula (p-value <0.001) [11]. The present study showed higher concordance compared to Lee J et al., and Meeusen JW et al., [5,44]. Lee J et al., showed concordance of 78.2% for Frieldwald's equation and 82.0% for Martin's formula [5]. Meeusen JW et al., found that overall concordance results as 76.9% for Frieldward's formula versus for 77.7% Martin's formula [44]. Possible explanation for difference in concordance is racial differences and related difference in dietary patterns. This could be postulated to impact TG:VLDL-C ratio.

The present study shows, among five different formulas Martin's formula showed best performance with correlation 0.9979, the lowest mean difference 0.31, lowest percentage of error 0.23% and best concordance 90.90%. Results of the present study are consistent with the results previously reported by Lee J et al., Martin SS et al., and Reiber I et al., [5,11,45]. Tomo S et al., showed that Martin's formula appeared to more precisely calculate LDL-C in type 2 diabetes when compared with the traditional Friedewald's formula [46]. Martin SS et al., looked into 1,310,440 total patients and 191,333 patients with Friedewald's LDL <70 mg/dL and noted that a greater difference in the Friedewald-estimated versus directly measured LDL occurred at lower LDL and higher TG levels [11].

As Friedewald's formula has three analytes there is an increased risk of analytical error exceeding NCEP recommended criteria (>±12%). Friedewald's formula uses a fixed factor of 5, but actual ratio is going to vary for wide range of cholesterol and triglyceride levels. Because of these limitations of Friedewald's formula, many researchers invented new formula's. New formulas did not perform well compared to Friedewald's formula. However, as Martin's formula use adjustable factor for TG:VLDL-C ratio found to be more accurate than Friedewald's formula [14].

The traditional calculation of LDL-C with the Friedewald's formula tends to significantly underestimate LDL-C levels in very high and high-risk treatment targets, especially when triglycerides exceed 400 mg/dL [45]. The present analysis shows that LDL-C estimation using the Martin's/Hopkins formula which is validated by the β-quantification method, yields a more accurate LDL-C value than that calculated by the Friedewald's formula.

In summary, higher correlation and linear regression co-efficients, higher agreement and smaller differences between Martin's formula and directly measured LDL values compared to Frieldwald's formula, Vujovic's formula, de Cordova's formula and Puavilai's formula values were encountered, in all the groups.

Limitation(s)

The present study also had several limitations that need to be addressed. Firstly, the β-quantification method, which is considered the gold standard method for measuring LDL-C, has not been used. Secondly, the study needs to be validated within a larger study population. Thirdly, instead of calculating adjustable factor for Indian population, in Martin's formula, the present study used calculator and there is a possibility that adjustable factor for Indian population may be different.

CONCLUSION(S)

In the present study, Martin's formula appeared to be more accurate compared to other formulas at all levels of triglyceride. Martin's formula could be cost-effective alternative to direct LDL-C measurement, which may be readily adoptable in clinical laboratories. Next to Martin's formula, at triglyceride >400 mg/dL, Puavilai's formula, performed best. Many laboratories globally use Friedewald's formula as alternative to direct method for LDL-C estimation. A cost-benefit analysis investigating the cost incurred from directly measuring LDL-C and the societal cost or burden arising from erroneous Friedewald estimations and the relative benefits of direct measurements should be conducted. More studies using larger sample sizes, from different ethnic and geographical populations need to be conducted.

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