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Original Research Article

COMPARISON OF CALCULATED LOW DENSITY LIPOPROTEIN-CHOLESTEROL LEVELS BY FRIEDEWALD'S, MARTIN'S, SAMPSON'S EQUATION TO DIRECT METHOD : A STUDY ON CARDIOVASCULAR RISK ASSESSMENT TOOL

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ABSTRACT

Background: Accurate estimation of low-density lipoprotein cholesterol (LDL-C) is essential for cardiovascular risk assessment. Although direct measurement is considered the reference standard, it is not routinely available in all settings. The Friedewald's equation is widely used in clinical practice, but has limitations at higher triglyceride levels. Newer equations, including the Martin's and Sampson's equations, have been developed to improve accuracy, particularly in hypertriglyceridemia. So, this study aimed to compare LDL-C estimated using the Friedewald's, Martin's, and Sampson's equations with directly measured LDL-C and evaluates their performance across different triglyceride levels. **Materials and Methods:** This analytical cross-sectional study included 128 participants. LDL-C was estimated using Friedewald's, Martin's and Sampson's equations and compared with directly measured LDL-C. As the data were not normally distributed, comparisons were performed using the Wilcoxon signed-rank test. The effect of triglyceride levels on the accuracy of estimation was assessed using one-way ANOVA with Tukey's post-hoc analysis. Agreement between methods was evaluated using Bland–Altman analysis. **Results:** The Friedewald's and Martin's equations significantly underestimated LDL-C, when compared to directly measured LDL-C [*($W=5709$, $p<0.001$)* and *($W=4818$, $p=0.002$),* respectively]. In contrast, LDL-C estimates obtained using the Sampson equation did not differ significantly from directly measured LDL-C *($W=4566$, $p=0.085$).* Bland–Altman analysis demonstrated a larger negative bias and wider limits of agreement for the Friedewald;s equation, particularly at higher triglyceride levels, whereas Sampson's and Martin's equations showed relatively smaller bias. The percentage difference between calculated and direct LDL-C increased with rising triglyceride levels for all equations *($p < 0.001$),* with the greatest underestimation observed for the Friedewald's equation. **Conclusion:** The Friedewald's equation shows significant underestimation of LDL-C, particularly at higher triglyceride levels. Sampson-based equations demonstrate better agreement with directly measured LDL-C, while the Martin equation shows relatively stable performance across triglyceride levels. This study suggests that in subjects with higher triglyceride level may have direct method LDL-C estimation which may aid in correct stratification of atherosclerotic cardiovascular disease risk and treatment plan.
Keywords: Low-density lipoprotein cholesterol, Very low-density lipoprotein, cholesterol, Triglycerides, Cardiovascular risk, Martin's equation, Friedewald's equation, Sampson's equation

INTRODUCTION

Need for Cardiovascular Risk assessment in India is high as 27% of total deaths due to cardiovascular diseases and 45% of deaths aged 40–69 years by WHO report. Though Statins reduce Low-Density Lipoprotein-Cholesterol (LDL-C), its use requires careful risk–benefit assessment. LDL-C remains a key molecule to plaque formation and atherosclerotic cardiovascular disease (ASCVD). LDL-C levels can be calculated or measured directly. The gold standard method to measure LDL-C directly is ultracentrifugation precipitation-based procedure.[1] The extra cost of dLDL-C to the lipid panel and the poor analytical performance of some dLDL-C tests made calculated LDL-C use more. Nearly for 50 yrs LDL-C calculation done using Friedewald equation (F-equation).[2] The VLDL-C calculation and high TGL values disrupt accurate LDL-C calculation using F-equation as cholesterol to TGL ratio altered by other factors like Diabetes mellitus (DM), Metabolic syndrome (MS), Obesity.[3-4] This imprecision in LDL-C measurement may delay the use of the novel lipid lowering medications. F-equation underwent some modifications.[5] Martin et al developed an equation (Martin equation) based on the Vertical Auto Profile test, which is a vertical-rotor ultracentrifugation-based method.[6,7] In 2018, on reviewing the guidelines on CVD risk biomarkers, the new American College of Cardiology and American Heart Association recommended the Martin equation (M-equation) as the preferred LDL-C calculation method,[8] Its because of the LDL-C level accurately measured and proved the clinical potency of new drugs like proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors that markedly reduced LDL-C levels.[9,10] To enable more accurate risk classification by LDL-C level, M-equation used an adjustable factor for the TG:VLDL-C ratio.[11]

The Vertical Auto Profile method found to underestimate the VLDL-C level in high-TG samples by M-equation. As many patients with elevated TGL levels have suppressed LDL-C levels, also making already low levels of LDL-C even more difficult to accurately estimate it, because calculated VLDL-C has greater proportion of error. Both F-equation and M-equation tried to estimate LDL-C accurately but it's hard to adjust the proportion of error in VLDL-C calculation when metabolically LDL-C level is reduced when TGL level increases.[12,13] This urged the need to reduce the inaccuracy of calculated LDL-C due to elevated TGL levels.

In a patient population with high TGL, Sampson et al developed a new LDL-C equation using β-quantification results.[14] Samson's equation (S-equation) tried to calculate LDL-C level more accurately both in patients having normolipidemia and high TGL levels up to 800 mg/dL. Compared to other equations, S-equation able to correctly classify patients into different LDL-C treatment groups and improve CVD risk management. The greatest clinical benefit of Sampson et al equation may be for patients with low LDL-C levels. The M-equation is also more accurate than the Friedewald equation for such patients, which is the reason many clinical laboratories have switched to this equation. Low LDL-C levels were once rare but are much more common with the use of anti-PCSK9 therapy.[9] Given the high cost of anti-PCSK9 therapy, the initial eligibility of patients for this type of therapy critically depends on the accurate measurement of LDL-C levels. Even once a patient is using anti-PCSK9 therapy, it is important to accurately monitor LDL-C levels. Because of the overestimation of the VLDL-C level by the Friedewald equation and some of the other equations, these patients can have falsely low LDL-C results, which may discourage more aggressive lipid lowering therapy that has been shown to be beneficial in high-risk patients.

As per the guidelines, specific percent of LDL-C reductions should be monitored to assess therapeutic effects of statins. However, if LDL-C is inaccurately estimated, this may have important implications with goal reductions and patient classification. As clinical decision-making trends and guideline management continue to push towards lower LDL-C levels, the need for updated and accurate clinical tools becomes even more important.

Hence, in this study it is proposed to compare and analyse the calculated LDL-C values by Friedewald's, Martin's and Sampson's equations. Furthermore, analysed the difference in LDL-c value among patient with high triglycerides and low LDL-c level.

Aim of the Study

To compare low-density lipoprotein cholesterol (LDL-C) estimated using different calculation equations with directly measured LDL-C.

Objectives

- To compare LDL-C estimated using the Friedewald, Martin and Sampson equations with directly measured LDL-C.
- To evaluate the effect of triglyceride levels on the accuracy of LDL-C estimation across different equations.

MATERIALS AND METHODS

This analytical cross-sectional study that includes outpatient fasting complete lipid profile values for patients of > 18yrs of age processed in central biochemistry laboratory collected for a period of 6 months.

Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the formula:

1. Friedewald's equation:

LDL-C = (Total Cholesterol) − (HDL-C) − (TG)/5

2. Martin's equation:

LDL-C = (Total Cholesterol) − (HDL-C) − (TG)/ Novel factor

3. Sampson's Equation:

LDL-C = (TC/0.94) – (HDL-C/0.971) – [(TG/8.56) +(TG x Non–HDL-C)/2140)– (TG2 /16100)] – 9.44

Inclusion Criteria

- Individuals aged ≥ 18 years
- Participants with complete lipid profile parameters, including total cholesterol, triglycerides, HDL-C, and directly measured LDL-C

Exclusion Criteria

Samples with high triglycerides levels (more than 800 mg/dL) and non-HDL-C >300 mg/dL.

Data Collection

A total of 128 participants who underwent lipid profile testing during the study period were included in the analysis. The samples were processed in fully automated biochemistry analyser- Transacia XL 640 after internal quality control check. Estimation of Total Cholesterol, High Density Lipoprotein-Cholesterol, Low Density Lipoprotein –Cholesterol and Serum Triglyceride were done by colorimetric method. Using F-equation, M-equation and S-equation Low-density lipoprotein cholesterol (LDL-C) levels were calculated and all data were entered in excel sheet.

Data Analysis

Data were analysed using Epi Info v7.2.6.0. The. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), as appropriate. Assessment of normality was done using the Shapiro–Wilk test to indicate the percentage difference across triglyceride groups. Additionally, homogeneity of variances was confirmed using Levene's test for equality of variances. Wilcoxon signed rank test were run on the data to compare calculated LDL-C values with directly measured LDL-C. The one-way ANOVA was performed to compare the mean percentage difference across the triglyceride categories. Tukey's post-hoc analysis was performed to identify which triglyceride categories differed significantly for each equation. Bland–Altman Analysis Plot was prepared to show the agreement between direct LDL-C and LDL-C estimated using different calculation equations. The results gave the mean + SD and P values were considered significant if ≤0.05.

RESULTS

A total of 128 participants were included in the analysis. The baseline lipid characteristics of the study population are presented in [Table 1].

Table 1: Baseline lipid profile characteristics of the study participants (N=128)

VARIABLE	Mean	SD	Median	IQR
Total cholesterol	215.35	46.91	213.5	185-242
HDL-C	55.04	16.39	53	44-65
Triglycerides	160.84	107.1	127	88-184.5
Direct LDL-C	133.96	34.61	133	110-153.5

Table 2: Comparison of calculated LDL-C with directly measured LDL-C (N=128)

Method	Mean (SD)	Median (IQR)	W value	p value
Sampson	131.49 (36.06)	131 (108.5-155)	4566	0.085
Friedewald	128.22 (37.24)	128 (103.5-152.5)	5709	<0.001
Martin	131.99 (36.31)	130.5 (106.5-154.5)	4818	0.002

Inspection of Q-Q Plots and Shapiro Wilk test revealed that LDL-C values were not normally distributed across the methods of estimation. Therefore, the Wilcoxon signed rank test was run on the data to compare calculated LDL-C values with directly measured LDL-C. The median LDL-C estimated by the different equations was compared with the directly measured LDL-C [Table 2]. Among the evaluated equations, the F-equation significantly underestimated LDL-C when compared to the direct measurement (W = 5709, p < .001). The M-equation also showed a statistically significant underestimation of LDL-C when compared to the direct method (W = 4818, p =0.002). In contrast, the S-equation did not show statistically significant differences from directly measured LDL-C values (W = 4566, p = .085). These findings indicate that the S-equation produced LDL-C estimates closer to the direct LDL- C measurement, whereas the F-equation demonstrated the largest deviation.

Table 3: One-way ANOVA comparing percentage difference between calculated LDL-C and directly measured LDL-C across triglyceride categories

	Normal TGL <150 mg/dL (%) (N=73)	Elevated TGL 150-199 mg/dL (%) (N=27)	200-299 mg/dL (%) (N=14)	≥ 300 mg/dL (%) (N=14)	p value
Sampson	1.3 (4.98)	-2.84 (6.28)	-5.55 (5.05)	-16.04 (7.17)	<0.001
Friedewald	-0.34 (5.27)	-6.04 (7.72)	-9.27 (6.61)	-21.83 (12.96)	<0.001
Martin	-0.76 (4.23)	-2.09 (4.97)	-2.51 (3.89)	-6.8 (5.35)	<0.001

The percentage difference between calculated LDL-C and directly measured LDL-C was evaluated across four triglyceride (TG) categories <150 mg/dL, 150–199 mg/dL, 200–299 mg/dL and ≥300 mg/dL Assessment of normality using the Shapiro–Wilk test indicated that the percentage difference values were normally distributed across triglyceride groups. Additionally, homogeneity of variances was confirmed using Levene's test for equality of variances. Therefore, one-way ANOVA was performed to compare the mean percentage difference across the triglyceride categories. The mean percentage difference with standard deviation for each category is presented in Table 3. In this analysis, negative percentage values indicate that the calculated LDL-C underestimated the directly measured LDL-C, whereas positive values indicate overestimation. Across all categories, the percentage difference increased with rising triglyceride levels, and this trend was statistically significant ($p < .001$). The F-equation showed the largest negative bias, with the degree of underestimation increasing substantially with rising triglyceride levels. The mean percentage difference changed from −0.34% in individuals with TG <150 mg/dL to −21.83% in those with TG ≥300 mg/dL, indicating a marked decline in accuracy at higher triglyceride concentrations. Similarly, the S-equation also demonstrated increasing underestimation with higher triglyceride levels, although the magnitude of deviation was smaller than that observed with the F-equation. Among the evaluated equations, the M-equation demonstrated comparatively smaller deviations across triglyceride categories, although some degree of underestimation was still observed at higher triglyceride levels.

Tukey's post-hoc analysis was performed to identify which triglyceride categories differed significantly for each equation. The F-equation's post-hoc analysis indicates that the equation performs relatively well at lower triglyceride levels but demonstrates substantial underestimation at very high triglyceride concentrations. For the S-equation, the estimation accuracy declines mainly when triglyceride levels become markedly elevated.In contrast, the M-equation demonstrated fewer significant pairwise differences between triglyceride categories, suggesting relatively greater stability of LDL-C estimation across varying triglyceride levels compared with the other equations.

Overall, these findings indicate that the degree of underestimation in calculated LDL- C increases with rising triglyceride levels, with the most pronounced deterioration observed at triglyceride levels ≥300 mg/dL, particularly for the F-equation.

Post hoc Tukey test - significance

1. Sampson:

Tukey Post-Hoc Test – PD_Samp

		150 to 199	200 to 299	More than 300	normal
150 to 199	Mean difference	—	2.72	13.2	−4.14
	p-value	—	.448	< .001	.007
200 to 299	Mean difference		—	10.5	−6.86
	p-value		—	< .001	< .001
More than 300	Mean difference			—	−17.34
	p-value			—	< .001
normal	Mean difference				—
	p-value				—

2. Friedewald

Tukey Post-Hoc Test – PD_fried

		150 to 199	200 to 299	More than 300	normal
150 to 199	Mean difference	—	3.23	15.8	−5.71
	p-value	—	.517	< .001	.003
200 to 299	Mean difference		—	12.6	−8.93
	p-value		—	< .001	< .001
More than 300	Mean difference			—	−21.49
	p-value			—	< .001
normal	Mean difference				—
	p-value				—

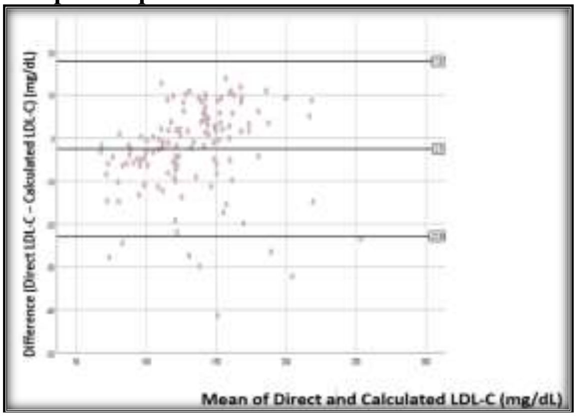
3. Martin

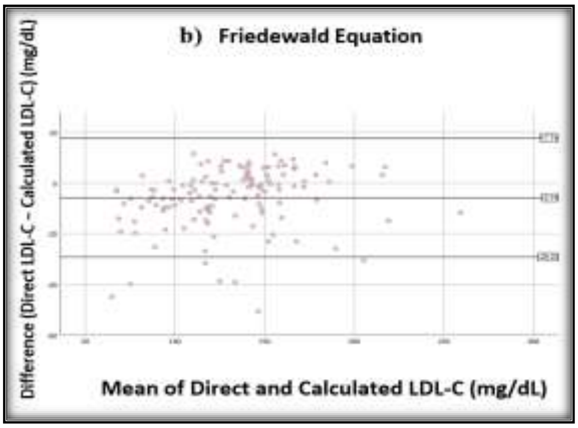
Tukey Post-Hoc Test – PD_ext martin

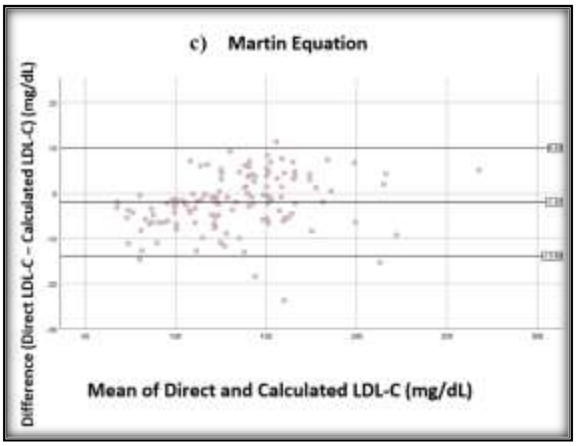
		150 to 199	200 to 299	More than 300	normal
150 to 199	Mean difference	—	0.406	4.70	−1.34
	p-value	—	.993	.010	.550
200 to 299	Mean difference		—	4.30	−1.74
	p-value		—	.060	.545
More than 300	Mean difference			—	−6.04
	p-value			—	< .001
normal	Mean difference				—
	p-value				—

Bland–Altman Analysis Plot Showing Agreement Between Direct LDL-C and LDL-C Estimated Using Different Calculation Equations

Sampson Equation







DISCUSSION

Among the evaluated equations, the F-equation significantly underestimated LDL-C when compared to the direct measurement ($W = 5709$, $p < .001$). This is in line with many previuos studies which shows F-equation low LDL-C values like AJ.Tremblay et al.(2004), J. Marniemi et al(1995) , D.A. Tighe et al.(2006).[15-17] LDL-C levels measured by the direct homogeneous assay had no difference between paired fasting and nonfasting samples and changes in postprandial samples were similar to those measured by β-quantification and also F-formula is known to underestimate LDL-C levels compared with β-quantification even at TGL levels <82mg/dL.[18]

The M-equation also showed a statistically significant underestimation of LDL-C when compared to the direct method ($W = 4818$, $p = 0.002$). These results are same as proposed by Youhyun Song et al. (2021), which states M-equation can still exhibit underestimation in conditions when low LDL-C, low triglycerides, and high HDL-C coexist.[19] In Type 2 Diabetes though F-equation might underestimate, M-equation gives accurate direct LDL-C, although it still showed minor underestimation in some studies.

In contrast, the S-equation did not show statistically significant differences from directly measured LDL-C values ($p = .085$). This results are in much agreement with many studies such as Li et al (2022), enlighting S-equation as an accurate and better method for calculating LDL-C specifically in subjects with high triglycerides (up to 800 mg/dL) or low LDL-C levels.[20] These findings indicate that the S-equation produced LDL-C estimates closer to the direct LDL- C measurement, whereas the F-equation demonstrated the largest deviation.

The one-way ANOVA showed the mean percentage difference increased with rising triglyceride levels across all category with statistical significance ($p < .001$). The F-equation showed the largest negative bias (−21.83% with TGL ≥300 mg/dL) that reduces accuracy at higher triglyceride concentrations. S-equation also demonstrated increasing underestimation with higher triglyceride levels (-16.04%with TGL>300mg/dL). Sampson et al also developed Modified sampson or Enhanced Sampson-equation incorporating apolipoprotein B (apoB) that gave even higher accuracy for low LDL-C levels which was studied by Coverdell et al and showed positive results.[21]

In our study M-equation gave comparatively smaller deviations across triglyceride categories with small degree of underestimation at higher triglyceride levels. Many studies , Ion Bogdan Mănescu et al,(2024) support that the extended Martin/Hopkins equation to provide a more accurate estimation of Low-Density Lipoprotein Cholesterol (LDL-C) compared to the Friedewald and Sampson equations, particularly in subjects with high triglyceride (TG) levels, ranging from 400 to 799 mg/dL.[22] Overall, these findings indicate that the degree of underestimation in calculated LDL- C increases with rising triglyceride levels, with the most pronounced deterioration observed at triglyceride levels ≥300 mg/dL, particularly for the Friedewald equation.

CONCLUSION

As the clinical management of patients at risk for cardiovascular disease (CVD) is critically dependent on their level of low-density lipoprotein cholesterol (LDL-C), derivation of a more accurate LDL-C equation for patients with hypertriglyceridemia and/or low LDL-C levels has practical value.

The ratio of cholesterol to TGs in VLDL-C can vary considerably depending on its size and other factors and this diminishes the accuracy of the F-equation. Both M-equation and S- equation provide an accurate estimate of LDL-C in normolipidemia subjects and when TGL level increased appropriate stratification of subjects required and correct extended or modified equation may be applied to calculate LDL-C levels.

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22. J Pers Med. 2024 Sep 20;14(9):1000. doi: 10.3390/jpm14091000 Low-Density Lipoprotein Cholesterol Gymnastics: Exploring the Advantages and Limitations of the Friedewald, Martin–Hopkins, and Sampson Equations for Personalized Lipid Management Ion Bogdan Mănescu 1,2,*, Liliana Demian 3,4, Minodora Dobreanu 1,3,4 Editors: Katharina Lechner, Patrick Müller. PMCID: PMC11433184 PMID: 39338254.