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RESEARCH ARTICLE

Comparison of the Accuracy of Calculated Low-Density Lipoprotein Cholesterol Versus Direct Measurement: A Cross-Sectional Study

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ABSTRACT

Background: Accurate assessment of lipid profiles particularly low-density lipoprotein cholesterol (LDL-C) is essential for the effective diagnosis and management of cardiovascular disease (CVD). While the Friedewald's formula remains the most commonly used method for estimating LDL-C, it has known limitations, especially in certain clinical scenarios. This has led to increased interest in alternative calculation methods.

Aim: To evaluate the accuracy of 12 calculated low-density lipoprotein cholesterol (LDL-C) estimation formulas in comparison with directly measured LDL-C levels.

Materials and Methods: Lipid profile records from 93 patients aged 18 years and older, with triglyceride levels below 400 mg/dL, were analysed. LDL-C levels were measured using the enzymatic method (CHE/CHO/POD) and also calculated using the following formulas: Friedewald, Anandaraja, De Cordova, Vujovic, Ahmadi, Puavillai, Chen, Hattori, Martin–Hopkins, Rao, DeLong, and Teerakanchana

Results: When comparing LDL-C values obtained by direct and calculated methods using the paired t-test, most calculated methods showed statistically significant differences from the direct method. However, the Chen and Martin methods demonstrated no significant difference, indicating close agreement with the direct assay. Overall, the calculated methods exhibited strong correlations with the direct method (r = 0.9142 to 0.996), except for the Ahmedi method, which showed a weak correlation (r = 0.450).

Conclusion: In conclusion, while most calculated LDL-C methods differed significantly from the direct assay, they generally showed strong agreement. The Chen and Martin formulas demonstrated the highest accuracy, with no significant differences and strong correlations, making them the most reliable alternatives. In contrast, the Ahmedi method showed poor correlation and limited clinical utility.

Keywords: Cholesterol, Lowdensity lipoprotein, Calculated formulas

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Introduction

Cardiovascular diseases (CVDs) remain the leading cause of death worldwide, accounting for about one-third of all fatalities and imposing a significant public health cost. Dyslipidemia is a major modifiable risk factor, with high low-density lipoprotein cholesterol (LDL-C) driving atherosclerosis and its clinical manifestations such as coronary heart disease (CHD), stroke, and peripheral vascular disease. As a result, reliable LDL-C testing is critical for determining cardiovascular risk, guiding lipid-lowering therapy, and monitoring treatment efficacy(1).

The gold standard method for estimating LDL-C is β-quantification, which requires ultracentrifugation and chemical precipitation. Although very precise, this procedure is labor-intensive, time-consuming, and necessitates specialized laboratory infrastructure, rendering it inappropriate for routine clinical treatment, particularly in resource-constrained environments. As a result, indirect estimating approaches have become widely accepted(2).

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The Friedewald formula (FF), which was introduced in 1972, is the most widely used method for estimating LDL-C due to its simplicity and cost-effectiveness. It calculates LDL-C by measuring total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C). However, despite its extensive use, FF has significant drawbacks. Its accuracy is reduced in conditions such as hypertriglyceridemia (TG > 400 mg/dL), non-fasting states, and Type III hyperlipoproteinemia. In these cases, FF may either underestimate or exaggerate LDL-C levels, potentially leading to incorrect therapeutic judgments(3).

To address these constraints, various alternative equations have been presented, including those created by Anandaraja, Chen, Vujovic, and Martin-Hopkins. These algorithms try to enhance LDL-C estimate by changing the association between triglycerides and very low-density lipoprotein cholesterol (VLDL-C), or by making population-specific changes. Notably, the Martin-Hopkins approach includes an adjustable factor for TG-to-VLDL conversion, which improves accuracy over a wider range of lipid profiles(4).

However, the performance of these formulas varies across populations. Variations in genetics, dietary habits, metabolic profiles, and dyslipidemia prevalence can all have an impact on their accuracy. As a result, investigations undertaken in various countries have produced contradictory findings about the reliability of these methodologies(5).

In this context, determining the agreement between estimated and directly measured LDL-C values is critical. Such assessments assist in determining the most accurate and clinically appropriate strategy for various demographics. This is especially significant in resource-constrained situations where access to direct LDL-C testing is limited and computed results remain the norm.

Materials& Methods

Design of study: Cross sectional study
Study Period: 1 Month
Total number of study subjects: n = 93

Inclusion Criteria:

Samples received in the Biochemistry laboratory of CDLS at RLJH & RC for routine lipid profile was collected, analyzed and direct low-density lipoprotein (LDL) cholesterol estimation was performed on these samples.

Exclusion Criteria

Patients with TG more than 400mg/dL, renal failure, hepatic diseases excluded from the study

Study Area

Patients visiting to R L JALAPPA hospital OPD or admitted in the wards

Statistical Analysis

Data were evaluated with IBM SPSS Statistics. Continuous variables were summarized as mean ± standard deviation (SD) after confirming normal distribution with suitable tests. The relationship between directly measured LDL-C and calculated LDL-C values obtained from various equations was examined using Pearson's correlation coefficient (r) to identify the degree and direction of the relationship between approaches.

The agreement between direct and computed LDL-C approaches was further evaluated using Cohen's kappa and mean percentage bias. These analyses will help to assess technique consistency and clinical interchangeability. A p-value of less than 0.05 was considered statistically significant in all comparisons.

Results

Table 1: Gender distribution

Gender	*Frequency*	*Percentage %*
Females	30	32.3
Males	63	67.7
Total	93	100.0

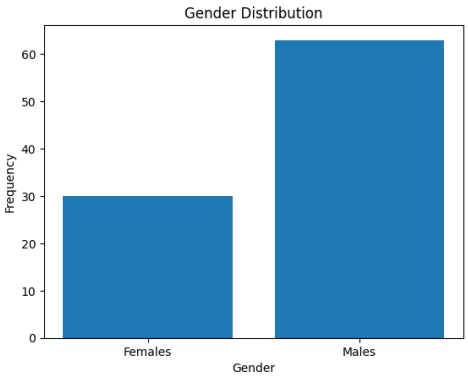

Figure 1: Gender distribution

Table 2: Frequency of subjects based on age

Age in years	*Number of subjects*	*Percentage (%)*
20-35 years	14	15.1%
36-50 years	47	50.5%
51-65 years	27	29.0%
>60 years	05	5.4%
Total	93	100%

Table 3: Age distribution of subjects with mean and Standard deviation

Particulars	*N*	*Minimum*	*Maximum*	*Mean*	*SD*	*Variance*
Age	93	23	70	48.26	11.265	126.889

Table 4: Lipid Profile parameters

Parameter	*Minimum*	*Maximum*	*Mean + SD*
Serum TC(mg/dL)	95	289	179.67± 42.15*
Serum TG(mg/dL)	53	347	167.74± 68.32*
Serum HDL-C (mg/dL)	19	58	36.19± 9.75*
Serum direct LDL-c(mg/dL)	58	198	112.28+35.68

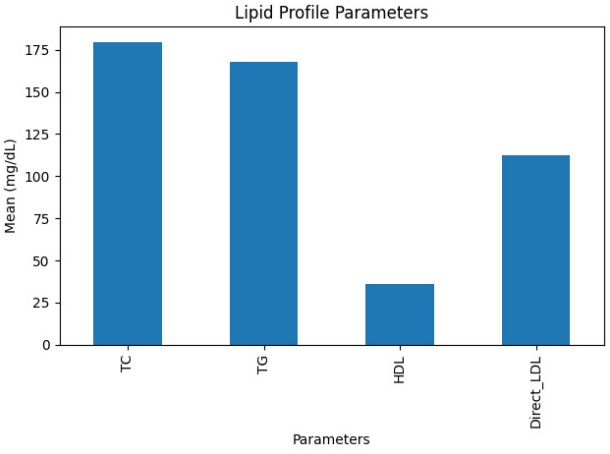

Figure 2: Lipid profile parameters

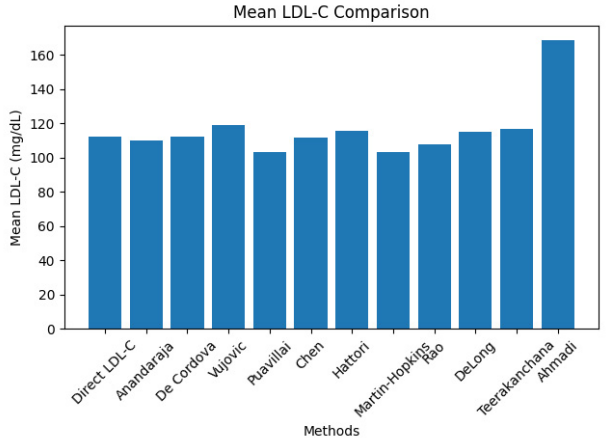

Figure 3: Mean LDL-C comparison

Table 5: Descriptive comparison of direct LDL and other calculated methods

Particulars	*N*	*Mean*	*SD*	*Std. error mean*
Direct LDL-C	93	112.28	35.68	-
Friedewald	93	109.93	34.67	3.59
Anandaraja	93	103.51	33.21	3.44
De Cordova	93	107.84	27.63	2.86
Vujovic	93	118.99	34.64	3.59
Ahmadi	93	168.37	57.02	5.91
Puavillai	93	115.52	34.60	3.58
Chen	93	112.36	31.38	3.25
Hattori	93	103.00	32.60	3.38
Martin–Hopkin	93	111.82	34.61	3.59
Rao	93	115.62	36.50	3.78
DeLong	93	116.64	34.60	3.58
Teerakanchana	93	115.18	31.97	3.31

When comparing the mean LDL-C values obtained by the direct and calculated methods using the paired t-test, the differences in means were statistically significant for most methods. However, the Chen and Martin methods showed no significant difference compared to the direct method (p-value not significant), indicating that these two methods closely agree with the direct assay in estimating LDL-C levels.

All the calculated methods showed good agreement with the direct method, indicating a high degree of correlation between these methods. The correlation coefficients ranged from 0.9142 to 0.996, except for the Ahmedi method, which showed a correlation of 0.450 (not significant).

Table 6: Comparison of direct LDL with other calculated methods

	Formulas	*Calculated Mean*	*Mean difference*	*p Value*
Direct LDL-C estimated Mean: 112.28	Friedewald	109.93	2.344	0.0001
	Anandaraja	103.51	8.763	0.0001
	De Cordova	107.84	4.437	0.0001
	Vujovic	118.99	-6.716	0.0001
	Ahmadi	168.37	-56.091	0.0001
	Puavillai	115.52	-3.247	0.0001
	Chen	112.36	-.0817	0.923
	Hattori	103.00	9.275	0.0001
	Martin–Hopkin	111.82	0.462	0.195
	Rao	115.62	-3.341	0.0001
	DeLong	116.64	-4.365	0.0001
	Teerakanchana	115.18	-2.905	0.0001

**Paired t test p value <0.005 is significant*

Discussion

The current study compared the efficacy of various computed low-density lipoprotein cholesterol (LDL-C) estimation methods to directly measured LDL-C, with the goal of identifying dependable and cost-effective alternatives for routine clinical use. The study population was primarily male (67.7%), with an average age of 48.26 ± 11.26 years, indicating a middle-aged cohort at higher risk for cardiovascular illnesses. The lipid profile values observed in this investigation were consistent with those published in similar groups, with mean total cholesterol and triglyceride levels indicating a moderately dyslipidemic group(6).

When comparing mean LDL-C levels, computed techniques differed significantly from directly measured LDL-C ($p < 0.005$). The Friedewald formula, the Anandaraja formula, the Vujovic formula, and others all underestimated or overestimated LDL-C. Notably, the Ahmadi formula significantly overstated LDL-C, with a high mean difference, indicating low reliability in this study sample. In contrast, the Chen formula and Martin-Hopkins approach demonstrated no statistically significant difference when compared to direct LDL-C ($p > 0.005$), indicating superior agreement and potential for clinical application(7)70, 7, 2024-05-17.; Abstract: Background: This study investigated whether directly measured small dense low-density lipoprotein cholesterol (D-sdLDL-C.

Table 7: Correlation of Direct LDL with other calculated methods

Formulas	*Correlation value (r Value)*	*p Value*
Friedewald	0.996	<0.0001
Anandaraja	0.973	<0.0001
De Cordova	0.914	<0.0001
Vujovic	0.989	<0.0001
Ahmadi	0.450	<0.0001
Puavillai	0.994	<0.0001
Chen	0.978	<0.0001
Hattori	0.996	<0.0001
Martin–Hopkin	0.996	<0.0001
Rao	0.996	<0.0001
DeLong	0.992	<0.0001
Teerakanchana	0.984	<0.0001

p value <0.005 is significant

Correlation analysis provided additional support for these findings. Most formulas showed a very strong positive association with direct LDL-C levels, with correlation coefficients (r) ranging from 0.914 to 0.996. Previous investigations have found a near-perfect correlation ($r \approx 0.996$) between the Friedewald, Hattori, and Martin-Hopkins methods(8). However, correlation does not equal

agreement. Despite the excellent correlation, multiple approaches revealed considerable mean differences, emphasizing the need of agreement analysis in addition to correlation. The Ahmadi formula had a weak correlation (r = 0.450), demonstrating its limited applicability(9).

This study's findings are consistent with previous publications indicating diversity in the performance of LDL-C calculation formulae across different populations. Dietary choices, genetic predisposition, and lipid distribution patterns can all have an impact on how accurate these equations are. The Chen and Martin-Hopkins approaches outperformed other methods in this investigation, implying that these formulas may better account for differences in triglyceride-rich lipoproteins(10).

In conclusion, whereas the majority of calculated methods exhibited a strong association with directly measured LDL-C, only the Chen and Martin-Hopkins methods showed good agreement without considerable bias. These approaches may serve as reliable alternatives to direct LDL-C measurement, especially in resource-constrained contexts where direct tests are not possible(11).

Conclusion

In conclusion, the current investigation shows that, while most computed LDL-C estimation methods have a strong positive association with directly measured LDL-C, considerable discrepancies in mean values limit their clinical interchangeability. The Chen formula and the Martin-Hopkins approach showed the best agreement with direct LDL-C values, with no statistically significant difference. These data imply that these two techniques outperform other formulas in terms of LDL-C estimate accuracy and reliability.

As a result, in situations when direct LDL-C measurement is not possible, the Chen and Martin-Hopkins procedures may be used for routine clinical applications. However, population-specific validation remains necessary before wider application.

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