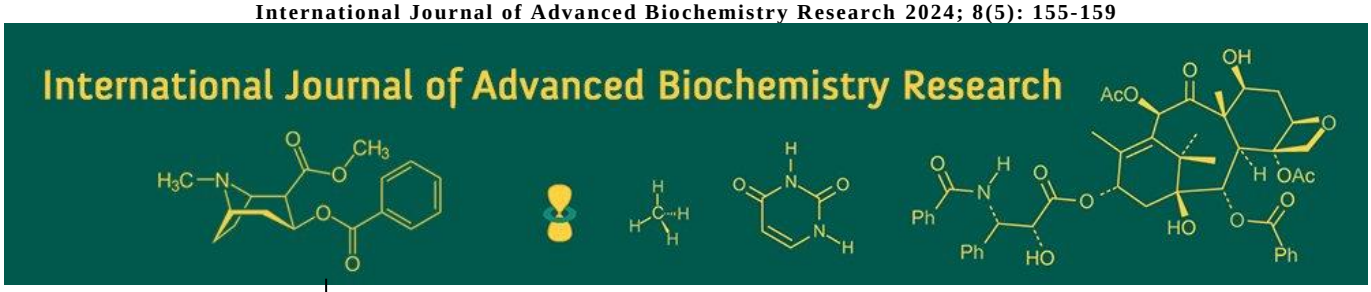

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A comparative analysis of direct LDL measurement and calculated LDL: Assessing accuracy and clinical implications

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Abstract
Background: Accurate assessment of low-density lipoprotein cholesterol (LDL-C) is crucial for managing atherosclerotic cardiovascular disease (ASCVD) risk. Traditional methods like the Friedewald equation have limitations, especially in hypertriglyceridemic or non-fasting conditions. Direct LDL-C measurement offers potential for greater accuracy.
Methodology: This prospective observational study, conducted from 2023 to 2024 at Pacific Medical College and Hospital, involved 110 patients aged 18-65 years. Ethical approval was obtained. Participants, not on lipid-lowering medications, provided blood samples after a 12-hour fast. Trained phlebotomists collected blood samples following standardized procedures. LDL-C levels were calculated using the Friedewald equation and directly measured using specialized assays. Participants were categorized based on triglyceride levels for comparative analysis.
Results: Significant differences in LDL-C levels were observed between calculated and direct measurement methods, especially in patients with high triglyceride levels. Group 3, with triglyceride levels exceeding 400 mg/dL, showed substantial disparities. The Bland-Altman analysis demonstrated acceptable agreement between methods, albeit with slight bias.
Conclusion: Meticulous selection of LDL-C measurement methods is crucial, particularly in patients with metabolic abnormalities. Triglyceride levels significantly influence LDL-C estimation accuracy. Alternative methods like direct LDL-C measurement, especially in patients with elevated triglyceride levels, can enhance cardiovascular risk management.

Keywords: LDL, ASCVD, Friedewald equation

Introduction
In the realm of cardiovascular health assessment, the accurate measurement of low-density lipoprotein cholesterol (LDL-C) stands as a cornerstone in evaluating and managing atherosclerotic cardiovascular disease (ASCVD) risk. Elevated levels of LDL-C have been consistently associated with an increased risk of ASCVD events, including myocardial infarction and stroke [1, 2]. Traditionally, LDL-C levels have been estimated using the Friedewald equation, which calculates LDL-C indirectly based on measurements of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides [3]. However, this method has inherent limitations, particularly in scenarios involving non-fasting samples or patients with hypertriglyceridemia, leading to potential inaccuracies in LDL-C estimation [4, 5].

In response to the limitations of calculated LDL-C, direct measurement methods have gained traction in clinical practice. Direct LDL measurement employs specialized assays that directly quantify LDL-C concentrations without reliance on calculation algorithms. These assays offer the promise of greater accuracy and reliability, especially in situations where calculated LDL-C may be compromised [6]. Direct LDL measurement has been shown to provide more precise results in various clinical scenarios, including individuals with high triglyceride levels or those with metabolic abnormalities [6, 7].

The comparative analysis of direct LDL measurement versus calculated LDL-C has emerged as a significant area of interest in cardiovascular research and clinical practice. Understanding the nuances, advantages, and limitations of each method is crucial for guiding clinical decision-making and optimizing patient care. This comparative analysis seeks to

evaluate the accuracy, reliability, and clinical implications of direct LDL measurement in contrast to calculated LDL-C estimation.

By synthesizing evidence from existing literature, this analysis aims to elucidate the strengths and weaknesses of both approaches. Studies comparing the two methods have demonstrated discrepancies in LDL-C values, particularly in populations with specific metabolic profiles or clinical conditions [8, 9]. Additionally, research has explored the impact of using direct LDL measurement on cardiovascular risk assessment and treatment decisions, highlighting potential implications for patient management strategies [10, 11].

Through a comprehensive review of relevant studies and clinical guidelines, this analysis will provide insights into the optimal approach for LDL-C assessment in different clinical settings. Moreover, it will address the potential implications of adopting direct LDL measurement for ASCVD risk stratification and therapeutic interventions. This comparative analysis aims to inform evidence-based recommendations and enhance the quality of care for individuals at risk of ASCVD by bridging the gap between research findings and clinical practice.

Methodology

This prospective observational study was conducted over one year from 2023 to 2024, involving 110 patients randomly selected from Pacific Medical College and Hospital, a tertiary care hospital. The present study obtained ethical approval from the institutional ethical committee before commencement.

Inclusion Criteria

- Adults aged 18 to 65 years, representative of the general adult population.
- Participants who had not been prescribed lipid-lowering medications, including statins, fibrates, or PCSK9 inhibitors, within the past three months to ensure accurate baseline lipid profiles.
- Individuals who had fasted for a minimum of 12 hours before blood sample collection to standardize lipid measurements.

Exclusion Criteria

- Patients with a documented history of liver disease, including hepatitis, cirrhosis, or hepatic steatosis, as these conditions may affect lipid metabolism and confound study results.
- Pregnant or lactating women, as pregnancy and lactation can lead to physiological changes in lipid levels.

Method of Data Collection

Blood samples were collected from eligible participants following standardized procedures by trained phlebotomists or healthcare professionals. Samples were obtained in the fasting state to minimize variability in lipid measurements.

The calculated LDL cholesterol levels were derived using the Friedewald equation, a commonly employed method for estimating LDL cholesterol levels in clinical practice when direct measurement was not available [3]. The Friedewald equation calculates LDL cholesterol using the following formula:

- LDL cholesterol (mg/dL) = Total cholesterol (mg/dL) − HDL cholesterol (mg/dL) − VLDL
- VLDL = Triglyceride value (mg/dL)/5

The analysis of the Lipid Profile was carried out with thoroughness and precision using the state-of-the-art COBAS INTEGRA-400 plus analyzer. Serum Total Cholesterol levels were determined using the Colorimetric assay method employing CHOD-POD [12], while serum HDL levels were assessed through homogeneous enzymatic colorimetric techniques [13]. Triglyceride levels were accurately quantified utilizing the Colorimetric endpoint GPO-PAP method [14]. Additionally, serum LDL-C and VLDL levels were computed utilizing Friedewald's Formula [3], showcasing a comprehensive and reliable approach to lipid profile assessment.

Measurement of LDL cholesterol was performed using a homogeneous enzymatic colorimetric assay on a Roche Cobas analyzer [15].

The research methodology involved categorizing participants into distinct groups based on their triglyceride (TAG) levels. Each case group (1, 2) comprised 30 individuals and Group 3 consisted of 20 patients, with TAG levels categorized as follows:

- Group 1 included participants with TAG levels ranging from 150 to 300 mg/dL,
- Group 2 encompassed individuals with TAG levels falling between 300 and 400 mg/dL, and
- Group 3 included participants with TAG levels exceeding 400 mg/dL.

Similarly, the control group consisted of 30 participants with TAG levels below 150 mg/dL. Statistical analysis was performed using industry-standard software, namely MedCalc version 14.8.1 and Microsoft Office 2016. All data were meticulously recorded and presented as mean values with accompanying standard deviations (mean ± SD). To assess the significance of various parameters, a Student's t-test was employed to compare findings between the case and control groups. A threshold of $p < 0.05$ was utilized to determine statistical significance, ensuring robustness and reliability in the interpretation of results.

Results

Before delving into the detailed analysis, Table 1 offers an overview of participant distribution across different age groups and study groups. The data indicate a predominant presence of participants aged 51 to 60 years across all groups. Notably, Group 3 exhibits a comparatively lower participant count compared to other groups, primarily due to the scarcity of patients with triglyceride levels exceeding 400 mg/dl.

Table 1: Distribution of Participants by Age Group and Group Type

Age (Years)	Control Group	Group-1	Group-2	Group-3	Total
21-30	2	2	2	1	7
31-40	7	6	6	6	25
41-50	6	6	6	8	26
51-60	8	8	8	5	29
61-70	7	8	8	0	23
Total	30	30	30	20	110

Table 2 provides a comprehensive comparison of metabolic parameters among different study groups, with a particular focus on LDL-C measurement methods. Notably, Group 3 consistently exhibits higher mean values across most parameters, suggesting potential metabolic dysregulation and increased cardiovascular risk compared to the control group and other case groups. Of significant importance is the comparison between LDL-C levels calculated by Friedewald's formula and measured directly, revealing discrepancies, especially in Group 3, where a strong negative correlation is observed.

This highlights the importance of validating LDL-C measurement methods to ensure accurate lipid assessment in clinical practice and research settings. These findings underscore the necessity for meticulous methodological considerations in lipid assessment to better understand and manage cardiovascular risk factors.

Table 2: Comparative Analysis of Parameters among Study Groups

Parameters	Control Group (Mean ± SD)	Group-1 (Mean ± SD)	Group-2 (Mean ± SD)	Group-3 (Mean ± SD)
Age (years)	51.30 ± 13.49	51.90 ± 13.64	47.70 ± 11.43	48.50 ± 11.76
Total cholesterol (mg/dl)	169 ± 28.97	219.18 ± 47.46	230.90 ± 35.41	248.80 ± 61.08
HDL-C (mg/dl)	45.55 ± 10.55	42.40 ± 11.72	36.62 ± 4.30	37.48 ± 6.74
Triacylglycerol (mg/dl)	101.11 ± 23.66	248.74 ± 32.36	346.89 ± 24.63	533.20 ± 84.25
LDL-C (Calculated) (mg/dl)	106.40 ± 25.43	119.79 ± 42.16	111.47 ± 34.60	112.63 ± 55.66
LDL-C (Measured) (mg/dl)	124.30 ± 28.60	120.65 ± 45.99	119.15 ± 36.29	173.60 ± 53.02
VLDL (mg/dl)	19.18 ± 4.51	51.49 ± 6.55	66.09 ± 5.76	101.88 ± 17.07
TC/HDL	4.03 ± 0.98	5.04 ± 1.26	6.49 ± 1.42	6.62 ± 1.74
LDL/HDL	2.33 ± 0.89	3.22 ± 0.99	3.27 ± 1.10	2.95 ± 1.08

Table 3 provides a comparative analysis of LDL-C measurement methods between the control and case groups, offering valuable insights into lipid assessment practices. While LDL-C levels calculated by Friedewald's formula and measured directly generally show agreement in the control group and Group-2, indicated by moderate positive correlations of 0.235 and 0.100 respectively, Group-3 presents a distinct scenario. Here, a strong negative correlation with a Pearson's correlation coefficient of 0.896 suggests significant discrepancies between the two measurement methods.

Table 3: Comparison of LDL-C Measurement Methods: Control vs. Case Groups by Pearson's Correlation Analysis

Group	Age (Years)	LDL-C (Friedewald's) (mg/dl)	LDL-C (Direct) (mg/dl)	Mean difference (mg/dl)	Pearson's correlation Coefficient [r]	95% confidence Interval for r	P Value
Control group	50.8 ± 13.49	106.40 ± 25.43	124.30 ± 28.60	-17.90	0.235	(-0.828, 0.642)	0.600
Group-1	49.40 ± 12.58	119.79 ± 42.16	120.65 ± 45.99	-0.86	0.013	(-0.639, 0.660)	0.966
Group-2	51.95 ± 12.17	111.47 ± 34.60	119.15 ± 36.29	-7.68	0.100	(-0.585, 0.744)	0.741
Group-3	47.55 ± 10.88	112.63 ± 55.66	173.60 ± 53.02	-60.97	0.896	(-0.988, -0.603)	0.00

Notably, the mean difference of -60.97 mg/dl in LDL-C levels between the methods in Group-3 starkly contrasts with other groups. These inconsistencies may stem from factors such as triglyceride levels, impacting the accuracy of Friedewald's formula. These findings underscore the pivotal role of meticulous validation and selection of LDL-C measurement methods in clinical practice, particularly in patients with metabolic abnormalities. Ensuring accurate lipid assessments is paramount for effective management and mitigation of cardiovascular risk factors, ultimately enhancing patient care and outcomes.

The Bland-Altman plot, a critical method for evaluating agreement between measurement techniques, was utilized to assess concordance in LDL-C measurement methods. This graphical tool plots the average of two measurements against their difference. The analysis revealed a mean difference of -23.0 mg/dl and limits of agreement from -72.4 mg/dl to 26.4 mg/dl.

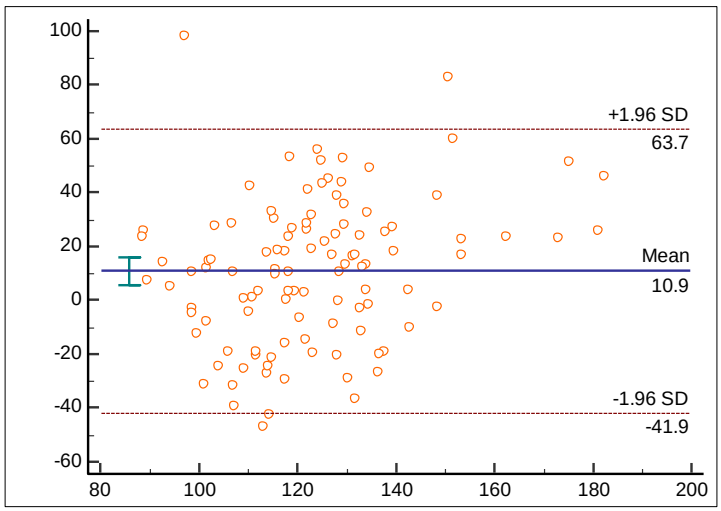

Fig 1: Mean difference between direct LDL and calculated LDL

Despite a slight systematic bias, the Bland-Altman plot demonstrated acceptable agreement between the direct measurement and Friedewald's formula. These findings emphasize the significance of employing meticulous validation and selection of LDL-C measurement methods in clinical practice to ensure accurate lipid assessments and effective management of cardiovascular risk factors [16].

Discussion

This study meticulously assessed the performance of direct LDL-C measurement compared to Friedewald's formula in evaluating patients at risk for coronary heart disease (CHD). The comparison revealed significant discrepancies between the two methods, particularly in scenarios where accuracy is crucial, such as in patients with elevated triglyceride levels.

Analysis across different study groups revealed intriguing insights into the relationship between lipid profiles and cardiovascular risk. Notably, Group 3, characterized by higher triglyceride levels exceeding 400 mg/dl, exhibited marked discrepancies between LDL-C levels calculated by Friedewald's formula and those measured directly. This underscores the limitations of Friedewald's formula, especially in contexts with elevated triglyceride levels, potentially leading to misclassification of cardiovascular risk.

The observed discrepancies between LDL-C measurement methods have profound implications for clinical practice, emphasizing the need for meticulous validation and selection of measurement techniques. Accurate lipid assessment is fundamental for risk stratification and treatment decisions in CHD management.

Several studies have investigated the accuracy and limitations of different methods for measuring LDL cholesterol (LDL-C) in clinical settings. Martin *et al*. (2013) identified significant discrepancies between directly measured LDL-C and the Friedewald formula, particularly in patients with elevated triglyceride levels. Their analysis found a mean difference of -23.0 mg/dl, with limits of agreement ranging from -72.4 mg/dl to 26.4 mg/dl [17].

Dintshi *et al*. (2022) compared measured LDL-C with calculated values using both the Friedewald and Martin-Hopkins formulae in diabetic adults. Their study revealed a mean positive bias of 14% for the Friedewald formula and 10.24% for the Martin-Hopkins formula, highlighting substantial differences between measured and calculated LDL-C values [18].

Similarly, Kannan *et al*. (2014) found a strong positive correlation (r = 0.89) between LDL-C calculated using the Friedewald formula and directly measured LDL-C. However, they observed median differences of 14.8 mg/dl, 12.2 mg/dl, 6.2 mg/dl, and 0.4 mg/dl across different triglyceride strata, indicating variability in accuracy based on triglyceride levels [19].

Bharathan *et al*. (2022) also reported a strong correlation (r = 0.94) between Friedewald-calculated and directly measured LDL-C but noted underestimation by the Friedewald formula, particularly in patients with higher triglyceride levels, age, female sex, and BMI. They found that many patients with coronary heart disease (CHD) did not meet LDL-C goals when using the Friedewald formula due to these discrepancies. The mean differences varied across different triglyceride levels, with increasing deviations observed in higher triglyceride strata [20].

The findings underscore the imperative for clinicians to consider alternative measurement methods, such as direct LDL-C measurement, particularly in patients with metabolic abnormalities and elevated triglyceride levels.

Triglyceride levels emerged as a critical determinant of the accuracy of LDL-C estimation by Friedewald's formula. The significant discrepancies observed, particularly at triglyceride levels exceeding 400 mg/dl, highlight the formula's limitations in accurately estimating LDL-C levels in such contexts. This underscores the importance of considering triglyceride levels in LDL-C measurement and the need for alternative methods in patients with elevated triglycerides to ensure accurate lipid assessment and effective cardiovascular risk management.

The Bland-Altman analysis provided further insight into the agreement between LDL-C measurement methods, revealing acceptable concordance despite slight systematic bias. The analysis underscored the variability in discrepancies, particularly at higher LDL-C levels, emphasizing the importance of meticulous methodological considerations in LDL-C measurement to ensure accurate lipid assessments and effective cardiovascular risk management.

Limitations and Future Directions

While the study contributes valuable insights into LDL-C measurement methods, it is not without limitations. The small sample size and lack of consideration for participant comorbidities may have influenced LDL-C levels, warranting caution in generalizing the findings. Future research should focus on larger studies with diverse participant populations to further elucidate the role of LDL-C measurement methods in cardiovascular risk assessment and management.

Conclusion

In conclusion, our study highlights the importance of meticulous methodological considerations in LDL-C measurement to ensure accurate lipid assessments in patients at risk for CHD. The findings underscore the limitations of Friedewald's formula, particularly in patients with elevated triglyceride levels, and emphasize the need for alternative methods, such as direct LDL-C measurement, to enhance cardiovascular risk stratification and treatment strategies, ultimately improving patient care and outcomes in CHD management.

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