



Evaluation of effect of serum triglyceride and total cholesterol on calculated LDL levels.

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Abstract

Background

Low-density lipoprotein cholesterol (LDL-C) is a key marker for cardiovascular risk assessment. The Friedewald formula is widely used for indirect LDL estimation; however, its accuracy is influenced by serum triglyceride (TG) and total cholesterol (TC) levels. This cross-sectional analytical study evaluated the effect of serum TG and TC on calculated LDL-C compared with direct measurement.

Materials and Methods:

A total of 495 fasting serum samples (normal, n=284; dyslipidemic, n=211) were analyzed. Lipid parameters, including total cholesterol, HDL-C, TG and direct LDL-C, were measured using an automated enzymatic method. Indirect LDL-C was calculated using the Friedewald formula. Samples were categorized into five groups based on TG and TC levels. Agreement between direct and calculated LDL-C was assessed using Pearson's correlation and percentage bias.

Results:

A strong correlation between direct and calculated LDL-C was observed in groups with TG <400 mg/dL (r ranging from 0.94 to 0.95), while reduced correlation was noted at TG >400 mg/dL (r=0.871). The percentage of LDL-C underestimation increased with rising TG levels (6% in Group A to 100% in Group D). In contrast, samples with elevated TC (>200 mg/dL) demonstrated 54% overestimation. Variability and bias increased significantly at TG levels >250 mg/dL.

Conclusion:

The Friedewald formula shows reduced reliability with increasing triglyceride levels, particularly beyond 250 mg/dL and is unreliable at levels >400 mg/dL. Elevated total cholesterol also contributes to estimation errors.

Recommendation:

Direct LDL-C measurement should be preferred in patients with triglyceride levels >250 mg/dL or elevated total cholesterol to ensure accurate cardiovascular risk assessment.

Keywords : serum triglyceride, total cholesterol, low density lipoproteins, biochemistry, medical, research

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Introduction:

Dyslipoproteinemia is diagnosed in most patients using plasma lipid and lipoprotein cholesterol concentration. Evaluation of the hyperlipidemic patient can include measurements of plasma cholesterol, VLDL, LDL, HDL and TGL levels.

Various technologies have been used to measure the plasma lipids or lipoproteins and lipoprotein subfraction

including enzymatic, immunochemical and chemical precipitation reagents and physical methods such as ultracentrifugation, electrophoresis and column chromatography.

For more than 20 years, the Centre for Disease Control and Prevention (CDC) Standardization programs for lipids and lipoproteins had maintained reference methods for cholesterol programs for cholesterol, triglycerides and

HDL cholesterol and had operated standardization programs [1]. These reference methods are the "gold standards" or accuracy targets for these common analytes. However, these reference methods are complex, time consuming, at least partially manual and required a high level of expertise for reliable operation. Consequently, simpler and more practical methods have evolved for routine clinical use.

Since the LDL – Cholesterol directly correlates with CHD, the accuracy of LDL-c levels either by direct measurement or by calculation is of utmost importance. The most common approach for determining LDL-c in the clinical laboratory is the Friedewald equation [2], which estimates LDL-c from measurements of total cholesterol, TGL and HDL-c.

The accuracy of the Friedewald equation has been extensively reviewed and the equation sometimes leads to contradictory results: despite that, it is recommended for routine use. This equation was validated by Warnick et al [3] who confirmed that the equation is valid when the triglyceride level is less than 400 mg/dL.

Three essential limitations are known for the Friedewald equation; when chylomicrons are present, in patients with type III hyperlipidemia and when TGL exceeds 400 mg/dL [4]. At high TG concentrations, the factor that TG/5 as an estimate of VLDL cholesterol concentration is not appropriate because such samples can also contain chylomicrons, chylomicron remnants, or VLDL remnants, all of which have higher TG/cholesterol ratios. Under these circumstances, the use of the factor (TG/5) would overestimate VLDL cholesterol and therefore underestimate LDL-c.

When triglycerides were <200 mg/dL, >90% of estimated LDL-c values were acceptable, (within $\pm 10\%$ of measured values). But the error becomes unacceptably large (i.e.) >10% at triglyceride concentrations greater than 400 mg/dL [3]. But when the Friedewald equation is used in patients with type III hyperlipoproteinemia, the opposite error can occur. Fortunately, both of these conditions are uncommon. Plasma from fasting subjects does not normally contain chylomicrons and even if present, chylomicrons can be observed visually as a floating "cream" layer in samples, that have been allowed to stand undisturbed at 4°C overnight. Also, the prevalence of type III hyperlipoproteinemia is only about 1 to 2% per 1000 persons in the general population. Thus the Friedewald equation will be reasonably reliable in the majority of patients.

Although calculating LDL-c from the Friedewald equation has shown a good correlation with the direct measurement of LDL-c, one of the studies showed that LDL-c level from the direct measurement was higher than the calculated one, if the serum TGL is higher than 300mg/dL [5]. It is also shown that the levels of LDL-c are less accurate if serum TGL levels are higher than 200mg/dL [6]. Apart from this low serum TGL may also positively affect the LDL-c calculation and lead to error. So, LDL-c levels should be measured directly [7]. From these reports, it is well known that the concentration of serum TGL is not reliable for deciding when the Friedewald formula can be used confidently and when not [8].

It is noticeable that when serum TGL is low and total cholesterol levels are undesirably high, Friedewald's equation may overestimate low density lipoprotein cholesterol [9]. Although convenient, Friedewald's calculation suffers from several well established limitations, which led an Expert Panel convened by the NCEP to recommend the development of accurate direct LDL cholesterol methods [10]. Earlier direct methods had limitations for general use. Recently, a new generation of homogenous methods capable of full automation has been introduced that uses specific reagents of various types to selectively expose and directly measure the cholesterol associated with LDL.

Thus, it is evident from aforementioned studies that the accuracy of LDL estimation by the Friedewald formula is affected by serum triglyceride level. This persuaded us to examine this formula and compare it with direct LDL estimation in a tertiary care hospital with the hypothesis that increased serum triglyceride & total cholesterol concentration can have its influence on the Friedewald formula.

Materials and methods

Study design

This study was designed as a cross-sectional analytical study based on retrospective laboratory data. The analysis compared calculated LDL-C using the Friedewald formula with directly measured LDL-C across varying levels of serum triglyceride and total cholesterol.

Study setting

The study was conducted at the Central Laboratory Services of a tertiary care teaching hospital in Tamil

Nadu, India. The laboratory caters to both inpatient and outpatient populations, including individuals undergoing routine health screening and patients with suspected or diagnosed metabolic and cardiovascular disorders. The facility is equipped with automated clinical chemistry analyzers and follows standardized internal and external quality control protocols, ensuring reliability of biochemical measurements.

Sampling procedure

A consecutive sampling method was employed. All eligible fasting lipid profile samples processed in the laboratory during the study period were included until the required sample size (n = 495) was achieved.

Sample size determination

The sample size of 495 was determined based on feasibility and availability of complete lipid profile records during the study period. Additionally, the sample size was considered adequate to detect a moderate correlation ($r \geq 0.3$) between calculated and direct LDL-C values with a statistical power of 80% and a significance level of 5%.

Participant selection (Eligibility criteria)

Fasting serum samples from individuals aged ≥ 18 years with complete lipid profile parameters (total cholesterol, triglycerides, HDL-C and LDL-C) were included.

Inclusion criteria:

- Fasting samples with complete lipid profile data
- Samples processed using standardized automated methods

Exclusion criteria:

- Non-fasting samples
- Samples with missing or incomplete lipid parameters
- Hemolysed or lipemic samples affecting analytical accuracy

Definition and measurement of variables

Serum lipid parameters including total cholesterol, triglycerides (TG), HDL-C and direct LDL-C were measured using standardized enzymatic methods on a fully automated clinical chemistry analyzer (Dimension® system). Internal quality control was maintained using two-level control materials.

Calculated LDL-C was derived using the Friedewald formula:

$$\text{LDL-C} = \text{Total Cholesterol} - (\text{HDL-C} + \text{TG}/5)$$

Samples were categorized into five groups based on triglyceride and total cholesterol levels:

- Group A: TG <150 mg/dL
- Group B: TG 151–250 mg/dL
- Group C: TG 251–400 mg/dL
- Group D: TG >400 mg/dL
- Group E: TG <150 mg/dL with total cholesterol >200 mg/dL

Agreement between calculated and direct LDL-C was classified as:

- Satisfactory: within ± 10 mg/dL
- Underestimation: < -10 mg/dL
- Overestimation: > +10 mg/dL

Bias control

Selection bias was minimized by including consecutive eligible samples over the study period. Measurement bias was reduced by using standardized automated assays, regular calibration of instruments and adherence to internal quality control procedures. All analyses were performed using the same analyzer to maintain consistency.

Statistical analysis

Descriptive statistics were expressed as mean \pm standard deviation (SD) and coefficient of variation (CV%). The correlation between calculated and direct LDL-C values was assessed using Pearson's correlation coefficient (r). Agreement and bias were evaluated based on percentage underestimation and overestimation across groups.

Statistical analysis was performed using SPSS version 20.0 and Microsoft Excel, with a significance level set at $p < 0.05$.

Ethical consideration

The study protocol was reviewed and approved by the Institutional Ethics Committee of PSP Medical College Hospital and Research Institute. As this study involved retrospective analysis of anonymized laboratory data, informed consent was waived.

Results

Participant flow

A total of 538 lipid profile records were initially screened from the laboratory database during the study period. Among these, 43 samples were excluded due to incomplete lipid profile data ($n=21$), non-fasting status ($n=12$) and sample quality issues such as hemolysis or lipemia ($n=10$).

Finally, 495 samples were included in the analysis and categorized into five groups based on triglyceride and total cholesterol levels: Group A ($n=284$), Group B ($n=115$), Group C ($n=40$), Group D ($n=6$) and Group E ($n=50$).

Descriptive characteristics of study samples

The demographic distribution and lipid profile characteristics of the study population are presented in Table 1 and Table 3.

Table: 1 demographic detail of the study subjects

GROUP	LIPID CONCENTRATION (mg/dl)	AGE RANGE (in yrs)	NUMBER OF SAMPLES
GROUP A	TG < 150	13 – 84	$n = 284$ (M = 199; F = 85)
GROUP B	TG 151 - 250	26 – 82	$n = 115$ (M = 72; F = 43)
GROUP C	TG 251 - 400	38 – 72	$n = 40$ (M = 27; F = 13)
GROUP D	TG > 400	53 – 70	$n = 6$ (M = 4; F = 2)

GROU P E	TOTAL CHOL > 200	22 – 88	$n = 50$ (M = 27; F = 23)
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Table: 3 lipid profile of various groups

GROUP	TRIGLYCERIDE (mg/dl)		TOTAL CHOLESTEROL (mg/dl)		HDL (mg/dl)	
	MEAN ± SD	CV %	MEAN ± SD	CV %	MEAN ± SD	CV %
GROUP A	148.25 ± 30.95	20.23	197.7 ± 22.92	23.45	35.4 ± 7.19	20.31
GROUP B	178.96 ± 42.76	23.89	190.70 ± 29.38	15.40	31.4 ± 8.53	27.15
GROUP C	189.15 ± 47.03	24.86	282.2 ± 45.53	16.13	31.7 ± 12.3	38.86
GROUP D	237.33 ± 42.94	18.09	632 ± 41.67	6.59	27.5 ± 6.78	24.65
GROUP E	219.64 ± 13.03	5.93	112.54 ± 25.37	22.54	43.1 ± 10.1	23.45

The majority of samples belonged to Group A (TG <150 mg/dL), followed by Group B. Mean lipid values varied across groups, with higher variability (SD and CV%) observed in patient samples compared to quality control samples (Table 4).

Table: 4 Serum lipid profile in quality control sample

S.NO	SERUM LIPID	NORMAL		ABNORMAL	
		MEAN ± SD	CV %	MEAN ± SD	CV %
1	TOTAL CHOLESTEROL (mg/dl)	231.27 ± 7.66	3.31	89.93 ± 2.10	2.33
2	TRIGLYCERIDE (mg/dl)	175.6 ± 6.01	3.41	83.93 ± 3.94	4.70
3	HDL (mg/dl)	66.47 ± 4.10	6.17	31.93 ± 1.06	3.31

4	LDL (mg/dl)	114.67 ± 5.53	4.82	48.73 ± 1.56	3.20
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Standard deviation and CV % in patient's sample in all groups is higher than that of Quality control samples

Comparison of direct and calculated LDL-C

The comparison between directly measured LDL-C and calculated LDL-C across different groups is summarized in Table 2.

Table: 2 effects of serum triglyceride and cholesterol concentration on calculated and direct LDL

LIPID	No of samples (n)	DIRECT LDL (mg/dl)		CALCULATE D LDL (mg/dl)	
		MEAN ± SD	CV %	MEAN ± SD	CV %
GROU P A TG (< 150 mg/dl)	n= 284	90.50 ± 21.06	23.27	93.24 ± 22.16	23.76
GROU P B TG (151 - 250 mg/dl)	n= 115	112.80 ± 35.54	31.50	109.81 ± 37.35	34.01
GROU P C TG (251 - 400 mg/dl)	n= 40	110.68 ± 37.25	33.65	100.96 ± 45.22	44.79
GROU P D TG (> 400 mg/dl)	n= 6	115.17 ± 27.49	23.86	82.60 ± 34.84	42.17
GROU P E TC (> 200 mg/dl)	n= 50	144.90 ± 17.17	11.84	153.66 ± 13.80	8.98

A close agreement between the two methods was observed in Group A and Group B, whereas increasing divergence was noted in Groups C and D. In Group E

(elevated total cholesterol), calculated LDL-C values were higher than direct LDL-C.

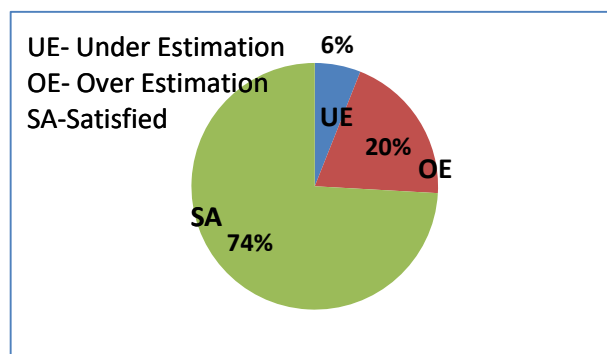
Underestimation and overestimation of LDL-C

The percentage of underestimation of calculated LDL-C increased progressively with rising triglyceride levels.

- Group A: 6% underestimation
- Group B: 24% underestimation
- Group C: 45% underestimation
- Group D: 100% underestimation

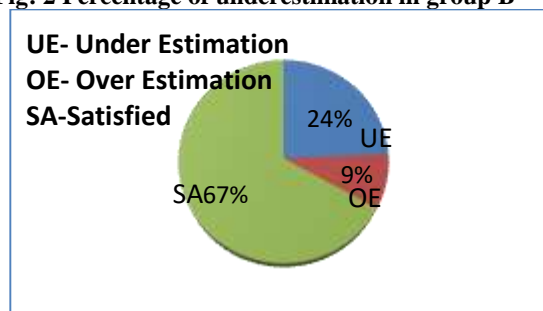
These findings are illustrated in Figures 1–4.

Fig: 1 Percentage of underestimation in group a



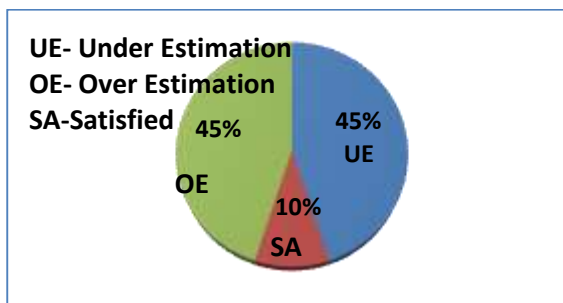
Among 284 samples, only 6 % was underestimated

Fig: 2 Percentage of underestimation in group B



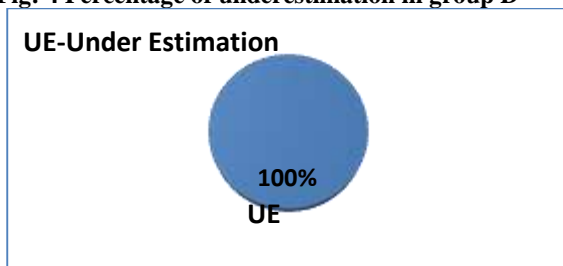
Among 115 samples percentage of underestimation was 24 %

Fig: 3 Percentage of underestimation in group C



Among 40 samples percentage of underestimation was 45 %

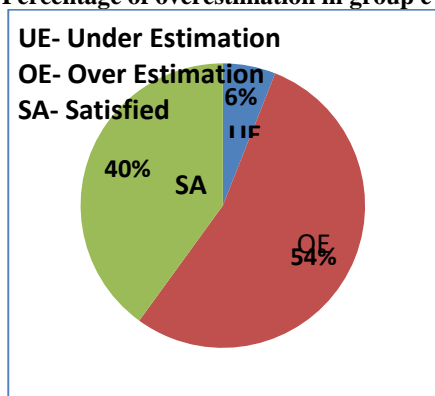
Fig: 4 Percentage of underestimation in group D



100 % of the samples underestimated by Friedewald formula

In contrast, Group E demonstrated 54% overestimation, as shown in Figure 5.

Fig: 5 Percentage of overestimation in group e



Group E shows an overestimation of about 54 %

Correlation analysis

Correlation between calculated LDL-C and direct LDL-C across different triglyceride levels is depicted in Figures 6-10.

A strong positive correlation was observed in:

- Group A (r = 0.941)
- Group B (r = 0.9544)
- Group C (r = 0.9447)

Fig: 7. Correlation between direct and calculated LDL in group A (TG < 150 mg/dl) r = 0.941

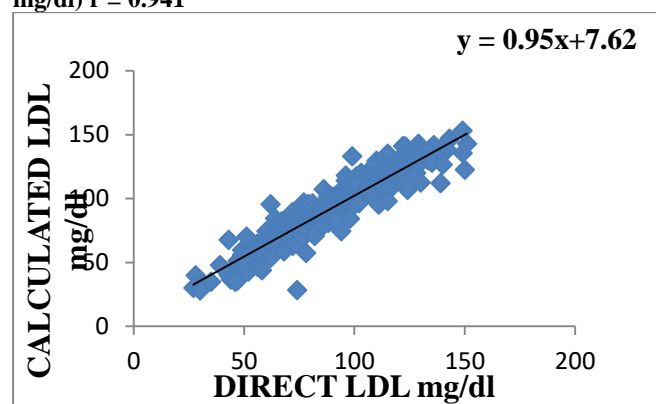


Fig: 8 Correlation between direct and calculated LDL in group B (TG 151 – 250 mg/dl) r = 0.9544

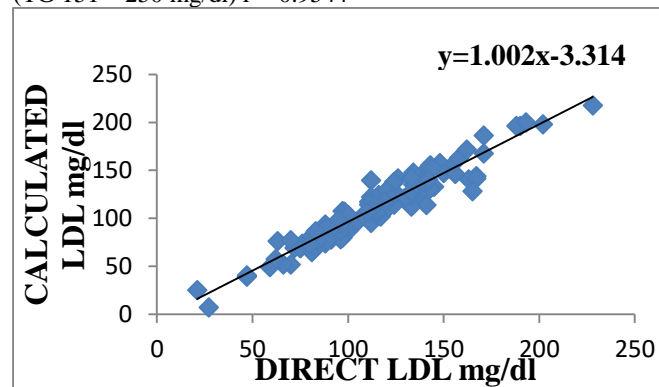
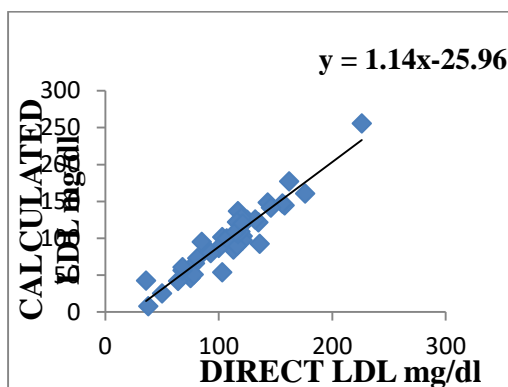


Fig: 8 Correlation between direct and calculated LDL in group C (TG 251 - 400 mg/dl) r = 0.9447



The acceptability of LDL-C estimation using the Friedewald formula decreased from 74% in Group A to 40% in Group E, indicating reduced reliability with increasing triglyceride and total cholesterol concentrations.

Correlation between lipid parameters

Correlation between lipid parameters is presented in Table 5. A significant positive correlation between total cholesterol and LDL-C was observed in all groups except Group D. The correlation between HDL-C and LDL-C was inconsistent across groups.

Reduced correlation was noted in:

- Group D ($r = 0.8719$)
- Group E ($r = 0.7141$)

Fig: 9. Correlation between direct and calculated LDL in group D (TG > 400 mg/dl) $r = 0.8719$

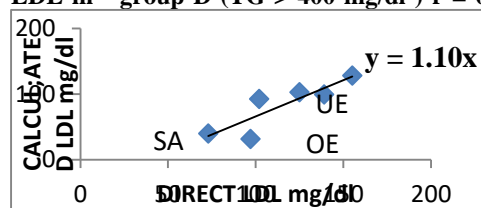
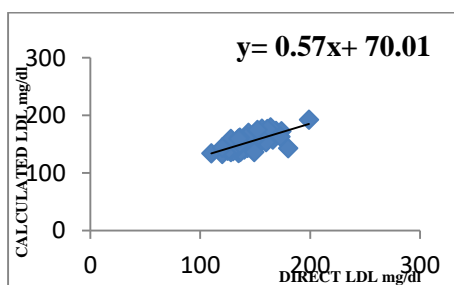


Fig:10: Correlation between direct and calculated LDL in group E (total chol > 200 mg/dl) $r = 0.7141$



Constant error or bias found very high in Group E

Bias and variability analysis

An increasing trend of constant error (bias) was observed with rising triglyceride levels, particularly in Groups C and D. Group E showed a higher degree of bias associated with elevated total cholesterol levels.

Table: 5 Correlations between parameters

Groups	"r" value between total cholesterol & LDL	"r" value between HDL & LDL
GROUP A	0.915	0.083
GROUP B	0.95	0.482
GROUP C	0.94	0.277
GROUP D	0.32	0.094
GROUP E	0.42	-0.484

Significant positive correlation was obtained in all groups except group D

Discussion

The Friedewald formula had been evaluated and published in various populations [10, 11]. Many studies had demonstrated its limitations and alternatives had been proposed involving different factors or more sophisticated formulas [12]. In some cases, specific models had been recommended for population subsets by categories of age, gender and concentrations of triglyceride and cholesterol [13, 14]. Direct measurement of serum LDL cholesterol was the most accurate. However, the use of the Friedewald equation was becoming even more widespread, because of its simplicity and as it is cost-effective. This study aimed to evaluate the accuracy of Friedewald formula.

According to the American College of Pathologists (CAP) survey, < 6% of participating laboratories in the US, used the homogenous methods and < 2% used the LipiDirect magnetic precipitation procedure. In the remaining laboratories, 92.7% used the Friedewald calculation [15]. Since, the homogenous assay was a more convenient β -quantification method; To investigate the Friedewald formula by analyzing 495 samples. Most recommendations of NCEP are related to lower LDL-c levels. The method of LDL-c measurement is also a

contributor to the concentration of LDL-c in an individual. The percentage of underestimation or overestimation of LDL-c helps in identifying the person at risk for CVD, whether treatment is required and or the response to treatment.

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The present study examined the percentage of underestimation of calculated over direct LDL-c at different concentrations of serum TGL. The reliability of Friedewald formula was less at serum triglyceride concentration >250mg/dl and above 400mg/dl, it 100% devaluated the LDL – Cholesterol concentration. Besides increased total cholesterol concentration had its influence on the Friedewald formula. The presented data supported the study of Wang et al [9] and the comments of Seyed Ali et al [7], that the serum total cholesterol level had an impact on LDL-c estimation. The acceptability percentage of LDL-c estimation by Friedewald formula decreased proportionately from group A to group E (74 % in group A to 40 % in group E). This was in concordance with the results of Benlian et al [16] and Nauck et al [17] where the accuracy declined with TGL concentration over 200 mg/dl, when using the Friedewald equation for calculated LDL-c.

Standard deviation and CV % was found to be higher in patient's sample in all groups than that of quality control samples. One plausible explanation for this could be the existence of wide intraindividual variation.

A notion between calculated LDL-c and direct LDL-c and an increasing percentage of underestimation of calculated LDL-c in the study group supported the fact that higher triglyceride concentration influenced the serum LDL-c measurement [22].

Cardiovascular diseases had become the leading cause of morbidity and mortality worldwide. Epidemiological studies showed a positive relationship between total cholesterol concentrations and mortality for coronary heart disease. Total cholesterol did not accurately predict the risk of CHD in many patients, because it is the sum of all cholesterol not only carried by atherogenic lipoproteins but also anti atherogenic lipoprotein [18]. However, it was of interest to know the correlation existing between lipids in our study. With the exception of group D, a significant positive correlation existed amidst all other groups. The results obtained for correlation studies could be explained based on the number of factors influencing the levels of lipids such as concentration of lipoproteins, the activity of enzymes involved in lipoprotein metabolism and the effects of treatment on the individuals [23].

Generalizability

The findings of this study are applicable to routine clinical laboratory settings where the Friedewald formula is commonly used for LDL-C estimation. As the study population included both normolipidemic and dyslipidemic individuals from a tertiary care center, the results reflect a spectrum of lipid profiles encountered in daily practice. The observed decline in accuracy of calculated LDL-C at higher triglyceride levels is consistent with known biochemical limitations of the Friedewald equation, suggesting that these findings can be extrapolated to similar hospital-based populations and diagnostic laboratories using automated enzymatic assays.

However, extrapolation to community-based populations or non-fasting samples should be done cautiously, as lipid distributions and pre-analytical conditions may differ. In addition, laboratories using different analytical platforms or assay methodologies may observe minor variations in absolute values, although the overall trend of reduced reliability at elevated triglyceride levels is expected to remain consistent.

Limitations

This study has certain limitations that should be considered while interpreting the results. First, the retrospective nature of data collection limited control over pre-analytical variables such as dietary status beyond documented fasting and potential biological variability. Second, the sample size in the highest triglyceride group (TG >400 mg/dL) was relatively small, which may affect the robustness of correlation estimates in this subgroup. Third, the study was conducted in a single tertiary care center, which may limit external validity across different geographic or primary care settings.

Furthermore, potential confounding factors such as comorbid conditions, medication use (e.g., lipid-lowering therapy) and metabolic status were not analyzed, as the study relied solely on laboratory data. Although standardized automated assays and quality control procedures were followed, analytical variability inherent to direct LDL-C measurement methods cannot be completely excluded.

Conclusion:

The original Friedewald calculation has generally prevailed for use in routine laboratories even though there is a well-established limitation, the many suggested modifications and the introduction of various direct

methods for LDL-c. In this study, the effect of serum TGL on calculated LDL-c in comparison with direct LDL-c reveals

- A reliable estimation of LDL -c was obtained when serum TGL < 250 mg/dl
- Not acceptable at TGL >250 mg/dl
- There is an influence of total cholesterol on LDL-c estimation

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List of abbreviations

- LDL-C: Low Density Lipoprotein Cholesterol
- HDL-C: High Density Lipoprotein Cholesterol
- TG: Triglycerides
- TC: Total Cholesterol
- VLDL: Very Low Density Lipoprotein
- CHD: Coronary Heart Disease
- CV: Coefficient of Variation
- SD: Standard Deviation
- NCEP: National Cholesterol Education Program
- CAP: College of American Pathologists

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Conflict of interest

The authors declare no conflict of interest.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. Due to institutional policies and patient confidentiality, the data are not publicly available.

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