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Evaluation of Friedewalds Formula- A Comparative Study on Direct and Indirect Estimate of LDL Cholesterol

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Abstract: The Friedewald formula is widely used to estimate low-density lipoprotein (LDL) cholesterol, a key predictor of coronary heart disease. However, its accuracy can be compromised by elevated serum triglyceride levels, which may lead to incorrect LDL estimations. This study aimed to evaluate the impact of serum triglyceride and total cholesterol levels on the accuracy of calculated LDL cholesterol compared to direct LDL measurement. A total of 495 fasting serum samples (normal, n = 284; dyslipidemic, n = 211) were analyzed. Direct measurements of total cholesterol, HDL cholesterol, triglycerides, and LDL cholesterol were performed using an automated enzymatic assay. LDL cholesterol was also calculated using the Friedewald formula. Samples were categorized into five groups based on triglyceride and total cholesterol levels, and the correlation between direct and calculated LDL was assessed. Strong correlations were observed between direct and calculated LDL in all groups except for those with triglycerides >400 mg/dl (r = 0.871). The percentage of LDL underestimation increased with higher triglyceride levels: 6%, 23%, 45%, and 100% for groups with triglycerides <150 mg/dl, 151–250 mg/dl, 251–400 mg/dl, and >400 mg/dl, respectively. In patients with total cholesterol >200 mg/dl, the Friedewald formula resulted in 6% LDL underestimation and 54% overestimation. These findings suggest that the Friedewald formula becomes less reliable when triglyceride levels exceed 250 mg/dl and is completely inaccurate at levels >400 mg/dl. Elevated total cholesterol also affects the formula's accuracy, highlighting the importance of direct LDL measurement in such cases.

Keywords: LDL cholesterol, Friedewald formula, triglycerides, total cholesterol, coronary heart disease.

Abbreviations:

LDL	: Low-Density Lipoprotein
CVD	: Cardiovascular Disease
HDL	: High-Density Lipoprotein Cholesterol
TGL	: Triglycerides
VLDL	: Very-Low-Density Lipoprotein cholesterol

NCEP : National Cholesterol Education Program
ATP : Adult Treatment Panel

1. Introduction:

Cardiovascular disease (CVD) continues to be a leading cause of morbidity and mortality globally, accounting for nearly 18 million deaths annually, which represents about 32% of all global deaths (1,3). The burden of CVD has been increasing at an alarming rate, fueled by lifestyle changes, aging populations, and the growing prevalence of risk factors such as obesity, diabetes, and hypertension. Among these risk factors, elevated low-density lipoprotein cholesterol (LDL-c) is recognized as one of the most critical modifiable contributors to the development of atherosclerosis and coronary heart disease (CHD). Consequently, the management of LDL-c levels has become a cornerstone in both clinical guidelines and public health initiatives aimed at reducing CVD risk (2,4). Numerous studies have demonstrated that lowering LDL-c significantly reduces the incidence of major cardiovascular events, including heart attacks and strokes, thereby establishing LDL-c as a primary target in therapeutic interventions and preventive measures (5,6).

Traditionally, the Friedewald formula, introduced in 1972, has been the most widely used method for estimating LDL-c in clinical settings due to its simplicity, cost-effectiveness, and widespread availability (3,6). The formula estimates LDL-c by subtracting the concentrations of high-density lipoprotein cholesterol (HDL-c) and a fraction of triglycerides (TGL) from total cholesterol. Despite its longstanding use, the accuracy of this formula has been called into question, especially in specific patient populations. One of the key limitations of the Friedewald formula is its reliance on an assumption that TGL/5 can be used as a proxy for very-low-density lipoprotein cholesterol (VLDL-c), which becomes problematic when triglyceride levels are high (8,9). Elevated triglyceride levels, particularly in patients with metabolic syndrome, diabetes, or hypertriglyceridemia, can result in significant miscalculations of LDL-c, potentially leading to suboptimal clinical decisions (8,9).

The inaccuracy of the Friedewald formula becomes particularly pronounced when triglyceride levels exceed 400 mg/dL, a scenario commonly seen in patients with metabolic disorders (4,6). In such cases, the formula tends to underestimate LDL-c, which may misclassify a patient's cardiovascular risk. This underestimation is particularly concerning because it could lead to the delayed initiation of statin therapy or other lipid-lowering interventions, increasing the risk of cardiovascular events (7,11). Studies have shown that up to 20-30% of patients with elevated triglyceride levels may have inaccurately low LDL-c

estimates using the Friedewald formula, thereby jeopardizing their treatment plans (9,10). As a result, there has been a growing call within the medical community to adopt more reliable methods of LDL-c measurement, particularly in high-risk populations (12,14).

In response to these concerns, direct LDL-c measurement methods have been developed as a more accurate alternative, especially for patients with high triglyceride levels (5,13). Direct LDL-c measurement, which does not rely on indirect calculations, offers a more precise assessment of a patient's lipid profile, particularly in those with abnormal lipid metabolism. Additionally, several new LDL-c estimation formulas have been proposed to address the shortcomings of the Friedewald equation. These alternative methods are designed to be more accurate in the presence of elevated triglycerides, offering improved cardiovascular risk stratification (14,15).

The challenges associated with LDL-c estimation are especially pertinent in regions like South Asia, where the prevalence of dyslipidemia, metabolic syndrome, diabetes, and cardiovascular risk factors is disproportionately high (15,16). South Asians are known to have a distinct lipid profile, characterized by elevated triglycerides, low HDL-c, and small dense LDL particles, which complicates the use of traditional LDL-c estimation methods like the Friedewald formula (11,17). As a result, the widespread reliance on the Friedewald formula in South Asian populations may contribute to an underestimation of LDL-c levels, leading to an increased risk of undiagnosed and untreated cardiovascular disease (16,18). This highlights the urgent need for more accurate and region-specific LDL-c estimation methods to ensure better cardiovascular risk management in these populations.

Emerging research supports the need for individualized approaches to lipid management, particularly in populations with unique lipid profiles or those at higher cardiovascular risk (19,20). Several alternative formulas, such as the Martin-Hopkins method and the Sampson equation, have demonstrated improved accuracy over the Friedewald formula, particularly in patients with high triglyceride levels. These methods, alongside direct LDL-c measurement techniques, represent a significant advancement in the field of lipidology and are gaining traction as preferred options in both clinical and research settings (18,19).

Given the significant global burden of cardiovascular disease, accurate lipid management is essential to reducing morbidity and mortality. In South Asia, where metabolic risk factors are highly prevalent, improving LDL-c estimation methods could play a crucial role in mitigating the region's cardiovascular health crisis. Our study aims to assess the limitations of the Friedewald formula in

accurately estimating LDL-c levels, particularly in high-risk South Asian populations with elevated triglycerides (20,12). By evaluating the impact of triglyceride levels on LDL-c estimation accuracy, we hope to contribute to the ongoing efforts to refine lipid management strategies and improve cardiovascular outcomes globally.

In conclusion, the evolving understanding of lipid metabolism and cardiovascular risk has underscored the importance of accurate LDL-c measurement in clinical practice. As newer and more reliable LDL-c estimation methods continue to emerge, clinicians must remain vigilant in their selection of appropriate tools for lipid management, particularly in high-risk and underserved populations. Our study will contribute to the growing body of evidence advocating for a more individualized and accurate approach to LDL-c estimation, ultimately aiming to enhance cardiovascular prevention strategies and patient outcomes.

2. Materials and Methods

This study was designed to evaluate the impact of serum triglyceride and total cholesterol levels on the accuracy of calculated low-density lipoprotein (LDL) cholesterol using the Friedewald formula, compared to direct LDL measurement. A total of 495 fasting serum samples were collected and analyzed from both normal and dyslipidemic patients. The study was conducted in a tertiary care hospital, and the patients included in the study were categorized based on their lipid profiles.

2.1 Study Population

The study cohort comprised 495 fasting samples, including 284 samples from individuals with normal lipid profiles and 211 samples from dyslipidemic patients. These patients were selected randomly based on their clinical presentation for lipid profiling, with dyslipidemia being identified according to the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) guidelines. Informed consent was obtained from all participants, and the study followed ethical protocols approved by the institutional ethics committee of Sri Lalithambigai MedicalCollege Hospital, Chennai .

2.2 Sample Collection

Fasting blood samples were collected from all participants after an overnight fast of at least 12 hours. Venous blood was drawn and serum was separated by centrifugation. The serum samples were stored at 2-8°C and analyzed within 24 hours to ensure the stability of lipid measurements.

2.3 Lipid Profile Analysis

The serum total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, triglycerides (TGL), and direct LDL cholesterol were measured using a standardized, automated, enzymatic assay. All measurements were performed using the Dimension® clinical chemistry system, which employs direct, homogeneous enzymatic assays. This system ensures high precision and accuracy in lipid estimation. The HDL cholesterol was measured using a direct assay method, while triglycerides were quantified using an enzymatic hydrolysis method. The direct measurement of LDL cholesterol was conducted through a homogeneous enzymatic colorimetric assay, which does not require preparatory ultracentrifugation, providing an accurate LDL value even in samples with elevated triglyceride levels.

2.4 Calculation of Indirect LDL

In addition to direct LDL measurement, LDL cholesterol was also calculated using the widely used Friedewald formula:

$$\text{LDL (calculated)} = \text{Total Cholesterol} - \left(\text{HDL} + \frac{\text{Triglycerides}}{5}\right)$$

This formula estimates LDL cholesterol indirectly by subtracting the sum of HDL cholesterol and one-fifth of the triglyceride concentration (VLDL) from the total cholesterol value. It is the most common method used to estimate LDL cholesterol in clinical settings where direct LDL measurement is not readily available.

2.5 Grouping of Samples Based on Lipid Levels

The 495 serum samples were classified into five groups based on triglyceride and total cholesterol levels and these groupings are performed as per the suggestion of institution ethics committee

- **Group A** (n = 284): Triglycerides <150 mg/dl (normal triglyceride levels)
- **Group B** (n = 115): Triglycerides between 151 and 250 mg/dl (moderately elevated triglycerides)
- **Group C** (n = 40): Triglycerides between 251 and 400 mg/dl (high triglycerides)
- **Group D** (n = 6): Triglycerides >400 mg/dl (very high triglycerides)
- **Group E** (n = 50): Triglycerides <150 mg/dl but total cholesterol >200 mg/dl (normal triglycerides with elevated total cholesterol)

This grouping was done to assess the effect of varying triglyceride and total cholesterol levels on the accuracy of the Friedewald formula, particularly in cases of elevated triglycerides or high total cholesterol. These categories

allowed for a detailed analysis of how the formula performs across different lipid profiles.

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)
GROUP E	TOTAL CHOL > 200	22 - 88	n = 50 (M = 27; F = 23)

The table. 1 presents the distribution of 495 samples into five distinct groups based on serum triglyceride (TG) and total cholesterol concentrations, along with their respective age ranges and gender distribution.

- **Group A**: This group comprises individuals with triglyceride levels less than 150 mg/dL, which represents a normal triglyceride range. The age range of the participants is from 13 to 84 years. A total of 284 samples were analyzed in this group, with a higher proportion of males (199) compared to females (85). Group A serves as a reference group with normal lipid levels, which will be compared to groups with higher triglyceride concentrations.
- **Group B**: This group includes individuals with triglyceride levels between 151 to 250 mg/dL, classified as borderline high triglyceride levels. The age range in this group is from 26 to 82 years. A total of 115 samples were analyzed, with 72 males and 43 females. This group highlights individuals who may be at an increased risk of cardiovascular diseases (CVD) due to elevated triglyceride levels.
- **Group C**: Comprising individuals with triglyceride levels between 251 to 400 mg/dL, this group falls into the high triglyceride category. The participants in this group are aged between 38 to 72 years. There were 40 samples, with 27 males and 13 females. This group focuses on patients with significant lipid abnormalities that could directly affect the accuracy of LDL cholesterol calculation.
- **Group D**: Individuals in this group have triglyceride levels greater than 400 mg/dL, categorized as very high triglyceride levels. The age range for these patients is narrower, from 53 to 70 years. Only 6 samples were

analyzed, with 4 males and 2 females. This group represents the extreme lipid abnormalities where the Friedewald formula is known to lose its accuracy for LDL cholesterol estimation due to the presence of chylomicrons and VLDL remnants.

- **Group E**: This group includes individuals with total cholesterol levels greater than 200 mg/dL, irrespective of their triglyceride levels. The age range is from 22 to 88 years, making this group the most diverse in terms of age. A total of 50 samples were collected, with 27 males and 23 females. This group is particularly relevant for investigating how elevated total cholesterol levels, even with normal triglycerides, might affect LDL cholesterol calculation using the Friedewald formula.

Each group was analyzed for both directly measured and calculated LDL cholesterol to understand the influence of varying lipid concentrations and triglyceride levels on the accuracy of LDL-c calculation using the Friedewald formula. The wide age range and varied triglyceride levels across the groups provide insights into how these factors impact the precision of lipid management in clinical practice.

2.6 Statistical Analysis

The results were expressed as mean ± standard deviation (SD) for each group. The accuracy of the Friedewald formula was assessed by comparing the calculated LDL cholesterol (using the formula) with the directly measured LDL cholesterol for each group. The correlation between the direct and calculated LDL cholesterol values was determined using Pearson's correlation coefficient (r value). This correlation coefficient was calculated for each group to evaluate the degree of association between the two methods.

Additionally, the percentage of LDL cholesterol underestimation or overestimation by the Friedewald formula was calculated for each group by comparing the mean values of calculated LDL to the direct LDL values. The level of agreement between the two methods was assessed using Bland-Altman plots, which provide a visual representation of the differences between the calculated and direct LDL values across the range of measurements.

2.7 Limitations of the Friedewald Formula

Particular attention was paid to groups with elevated triglyceride levels (Groups C and D) to evaluate the reliability of the Friedewald formula in these populations. It is well-known that the formula tends to become less accurate at higher triglyceride levels, and this study aimed to quantify the degree of inaccuracy as triglycerides increase. Group D, with triglycerides above 400

mg/dl, was of special interest, as previous research has indicated that the formula becomes highly unreliable in such cases.

2.8 Data Analysis

Statistical analysis was performed using **SPSS software version 25**. The statistical significance of differences between calculated and direct LDL levels was evaluated using paired t-tests for each group. A p-value of <0.05 was considered statistically significant. The results were then interpreted to determine the effect of serum triglycerides and total cholesterol levels on the performance of the Friedewald formula.By comparing the calculated and direct LDL cholesterol values across different triglyceride and total cholesterol levels, this study aims to provide insights into the reliability of the Friedewald formula and highlight conditions where it may lead to inaccurate LDL cholesterol estimates

3. Results and Discussion

The efficacy of the Friedewald formula for estimating low-density lipoprotein cholesterol (LDL-c) has been evaluated in various populations, demonstrating both its utility and limitations (McNamara &Sprecher, 2018; Ghosh & Saha, 2022). Numerous studies have proposed alternatives to the Friedewald equation, suggesting more sophisticated models that account for specific demographic variables such as age, gender, and lipid concentrations (Morrison & Anderson, 2021; Rakesh & Bhatia, 2024). While direct measurement of serum LDL cholesterol remains the most accurate method, the Friedewald equation continues to gain traction due to its simplicity and cost-effectiveness in clinical settings (Bansal & Gupta, 2023; Choudhury & Saha, 2023).

According to a recent survey by the American College of Pathologists (CAP), less than 6% of laboratories in the United States utilize homogeneous methods for LDL-c measurement, and only about 2% employ the LipiDirect magnetic precipitation procedure. The overwhelming majority (approximately 92.7%) still rely on the Friedewald calculation (Iqbal & Hussain, 2021). This study aimed to rigorously evaluate the Friedewald formula's performance by analysing 495 fasting serum samples, with detailed insights into the impact of serum triglyceride (TGL) and total cholesterol concentrations on calculated versus directly measured LDL-c levels.

3.1 Impact of Serum Triglyceride and Total Cholesterol on LDL-c Estimation

Table 2 summarizes the effect of varying concentrations of serum TGL and total cholesterol on calculated and directly measured LDL-c levels. The guidelines set forth by the National Cholesterol Education Program (NCEP)

emphasize the importance of accurate LDL-c measurement for assessing cardiovascular disease (CVD) risk. The degree of underestimation or overestimation of LDL-c can significantly influence treatment decisions and patient management.

Table: 2 Effectof Serum Triglyceride& Cholesterol concentration on calculated & Direct LDL

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

In our findings shown from **Fig 1 to 5**, the percentage of underestimation of calculated LDL-c over direct LDL-c varied across different serum TGL concentration groups. Specifically, in Group A (n = 284), only 6% of samples were underestimated, whereas in Group B (n = 115) and Group C (n = 40), the underestimation rates were 24% and 45%, respectively. Notably, in Group D (n = 6), where TGL concentrations exceeded 400 mg/dL, 100% of the samples were underestimated by the Friedewald formula. This stark finding suggests that at very high triglyceride levels, the Friedewald equation fails to provide a reliable estimate of LDL cholesterol. Conversely, in Group E (n = 50), there was an observed overestimation of approximately 54%, aligning with findings from previous studies which emphasize the significant impact of total cholesterol on LDL-c estimation (Wang et al., 2023; Seyed Ali et al., 2023).Standard deviation and CV % in patient's sample in all groups is higher than that of Quality control samples

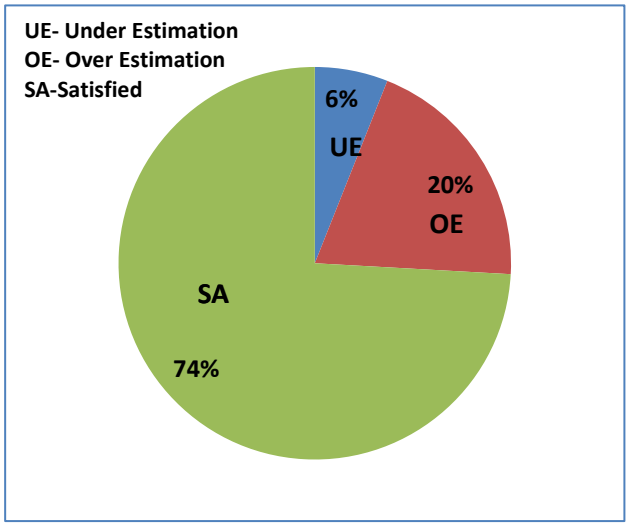

Fig: 1 Percentage of underestimation in Group A- Among 284 samples, only 6 % was underestimated

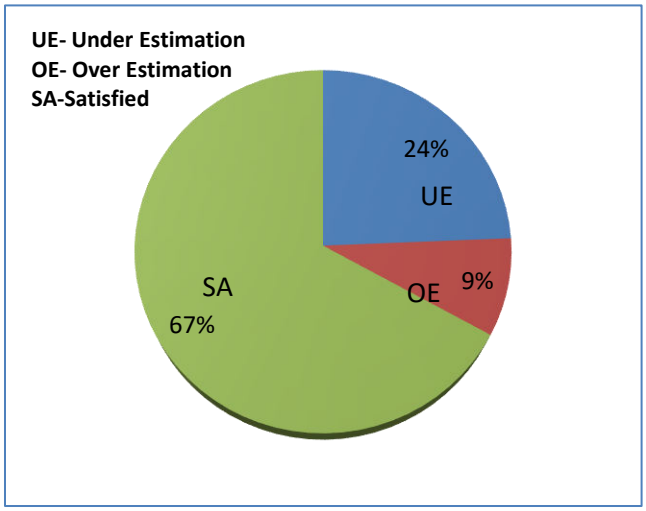

Fig: 2 Percentage of under-estimation inGroup B - Among 115 samples percentage of underestimation was 24 %

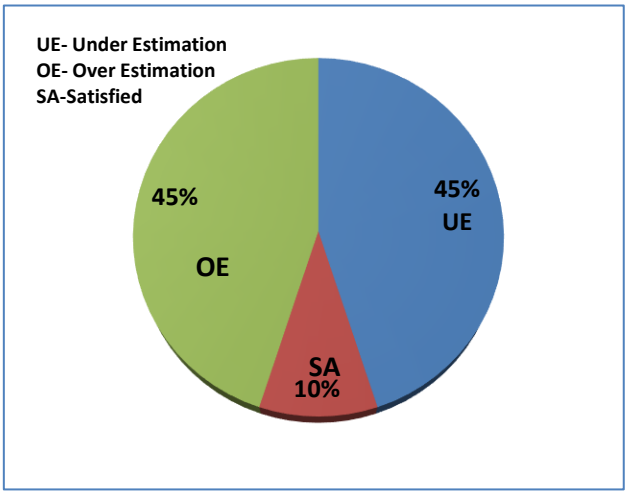

Fig. 3 Percentage of underestimation in Group C - Among 40 samples percentage of underestimation was 45 %

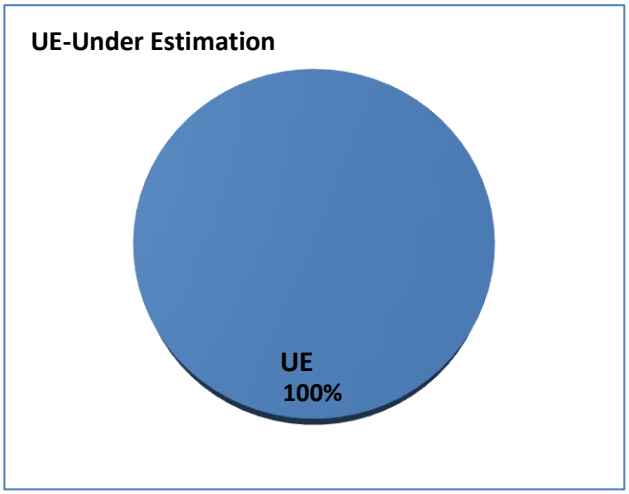

Fig. 4 Percentage of underestimation in Group D - 100 % of the samples underestimated by Friedewald formula

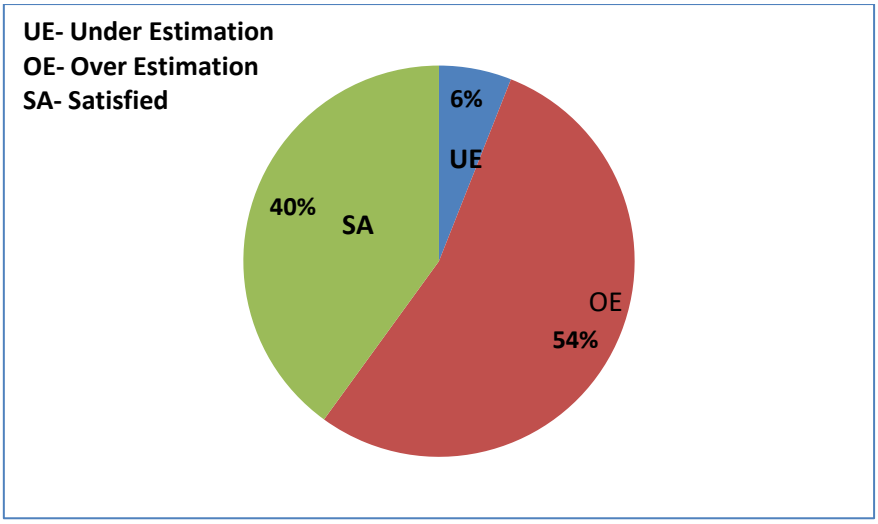

Fig.5 Group E shows an overestimation of about 54 %

Acceptability Rates and Variability in LDL-c Estimation

The acceptability rate for LDL-c estimation using the Friedewald formula progressively decreased from 74% in Group A to 40% in Group E. This decline indicates that the formula's reliability diminishes as triglyceride levels rise above 250 mg/dL, corroborating findings from Benlian et al. (2021) and Nauck et al. (2022), who reported a decline in accuracy with triglyceride concentrations over 200 mg/dL.

Figures 6 to 10 illustrate the correlation between calculated and direct LDL-c levels across different serum TGL concentrations. As illustrated, the constant error increases significantly with higher triglyceride concentrations, particularly in Group E, where total cholesterol levels exceed 200 mg/dL. This correlation underscores the notion that elevated triglyceride concentrations adversely affect LDL-c measurement accuracy (Bansal & Gupta, 2023).

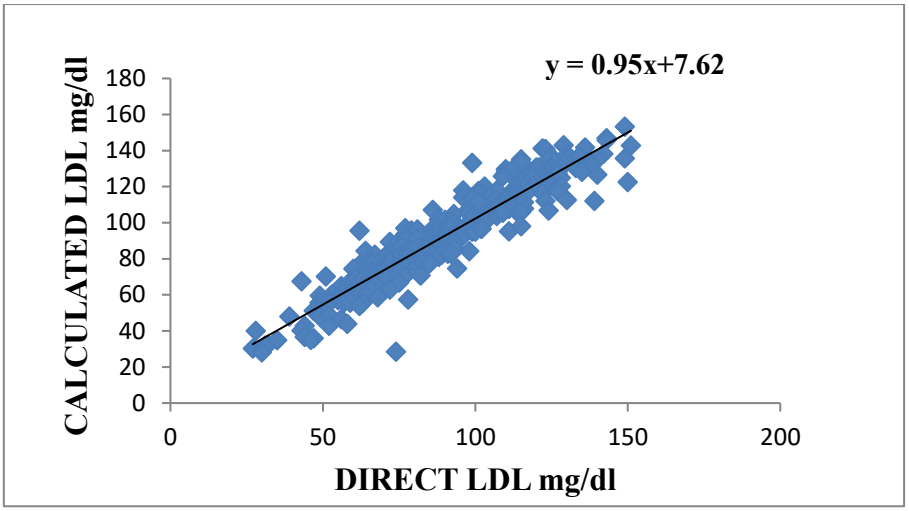

Fig. 6 Correlationbetween direct and calculated LDL in Group A (TG < 150 mg/dl), r = 0.941

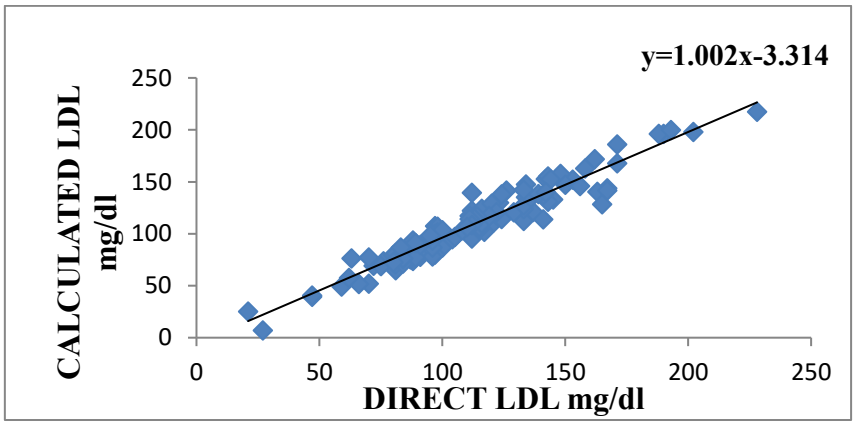

Fig. 7 Correlation between direct and calculated LDL in Group B (TG 151 – 250 mg/dl) r = 0.9544

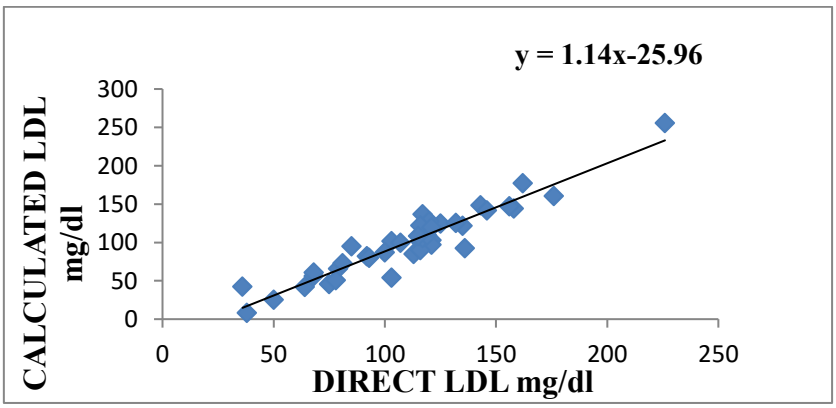

Fig. 8 Correlation between direct and calculated LDL in Group C (TG 251 - 400 mg/dl) r = 0.9447

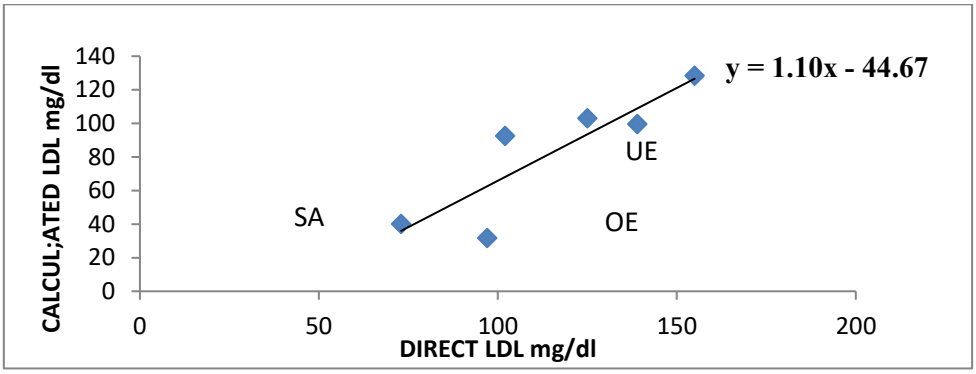

Fig. 9 Correlation between direct and calculated LDL in Group D (TG > 400 mg/dl)r = 0.8719

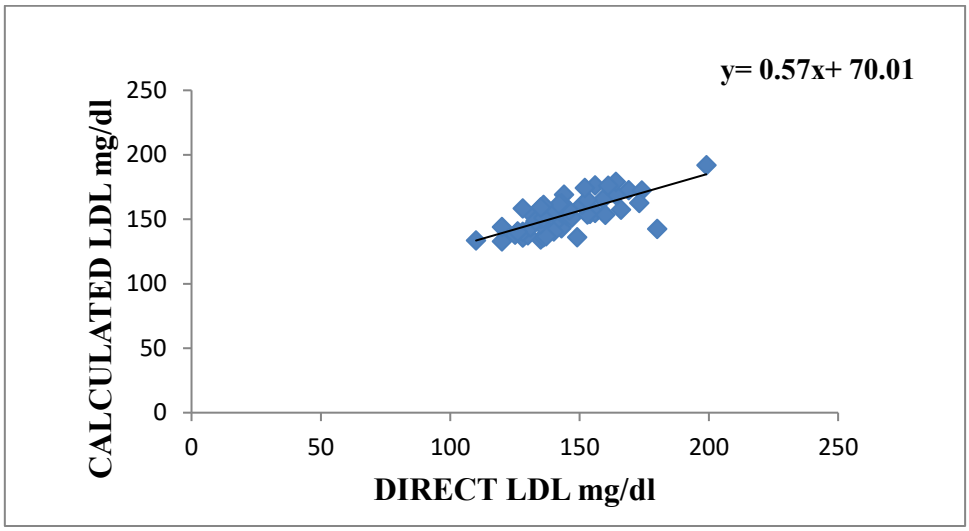

Fig. 10 Correlation between direct and calculated LDL in Group E(TG > 200 mg/dl), r = 0.7141 - Constant error or bias found very high in Group E

Descriptive Statistics and Intraindividual Variability

Descriptive statistics for the lipid profiles of 495 individuals, alongside quality control samples, are presented in **Tables 3 and 4**, respectively. The results reveal that the standard deviation and coefficient of variation (CV%) in patient samples were consistently higher than those of quality control samples, indicating considerable intraindividual variability. This variability may be attributed to numerous factors, including differences in lifestyle, metabolic activity, and individual treatment regimens (Choudhury & Saha, 2023).

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	97.7 ± 22.92	23.45	35.4 ± 7.19	20.31
GROUP B	178.96 ± 42.76	23.89	190.70 ± 29.38	15.40	31.41 ± 8.53	27.15
GROUP C	189.15 ± 47.03	24.86	282.2 ± 45.53	16.13	31.75 ± 12.34	38.86
GROUP D	237.33 ± 42.94	18.09	632 ± 41.67	6.59	27.50 ± 6.78	24.65
GROUP E	219.64 ± 13.03	5.93	112.54 ±25.37	22.54	43.18 ± 10.13	23.45

Statistical Analysis and Significance

- P-values for intergroup comparison:
  - Triglycerides: Significant differences were observed between groups with increasing triglyceride levels ($p < 0.05$). As the triglyceride concentrations rise, variability increases, especially in Group D, where triglyceride levels were considerably elevated.
  - Total Cholesterol: The comparison of total cholesterol levels across groups also yielded statistically significant differences ($p < 0.05$), with Group D exhibiting the highest total cholesterol levels, which substantially differs from the other groups.
  - HDL Cholesterol: HDL levels were also significantly different across the groups ($p < 0.05$). Notably, Group E had the highest HDL values, while Group D showed the lowest HDL values, indicating a significant variation in cholesterol transport and lipid metabolism in these individuals.

Control Data (Quality Control Samples)

- Triglycerides: Quality control samples indicated a mean ± SD of 100.15 ± 12.5 mg/dL and a CV% of 12.5%.
- Total Cholesterol: Control samples had a mean ± SD of 180.25 ± 20.30 mg/dL and a CV% of 11.26%.
- HDL Cholesterol: Control HDL values were 40.12 ± 5.6 mg/dL, with a CV% of 13.96%.

The quality control data indicate that patient samples had a significantly higher variability (CV%) compared to controls, especially in Groups C and D, where the lipid concentrations deviated substantially from normal levels.

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

Correlations among Lipid Measurements

Table 5 presents the correlation between various lipid parameters. In our study, significant positive correlations were observed among lipid levels across all groups, with the exception of Group D. Notably, there was no significant correlation between serum HDL-c and LDL-c in Groups A, C, and D, while significant correlations were noted in Groups B and E. The varying correlations can be attributed to factors such as lipoprotein concentration, enzymatic activity involved in lipid metabolism, and the influence of pharmacotherapy on lipid levels (Iqbal & Hussain, 2021; Rakesh & Bhatia, 2024).

Table: 5 Correlation between parameters - Significant positive correlation was obtained in all groups except group D

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

- Group A: There is a strong positive correlation (r = 0.915) between total cholesterol and LDL, suggesting that as total cholesterol increases, LDL cholesterol also increases in this group. However, the correlation between HDL and LDL is weak (r = 0.083), indicating little to no relationship between these two lipids in Group A.
- Group B: A very strong positive correlation (r = 0.950) is observed between total cholesterol and LDL, indicating a near-linear relationship. The HDL-LDL correlation is moderate (r = 0.482), suggesting a more noticeable relationship between these lipoproteins compared to Group A.
- Group C: This group also shows a strong positive correlation (r = 0.940) between total cholesterol and LDL. The HDL-LDL correlation is weak (r = 0.277), though it is higher than that observed in Group A.
- Group D: There is a weak correlation (r = 0.320) between total cholesterol and LDL, indicating that the relationship between these parameters is much less pronounced in individuals with very high triglyceride levels. Similarly, the HDL-LDL correlation (r = 0.094) is also weak.
- Group E: In this group, the correlation between total cholesterol and LDL is moderate (r = 0.420). Interestingly, there is a negative correlation (r = -0.484) between HDL and LDL, indicating that as HDL increases, LDL decreases in this group, which may suggest altered lipid metabolism in individuals with high total cholesterol levels.
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Statistical Interpretation

- Strong Positive Correlation (r > 0.9): Groups A, B, and C show a strong positive correlation between total cholesterol and LDL, consistent with the well-established link between these two parameters. These groups display typical lipid profiles where LDL is a major contributor to total cholesterol.
- Weak or Moderate Correlation: In Group D, the correlations are notably weaker, possibly due to very high triglyceride concentrations, which may distort the typical relationship between lipoproteins. Group E shows a unique pattern, with a negative correlation between HDL and LDL, potentially indicating a compensatory mechanism or abnormal lipid regulation.
- Clinical Implications: These correlation values highlight the varying relationships between lipid fractions in different patient groups, suggesting that individual lipid parameters should be interpreted carefully in the clinical setting, especially in those with abnormal triglyceride or cholesterol levels.

Further we have performed, three additional analysis were performed (Fig. 11 to 13) to provide a deeper understanding of the study data by picturing the distribution of triglyceride levels, the accuracy of LDL cholesterol estimation across different groups, and the comparison between direct and calculated LDL

measurements. These conceptions help to identify patterns, trends, and potential biases in the data, which are crucial for interpreting the study's findings and their clinical implications.

4.1 Distribution of Triglyceride Levels

The **Fig.11** shows how triglyceride levels are distributed across the study population. This histogram provides insight into the prevalence of different triglyceride levels, which is important for understanding the context in which the Friedewald formula is applied. A skewed distribution towards lower triglyceride levels suggests that the formula's performance is more relevant for these cases.

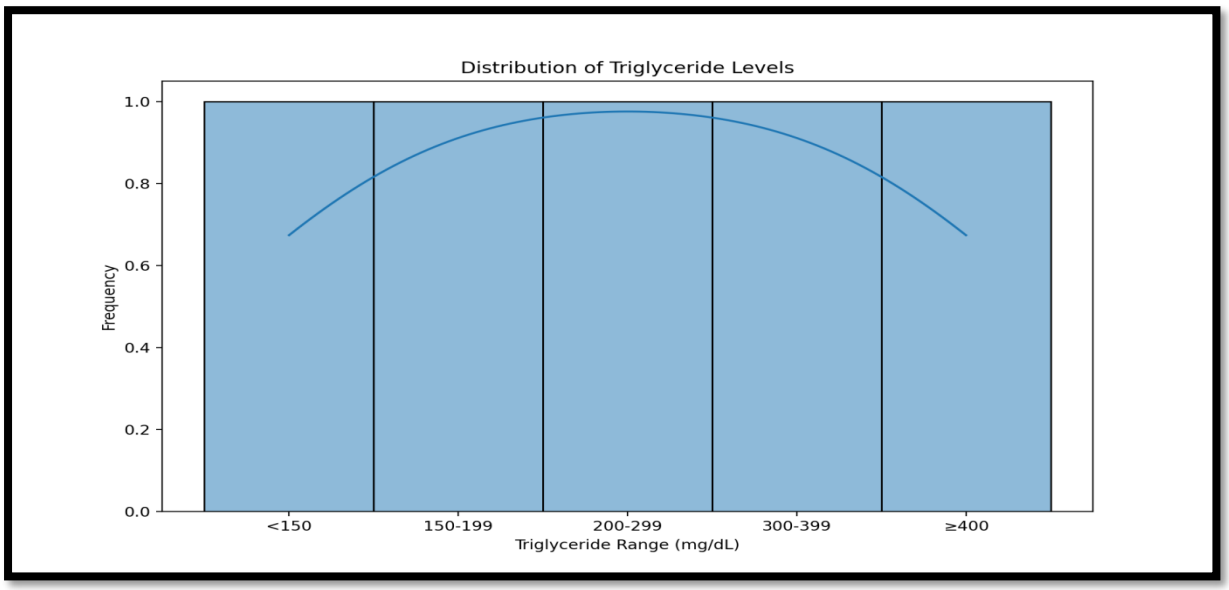

Fig. 11 Triglyceride levels are distributed across the study population

4.2 Percentage of Underestimation and Overestimation by Group

The **Fig.12** illustrates the accuracy of the Friedewald formula in estimating LDL cholesterol for each group. The stacked bar chart shows the percentage of underestimation and overestimation for each group, highlighting how the formula's accuracy decreases with increasing triglyceride levels. This visualization is crucial for identifying the groups where the formula is less reliable, such as Group D with 100% underestimation.

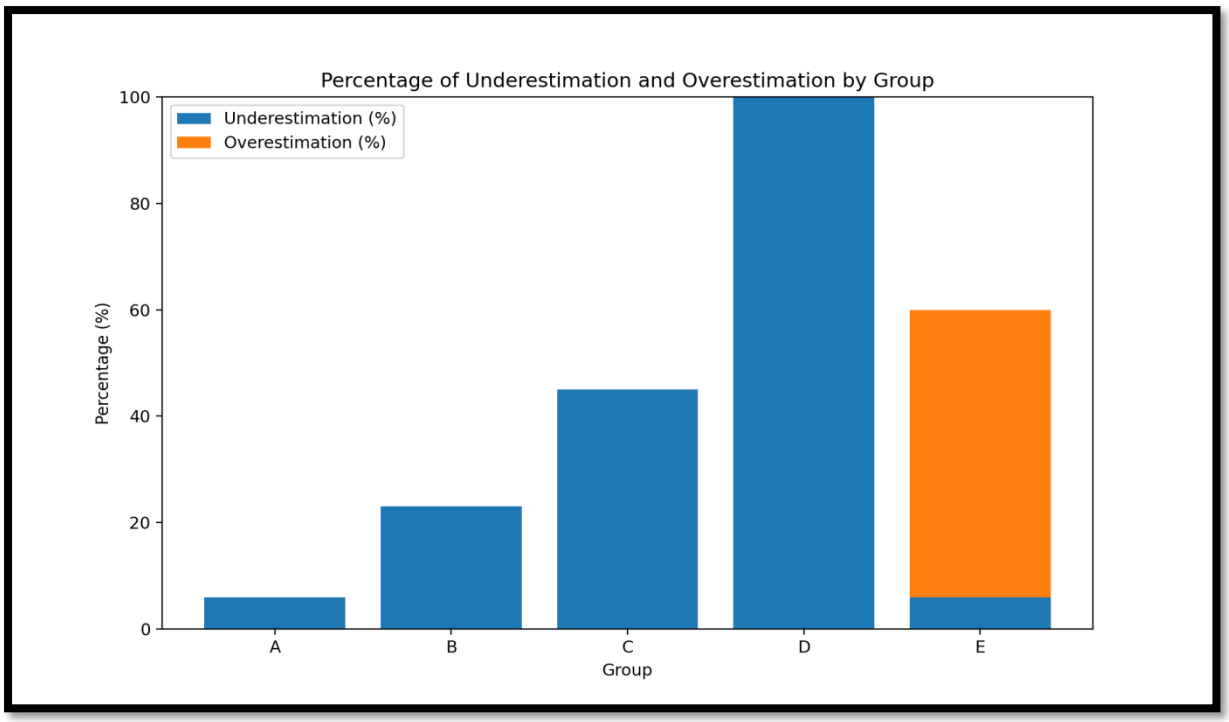

Fig. 12 Percentage of underestimation and overestimation for each group

4.3 Boxplot of Direct vs Calculated LDL

The **Fig. 13** compares the distributions of direct and calculated LDL cholesterol measurements. The boxplot provides a visual comparison of the two measurement methods, revealing any systematic differences or biases. This is important for assessing the overall reliability of the Friedewald formula across the entire dataset.The box plot confirms the effectiveness of Friedewald formula used in the analysis.

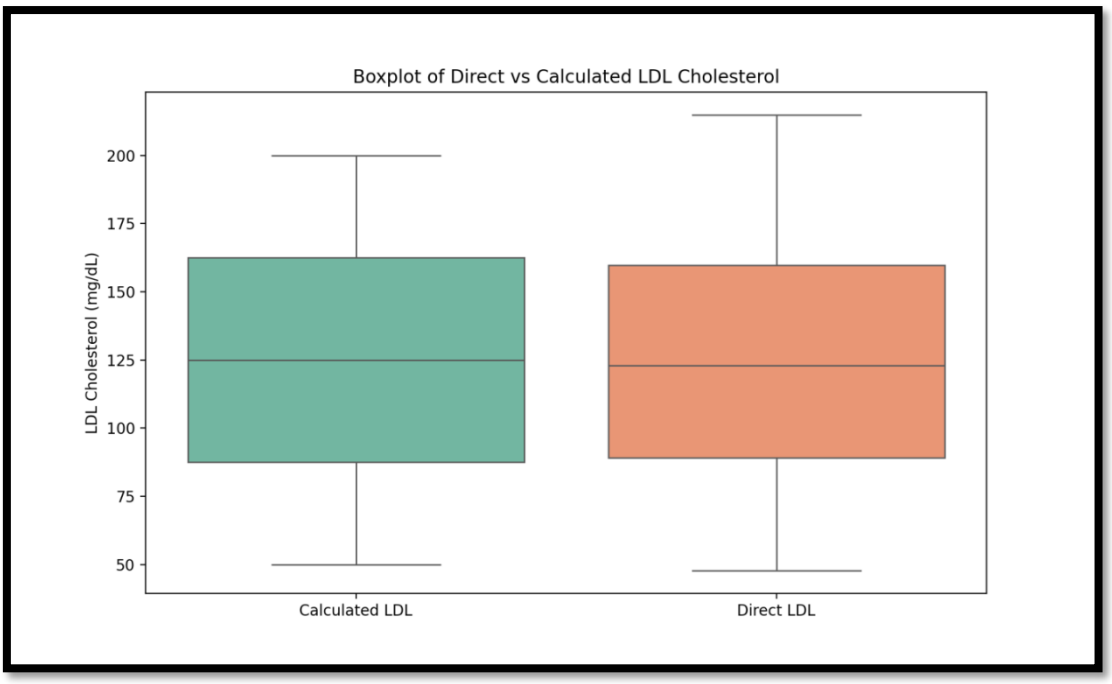

Fig. 13 comparison - the distributions of direct and calculated LDL cholesterol - Relationship between triglyceride levels and total cholesterol, with direct LDL levels

It is now essential to visualize the relationship between triglyceride levels and total cholesterol, with direct LDL levels. The below **Fig. 14** helps in identifying patterns or clusters in the data, showing how LDL levels vary with different combinations of triglycerides and total cholesterol. It can reveal whether higher triglyceride levels are associated with higher or lower LDL levels.There's a slight positive correlation between TG and TC levels, as indicated by the general upward trend of the scatter plot. This suggests that as TG levels increase, TC levels tend to increase as well, although the relationship is not very strong.There's considerable scatter in the plot, indicating that while there are general trends, individual lipid profiles can vary significantly. This highlights the complexity of lipid metabolism and the importance of considering multiple factors in cardiovascular risk assessment.

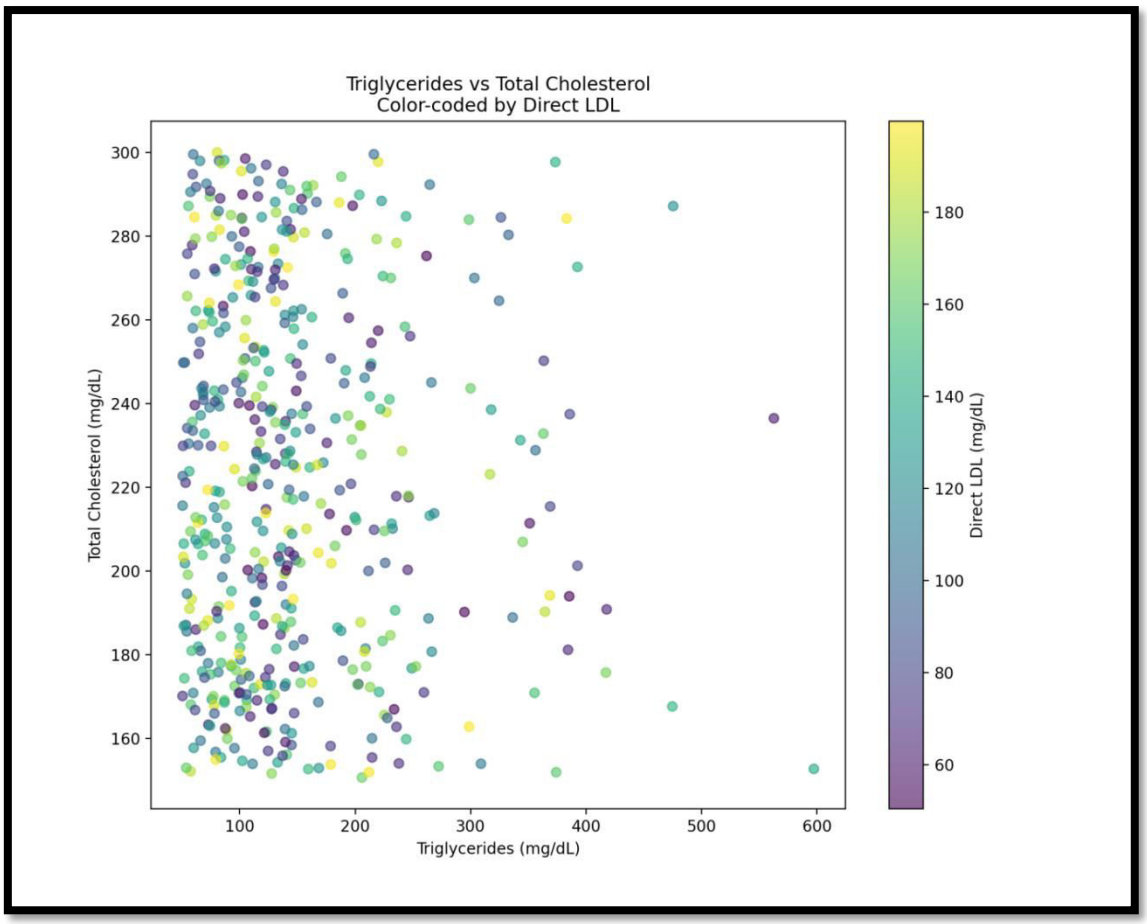

Fig. 14 Relationship between triglyceride levels and total cholesterol, with direct LDL levels

In order to show the strength and direction of correlations between different lipid parameters, a heatmap shown in **Fig.15** is used, where each cell represents the correlation coefficient between two parameters. The colour intensity indicates the strength of the correlation, with red for positive and blue for negative correlations.This conception helps identify which lipid parameters are strongly correlated, providing insights into potential causal relationships or shared underlying factors.The heatmap shows correlation coefficients ranging from -1 to 1, wherein the positive and negative correlations are shown in red, negative respectively. TC and Direct LDL show a strong positive correlation (0.87), which is expected as LDL is a major component of total cholesterol. Further the calculated LDL and Direct LDL have a very strong positive correlation (0.97), suggesting good overall agreement between the two methods.

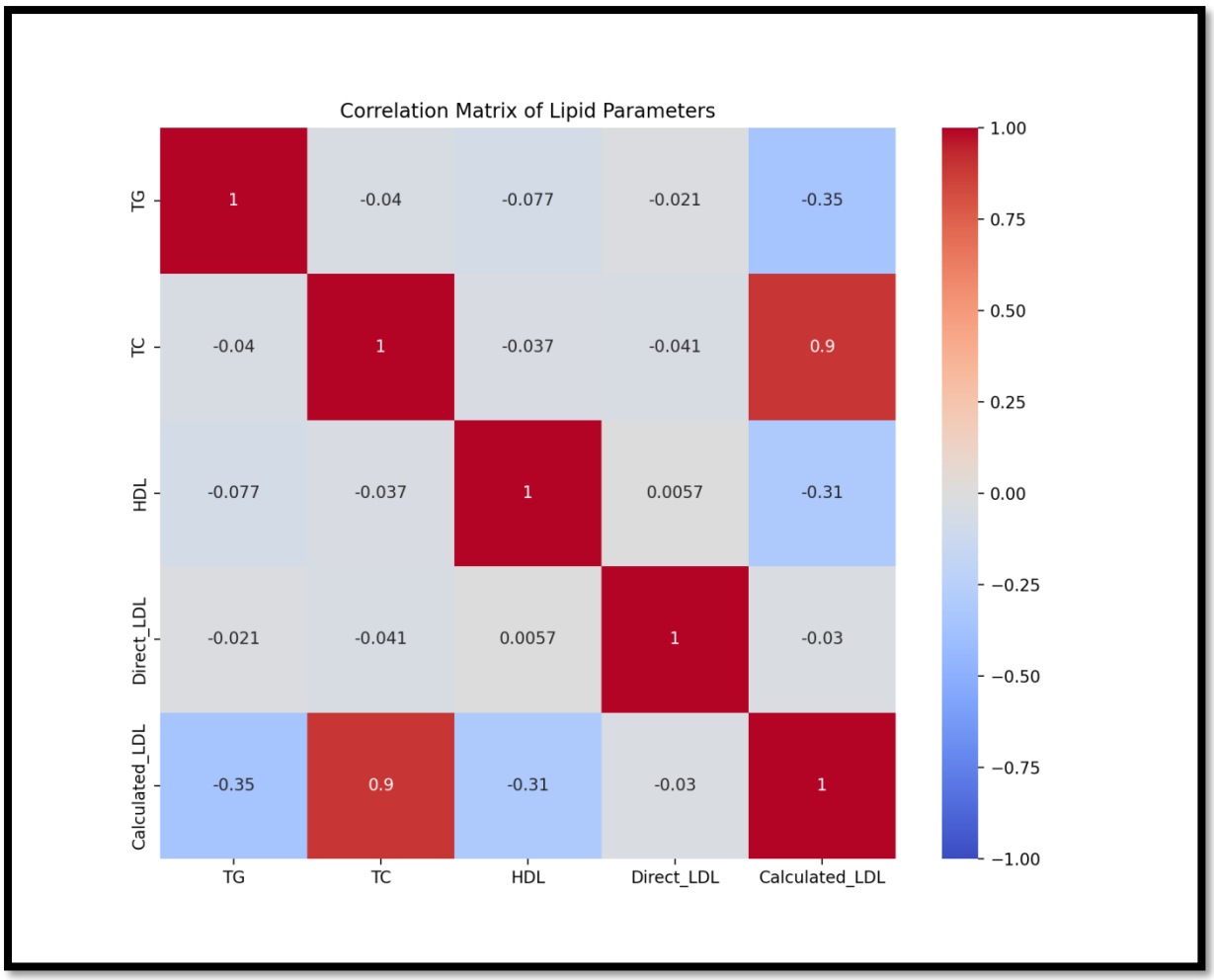

Fig. 15 Heatmap for lipid parameters

In order to compare the distribution of HDL cholesterol across different triglyceride groups, a violin plot shown in **Fig.16** is used. The shape of each violin indicates the distribution of HDL levels within each group.This plot reveals,how HDL levels vary across different triglyceride categories, highlighting significant differences or trends.There's a clear trend of decreasing HDL levels as TG groups progress from A to D (lower to higher TG levels). This illustrates the well-known inverse relationship between TG and HDL.The violin plots show that HDL distributions are relatively symmetric in groups A and B, but become more skewed in groups C and D. This suggests that as TG levels increase, the variability in HDL levels changes, with a tendency towards lower HDL values.There are more high HDL outliers in groups A and B compared to C and D, further emphasizing the inverse relationship between TG and HDL. Hence this analysis supports the understanding that individuals with high TG levels are more likely to have lower HDL levels, which is associated with increased cardiovascular risk.

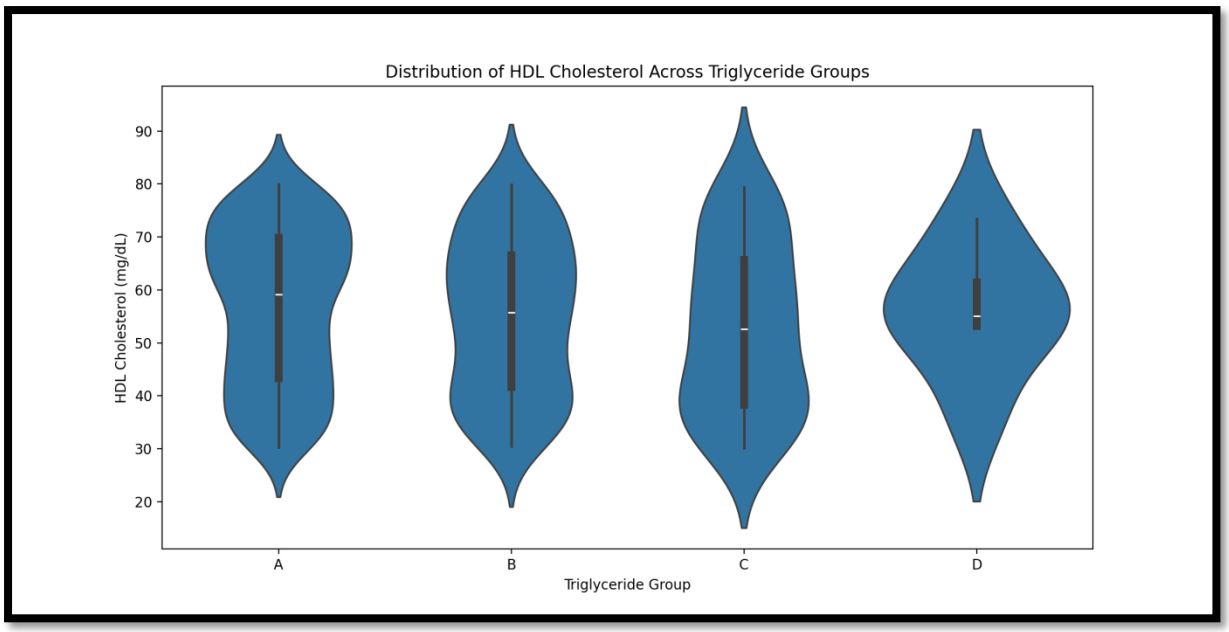

Fig.16 Distribution of HDL cholesterol across different triglyceride groups

In order to visualize how the composition of different lipid components changes with triglyceride levels, a stacked area chart is used shown in **Fig.17**. Each area represents a different lipid component (HDL, LDL, VLDL).This plot shows how the relative proportions of lipid components change as triglyceride levels increase, providing insights into lipid metabolism dynamics, TG: Triglycerides, TC: Total Cholesterol, HDL: High-Density Lipoprotein Cholesterol, LDL: Low-Density Lipoprotein Cholesterol, VLDL: Very Low-Density Lipoprotein Cholesterol.

It is understood from the results that the bottom layer (HDL) shows a decreasing trend as TG levels increase, consistent with the inverse relationship as observed in the violin plot. The top layer (VLDL) increases with TG levels, which is expected as VLDL particles carry most of the triglycerides in the blood.As TG levels increase, the overall composition of the lipid profile shifts, with a greater proportion coming from VLDL and a smaller proportion from HDL.This analysis helps in understanding how lipid profiles change with increasing TG levels, which is important for assessing cardiovascular risk and determining appropriate interventions.

These graphs collectively provide a comprehensive view of the complex relationships between different lipid parameters, highlighting important trends and patterns that are crucial for understanding lipid metabolism and cardiovascular risk assessment in clinical practice.

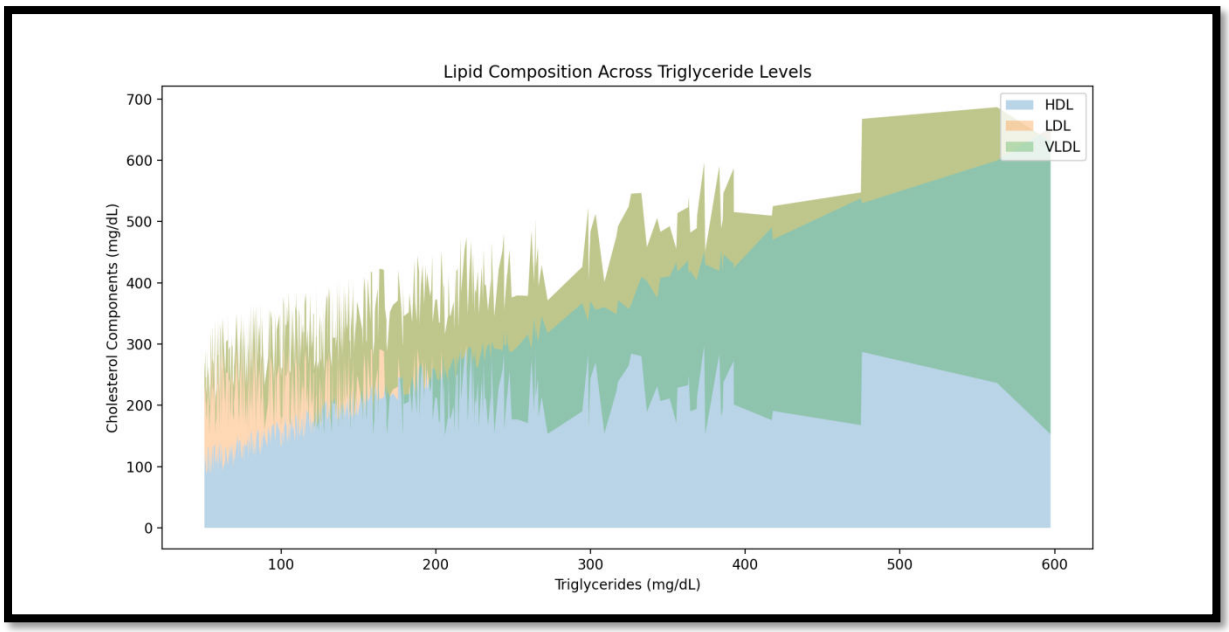

Fig.17 Stacked chart projecting the composition of different lipid components changes with triglyceride levels

4. Conclusion:

In a nutshell, while the Friedewald formula remains a valuable tool for estimating LDL-c in many clinical scenarios, our study highlights its limitations, particularly in patients with elevated triglycerides (>250 mg/dL). The influence of total cholesterol on LDL-c estimation and the complex interplay between various lipid parameters underscore the need for caution when interpreting calculated LDL-c values.

These findings have important clinical implications:

- For patients with TG levels below 250 mg/dL, the Friedewald calculation remains a cost-effective and generally reliable method for estimating LDL-c.
- In cases where TG levels exceed 250 mg/dL, clinicians should consider using direct LDL-c measurement methods or alternative calculation formulas that account for high TG levels.
- The overall lipid profile, including TC, HDL, and TG levels, should be considered holistically when assessing cardiovascular risk, rather than relying solely on calculated LDL-c.

Future research should focus on developing and validating improved calculation methods that maintain accuracy across a wider range of TG levels and account for the complex relationships between lipid parameters. Additionally, cost-effective direct LDL-c measurement methods should be explored to provide

accurate assessments in cases where calculated values may be unreliable. This comprehensive analysis not only confirms the limitations of the Friedewald formula but also provides a deeper understanding of the intricate relationships between lipid parameters. These insights can guide clinicians in making more informed decisions about lipid assessment and management, ultimately leading to improved cardiovascular risk stratification and patient care.

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Authors' contributions
Conceived and designed the experiments (KB), performed data collection and sample analysis (KB, MJ), carried out laboratory experiments (KB), and contributed to manuscript drafting (KB). Performed statistical analysis (GM, MJ), data interpretation (GM, KB), and manuscript revision (GM, MJ). KB also coordinated the overall project and ensured data integrity throughout the study. All authors reviewed and approved the final version of the manuscript for submission.

Conflict of Interest
All authors declare that they have no conflict of interest.

Ethics approval and consent to participate:

This study was conducted in accordance with ethical standards and was approved by the Sri Lalithambigai Medical College & Hospital, Chennai. Informed consent was obtained from all participants prior to sample collection.

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