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"Comparative Study Of Serum Low-Density Lipoprotein (LDL) Level By Direct Methods, And Indirect Methods."

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Abstract:
Background*: Elevated LDL cholesterol levels are linked to an increased risk of heart disease and stroke, making accurate LDL assessment vital for evaluating health risks and making treatment decisions. While both direct and indirect methods provide LDL values, their accuracy may be compromised in cases of high triglycerides. This study aims to compare direct and indirect techniques for measuring LDL cholesterol, specifically the Friedewald and Modified formulas, to assess their advantages and limitations in clinical risk assessment and decision-making.*
The present study *is to compare serum low-density lipoprotein (LDL) levels obtained through direct and indirect methods (Friedewald and Modified formulas).*
Materials and Methods*: This cross-sectional study involved 200 participants aged 20 to 60 years of all genders who were referred for lipid profile testing (OPD/IPD) at the Department of General Medicine. A direct homogeneous assay was used to measure LDL-C levels, while Friedewald's and the Modified formulas were employed to calculate LDL cholesterol levels.*
Results*: The study found variations in LDL-C measurements depending on the method used, especially as triglyceride levels increased. The direct method tended to yield higher LDL-C levels than either of the formulas. Despite this, the results from the direct method were closer to those from both formulas, with the Friedewald formula showing the lowest mean LDL-C.*
Conclusion*: This study compares the use of Friedewald's formula, the direct measurement, and the Modified formula for estimating LDL cholesterol. The Modified method proved to be more accurate, with higher diagnostic sensitivity and specificity, making it a more reliable tool for LDL estimation in clinical practice.*
Keywords: *low-density lipoprotein, calculation, Friedewald formula, Modified formula.*

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I. Introduction

Lipoproteins are particles that carry hydrophobic lipids such as cholesterol and triglycerides in the bloodstream. They consist of a hydrophobic core surrounded by apolipoproteins.[1] These lipoproteins are produced by the liver and intestines and vary in size and density depending on their lipid-to-protein ratio.[2,3] As the types of proteins and lipids in these particles change over time, lipoproteins undergo dynamic transitions. Lipoproteins, which can be spherical or discoidal, transport dietary triglycerides and cholesterol to the body's cells [4,5]. They are synthesized in the intestines and processed by lipoprotein lipase before being either absorbed or utilized for energy.[6] Chylomicron remnants, produced by lipoprotein lipase, are lipoproteins rich in cholesterol that are quickly cleared by the liver. These particles help transport dietary cholesterol and triglycerides to cells, where they are hydrolysed by lipoprotein lipase. The resulting fatty acids are either stored as triglycerides or used as energy.[7]

Very-low-density lipoproteins (VLDL), which range in size from 30 to 90 nm, carry cholesterol and triglycerides from the liver to other tissues. As lipoprotein lipase acts on VLDLs to break down triglycerides, intermediate-density lipoproteins (IDLs) are formed.[8] IDLs are temporary lipoproteins that emerge as VLDLs lose triglycerides in the capillaries, and they contain apoB-100. These IDLs are either removed by the liver via apoE binding or transformed into low-density lipoproteins (LDLs) after losing apoE and acquiring cholesterol esters. LDL is the primary lipoprotein in plasma and plays a key role in cholesterol transport, originating from VLDL breakdown. In conditions like familial hypercholesterolemia, malfunctioning receptors cause an accumulation of LDL.[9] High- density lipoproteins (HDLs), the smallest and densest lipoproteins, are produced by the liver. They transport cholesterol back to the liver for excretion and help prevent both acute and chronic cardiovascular issues, including the rupture of atherosclerotic plaques.[10]

Lipoprotein metabolism is crucial for regulating lipid movement in the blood and maintaining metabolic health, and it plays an essential role in preventing cardiovascular diseases while improving diagnostic and therapeutic approaches.[11] Circadian rhythms also regulate lipid metabolism at the cellular level, influencing processes like emulsification, digestion, and absorption. Free fatty acids within cells are used for energy, biosynthesis, and gene regulation.[12] High levels of LDL-C and low levels of HDL-C are associated with atherosclerotic cardiovascular disease (ASCVD).[13] LDL cholesterol, a critical component of lipoproteins and a precursor to steroids, is common in women and adults over 40, though children may have difficulty removing it due to lower-density LDL. LDL-C levels can be measured through direct or indirect methods, with direct methods offering higher specificity at a lower cost, and ultracentrifugation serving as the gold standard.[14] Many clinical labs utilize direct methods and automated systems, although they cannot fully automate the process. Homogeneous assays, which use detergents to selectively block specific lipoprotein classes, allow for precise LDL-C measurement, but they can be inconsistent, especially at lower concentrations. Despite the availability of homogeneous LDL-C assays, indirect methods like the Friedewald equation and modified formulas are still widely used in clinical settings due to the time and financial costs associated with ultracentrifugation.[15,16.17,18] The Friedewald formula, developed for epidemiological studies, is commonly used to estimate LDL-C levels, and it is also used to estimate total cholesterol (TC), HDL-C, and triglycerides (TG).[19,20] In 2013, Martin et al. proposed a modified version of the formula that uses a combination of triglycerides to calculate the VLDL-C ratio.[13] The modified Friedewald formula (MF-LDL-C) combines TC, triglycerides, and HDL-C to calculate LDL-C with more precision. While lipid ultracentrifugation is effective for measuring LDL, its cost and time limitations make it inefficient, which is why computational methods like the Friedewald equation are more commonly employed. Choi et al. developed a modified Friedewald equation based on cohort data from the Green Cross Research Institute, utilizing a triglyceride-to-VLDL-C ratio to address the limitations of the current equation.[21] A study showed that estimating plasma triglycerides using a factor of five is not sufficient for accurately estimating VLDL-C, outperforming eleven other formulas. This research aims to compare LDL levels measured by direct methods with those estimated using indirect methods such as the Friedewald and modified formulas. The objective of this study is to assess LDL cholesterol levels using both direct and indirect approaches.

II. Materials And Methods

Sample collection: A 3ml fasting venous blood sample was taken from patients in plain vials and subjected to centrifugation for serum separation and used for estimation of low-density lipoprotein (LDL) using a fully automated biochemistry analyzer VITROS 5600 integrated system in the Department of Biochemistry in Central Laboratory at NATIONAL INSTITUTE OF MEDICAL SCIENCE & RESEARCH, Jaipur, Rajasthan. These parameters were estimated using VITROS 5600 for Direct method and for Indirect method

1. **Friedewald Low-density lipoprotein cholesterol (LDL-CF) =** Total cholesterol (TC)- High-density lipoprotein cholesterol (HDL-C)- Triglyceride /5 in mg/dl.
2. **Modified formula estimated Low-density lipoprotein cholesterol (LDL-CM)** = Total cholesterol (TC)- High-density lipoprotein cholesterol (HDL-C)- Triglyceride /adjustment factor in mg/ dl. (Numbers from 4.0 to 6.5 with one decimal place were applied as adjustment factors).

Figures And Tables

Table 1: Descriptive statistics of LDL of patients at different levels of triglyceride by direct, Friedewald, and modified method

Variables		Minimum	Maximum	Median (IQR)	Mean ± SD
TG ≤ 100 mg/dl	**Direct**	32.9	157	88.75 (66.6-113.3)	90.57 ± 28.3
	Friedewald	34.4	150	85.4 (67.7-109.35)	89.1 ± 28.26
	Modified	37.5	153	88.15 (70.65-112.1)	91.73 ± 28.27
TG: 101– 200 mg/dl	**Direct**	36.5	181.5	111.8 (83.88-131.6)	108.4 ± 31.39
	Friedewald	38.4	191	107 (80.25-129.8)	106.1 ± 30.91
	Modified	43.5	197.1	112.7 (86.08-135.08)	111.5 ± 30.75
TG: 201– 300 mg/dl	**Direct**	30.1	218	115.1 (83.6-128)	105.35 ± 38.32
	Friedewald	9.2	217.6	104 (64.5-125.5)	96.6 ± 41.75
	Modified	18.5	225.8	112.25 (72.1-133.1)	104.63 ± 41.39
TG: 301– 400 mg/dl	**Direct**	26.4	274.5	123.35 (89.3-153.03)	126.22 ± 51.22
	Friedewald	13	329.8	93.9 (66.35-133.65)	104.05 ± 58.35
	Modified	14	367	105.05 (76.7-145.5)	115.9 ± 60.59

Table 2: Comparing LDL between direct, Friedewald, and modified methods by using Anova statistics

LDL	Direct	Friedewald	Modified	Anova	P - Value	Significance
TG≤ 100 mg/dl	90.57 ± 28.3	89.1 ± 28.26	91.73 ± 28.27	0.112	0.89393	All are not

TG: 101–200 mg/dl	108.4 ± 31.39	106.1 ± 30.91	111.5 ± 30.75	0.377	0.68634	significant
TG: 201–300 mg/dl	105.35 ± 38.32	96.6 ± 41.75	104.6 ± 41.39	0.679	0.50862	
TG: 301–400 mg/dl	126.22 ± 51.22	104.05 ± 58.35	115.9 ± 60.59	1.887	0.15522	

Table 3: Descriptive statistics of LDL by direct, Friedewald, and modified method

LDL	Minimum	Maximum	Median (IQR)	Mean ± SD
Direct Method	26.4	274.5	108.6 (78.04-131.25)	107.64 ± 40.1
Friedewald Method	13	329.8	97.4 (72.55-122.1)	98.95 ± 41.77
Modified Method	14	367	105.55 (77.5-129.13)	105.9 ± 42.9

III. Discussion

The present study was conducted in the Department of Biochemistry, National Institute of Medical Sciences & Research, Jaipur, Rajasthan, in association with the Department of General Medicine. It included 200 subjects between the ages of 20 and 60 from the OPD/IPD of General Medicine and our central biochemistry lab, NIMS Hospital. The study analyzed the mean LDL levels of patients at different triglyceride levels using direct, Friedewald, and modified methods. The Modified Method showed the highest mean LDL (91.73 mg/dL), followed by the Direct Method (90.57 mg/dL) and Friedewald Method (89.1 mg/dL). The median LDL values were similar, but the Modified Method had a slightly higher range. The Direct Method showed the highest mean LDL (105.35 mg/dL) at 201-300 mg/dL, while the Friedewald Method reported the lowest mean LDL (96.6 mg/dL). The median LDL values indicated that the Direct Method was the most accurate. Research has shown that various methods for estimating low-density lipoprotein cholesterol (LDL-C) levels perform poorly, particularly in patients with elevated triglyceride levels. The Friedewald equation, commonly used, has limitations, especially when TG levels are high. Studies have found that the Friedewald-estimated LDL-C is typically 18.4 mg/dL lower than directly measured LDL-C. **Reiber et al (2022)** found that the Friedewald equation's accuracy decreased as TG levels increased, highlighting the need for more reliable estimation methods. **Brindha DP et al (2020)** compared the accuracy of the Friedewald and modified Friedewald formulas in estimating LDL cholesterol, particularly in patients with varying triglyceride levels. The Direct Method consistently reports higher LDL levels compared to the Friedewald Method, especially in patients with higher triglyceride levels. Table no. 2, The study compared LDL levels across different triglyceride ranges using direct, Friedewald, and modified methods. Results showed no significant differences for all TG categories. For TG ≤ 100 mg/dl, the mean LDL was 90.57 ± 28.3, while for 100-200 mg/dl, it was 108.4 ± 31.39. For TG levels 201-300 mg/dl, the direct method had 105.35 ± 38.32, while the modified method had 115.9 ± 60.59. These results align with **Sajja A, et al. (2021)** findings that the Friedewald equation underestimates LDL-C with TG levels above 400 mg/dl, leading to underestimation. Table no. 3, The direct method, Friedewald, and modified methods for estimating LDL levels have been compared for their range, central tendency, and variability. The direct method has a minimum LDL value of 26.4 and a maximum of 274.5, with a median of 108.6 and a mean of 107.64 ± 40.1. The Friedewald method has a slightly wider range, with a median LDL value of 97.4 and a mean of 98.95 ± 41.77. The modified method has a minimum LDL of 14 and a maximum of 367, with a median of 105.55 and a mean of 105.9 ± 42.9. The results suggest that the direct method provides more consistent LDL estimates across a broader range of patients, highlighting the need for careful interpretation of LDL measurements, especially when triglyceride levels are elevated. According to the **Sung, Ki-Chul et al. (2020)** A study comparing Friedewald- calculated LDL-C levels with directly measured values in 145,043 Korean adults found that Friedewald-calculated levels were consistently 15 mg/dL lower than directly measured values. The Friedewald method showed high sensitivity and specificity, but in 82% of subjects, the LDL-C values differed by more than 10 mg/dL. The study also found substantial reclassification issues, particularly in patients categorized under the National Cholesterol Education Program risk levels. The low concordance between the two methods suggests the need for a more accurate and standardized approach to LDL-C measurement, especially in patients with elevated triglyceride levels. The study calls for a global consensus on the method of LDL-C measurement to ensure consistent and reliable lipid profiling across clinical settings.

IV. Conclusion

The comparison of LDL values obtained through the direct, Friedewald, and modified methods across various triglyceride (TG) levels reveals no significant statistical differences, as indicated by the high P-values for all TG categories. This suggests that, regardless of the triglyceride level, the three methods generally provide similar LDL measurements. While slight variations in mean and median values were observed, these differences are likely attributed to the specific assumptions and adjustments made by each method, especially in individuals with varying triglyceride levels.

The direct method exhibited relatively consistent LDL values, with a narrower interquartile range and a large standard deviation, indicating moderate variability. The Friedewald method had a broader range of LDL

values, suggesting potential inaccuracies, particularly at extreme LDL or triglyceride levels. The modified method showed similar variability to the direct method but with slight adjustments for higher triglyceride concentrations.

The findings support the use of all three methods interchangeably for LDL estimation in clinical settings, with the direct method potentially offering more reliable results, especially for individuals with elevated triglyceride levels. However, careful interpretation is essential when assessing LDL values, particularly in cases of high triglyceride concentrations, to ensure accurate risk stratification.

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