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Diagnostic Accuracy Study of Direct and Formula-Based Method for Estimation of LDL Cholesterol

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ABSTRACT

Introduction: Increased LDL-C is a well-known risk factor for cardiovascular disease. Also, LDL-C is used for risk stratification and in planning appropriate treatment. This requires the accurate measurement of LDL-C. Though β quantification is the reference method for LDL-C estimation it can't be used routinely. Even though homogenous assays are available, some clinical laboratories use the Friedewald formula, which has its disadvantages. Aim & Objectives: To identify which LDL-C formula best correlates with direct LDL-C among various levels of TG. Study Design: A retrospective diagnostic accuracy study. Materials and Methods: Complete lipid data of 15,094 was obtained from the hospital information system. Based on TG level data, it was grouped into TG <100 mg/dl (N=6022) and TG 100-200 mg/dl (9072). Direct homogenous assay was used to measure LDL-C. LDL-C was calculated using seven formulae (Friedewald, Hattori, de Cordova, Vujovic, Anandaraja, Chen, and Sampson). Results: Correlation between direct and formula-based LDL-C by ICC showed maximum correlation by Vujovic (0.978). Bland Altman plot between Vujovic and the direct method shows a bias of -0.38 and it may overestimate or underestimate by 23 mg/dl. Conclusion: LDL-C calculated using Vujovic showed maximum correlation with direct homogenous assay when TG <200 mg/dl.

Keywords: LDL Cholesterol, LDL Cholesterol Calculating Formula, Friedewald Formula, Hattori Formula, Anandaraja Formula, Chen Formula, de Cordova Formula, Vujovic Formula, Sampson Formula.

INTRODUCTION.

According to World Health Organization report in 2021, cardiovascular disease (CVD) accounted for 32% of global deaths in 2019, and raised low-density lipoprotein cholesterol (LDL-C) levels are a widely recognized predictor of risk for CVD(1,2). Additionally, a patient's risk categorization and initiation of appropriate treatment are both based on the serum LDL-C level(3). β Quantification is the gold standard method for estimating LDL-C and involves lipoprotein separation by preparative ultracentrifugation. However, Beta quantitation is not compatible with day-to-day use because of its high cost, arduous nature, need for ultracentrifugation, need for large sample volume, and need for expensive equipment. In 1998, LDL-C estimation using Homogenous direct assays was developed by manufacturers and approved by a network of laboratories under the Cholesterol Reference Method Laboratory Network (CRMLN)(4). However, the expense of these tests prevents most Indian laboratories from using them and makes them use formula-based LDL-C(5). Friedewald formula is the routinely used formula in clinical laboratories.

Adult Treatment Panel III (ATP III) guidelines of the National Cholesterol Education Programme (NCEP) also advise using the Friedewald formula to determine LDL-C(3).

Friedewald's formula uses Total cholesterol (TC), Triglyceride (TG), and High-density lipoprotein cholesterol (HDL-C) values to estimate LDL-C

$$LDL\text{-}C = TC - HDL\text{-}C - (TG / 5) \text{ in mg/dL}(6)$$

TG/5 denotes VLDL-C

A fasting sample is required as this equation assumes the ratio of TG to VLDL-C is constant of 5:1. As non-fasting samples contain chylomicrons (Carries TG to adipose tissue, skeletal muscle, and other peripheral tissues) and chylomicron remnants the ratio will be altered. Because of this, if data is obtained from a non-fasting sample and the Friedewald formula is used to estimate LDL-C, it overestimates VLDL-C and leads to LDL-C underestimation(7). This ratio is also altered in conditions such as Type II diabetes mellitus, nephrotic syndrome, liver disease, and chronic alcoholism, and not recommended to use this formula(8–10). In non-fasting individuals, when the TG is greater than 400 mg/dL or less than 100 mg/dL(11), or when the patient has type III or type I hyperlipoproteinemia, Friedewald's yields inaccurate results(12). To account for these issues, several LDL-C calculating formulae have been established(13–18). This study aims to identify which formula best correlates with direct LDL among various levels of TG.

MATERIALS AND METHODS

A diagnostic accuracy study taken on after obtaining an exemption from review from the Institutional Ethics Committee (IEC), JIPMER

Lipid profile data (TC, TG, HDL-C, LDL-C) and patient information such as age and gender were obtained from the Hospital Information System (HIS) portal retrospectively for a period from November 2021 to April 2023 from the clinical biochemistry laboratory, JIPMER.

TC and TG were estimated by the cholesterol oxidase peroxidase method(19) and glycerol kinase method(20,21) respectively. LDL-C(22) and HDL-C(23) were estimated using the homogenous enzymatic method in the Beckman Coulter autoanalyzer AU5800

Non-HDL-C = TC – HDL-C

Data obtained were entered into an Excel sheet. LDL-C concentrations are calculated using HDL-C, TC, TG, and Non-HDL-C concentrations with seven formulae mentioned in

Table 1: Comparison of LDL-C formulae

Author	Formula
Friedewald(6)	LDL-C = TC – (HDL-C) - (0.2×TG)
Hattori(13)	LDL-C = 0.94(TC) - 0.94(HDL-C) - 0.19(TG)
de Cordova(14)	LDL-C = TC - (HDL-C) × 0.7516
Vujovic(15)	LDL-C = TC - (HDL-C) - (TG/6.85)
Anandaraja(16)	LDL-C = (0.9 × TC) - (0.9 × TG/5) – 28
Chen(17)	LDL-C = [TC - (HDL-C)] × 0.9 - (TG × 0.1)
Sampson(18)	LDL-C = TC/0.948 - HDL-C/0.971 – [TG/8.56 + (TG × Non-HDL-C)/2140 - TG^2 /16100] - 9.44

Note: TC-total cholesterol, LDL-C-low density lipoprotein-cholesterol, HDL-C-high density lipoprotein-cholesterol, TG-triglyceride SPSS 20.0 was used to perform statistical analysis Since the data is non-normally distributed by the Kolmogorov-Smirnov test, data was presented as median with range. Data were grouped based on two variables namely age and TG levels into three and five groups respectively. The categorical variable (Age and Gender) was expressed in frequency and percentage. Intraclass correlation was used to see the correlation between direct and formula-based LDL-C methods. Bland Altman plot was plotted to assess the degree of agreement between the LDL-C estimation formulas and the direct LDL-C method was employed. The effect size was calculated by Cohen's d P value <0.05 were considered statistically significant

RESULTS

In this study, we analyzed the lipid profile data of 15,094 samples. Out of 15,094 samples, 7405 (49.0%) were females, and 7689 (50.9%) were males. The median age was 46 (32-58) years. Median TC, TG, HDL-C and LDL-C were 174(142-206) mg/dl, 111(84-144) mg/dl, 43(36-51) mg/dl and 112(88-138) mg/dl respectively shown in

Table 2: Demographic details

The total number of samples analyzed	15,094
Number of males	7689 (50.9%)
Number of females	7405 (49.0%)
Median age in years	46 (32-58)
Total cholesterol in mg/dl	174(142-206)
Triglycerides in mg/dl	111(84-144)
HDL-C in mg/dl	43(36-51)
LDL-C in mg/dl	112(88-138)

Based on the TG level data set was divided into 2 groups i.e., TG - <100 mg/dl, TG - 100-200 mg/dl. There were 6022, 9072 samples in each TG group respectively

Table 3: Comparison of direct LDL-C and formula-based LDL-C

Method	**Median with IQR**	**p-value**	**Effect size (Cohen's d)**
Direct	112(88-138)		
Friedewald	106.6(79.8-134.4)	<0.01	0.1
Hattori	100(74.8-126.1)	<0.01	0.3
de Cordova	96.9(75.9-119.5)	<0.01	0.4
Vujovic	112.6(85.6-141)	0.367	-0.009
Anandaraja	108(80.1-136.1)	<0.01	0.1
Chen	105.1(80.4-130.7)	<0.01	0.2
Sampson	108.6(81.5-136.9)	<0.01	0.1

Table 4: Comparison of direct LDL-C and formula-based LDL-C with TG <100 mg/dl

Method	**Median with IQR**	**p-value**	**Effect size (Cohen's d)**
Direct	101(79-124)		

Friedewald	98.4(74.4-124.4)	<0.01	0.04
Hattori	92.3(69.7-116.7)	<0.01	0.2
de Cordova	85.6(66.8-105.2)	<0.01	0.5
Vujovic	102.7(78.4-128.7)	<0.01	-0.1
Anandaraja	101.7(75.8-128)	0.852	-0.009
Chen	94.8(72.9-118.3)	<0.01	0.2
Sampson	99.2(74.3-125.62)	<0.01	0.02

Table 5: Comparison of direct LDL-C and formula-based LDL-C with TG 100-200 mg/dl

Method	Median with IQR	p-value	Effect size (Cohen's d)
Direct	121(97-146)		
Friedewald	112(84.4-140.6)	<0.01	0.2
Hattori	105(79-131.8)	<0.01	0.4
de Cordova	105.2(84.1-127)	<0.01	0.4
Vujovic	119.4(91.9-148.1)	<0.01	0.02
Anandaraja	112.4(83.7-141.5)	<0.01	0.2
Chen	112(87.1-137.6)	<0.01	0.2
Sampson	115(87.6-143.4)	<0.01	0.1

Correlation analysis between direct and formula-based LDL-C were performed by intraclass correlation. All formula-based LDL-C showed a better correlation with direct LDL-C (>0.7). Overall, a strong correlation was found between Vujovic LDL-C and Direct LDL-C (0.978) shown in Table 6. In TG - < 100 mg/dl and TG - 100-200 mg/dl groups strong correlation was shown by Vujovic formula 0.978 and 0.977 respectively shown in Table 7 and 8.

Table 6: Correlation analysis between direct LDL-C and formula-based LDL-C

Methods	Intraclass correlation coefficient	95%CI	p-value
Friedewald	0.971	0.949-0.981	<0.01
Hattori	0.953	0.619-0.983	<0.01
de Cordova	0.934	0.142-0.981	<0.01
Vujovic	0.978	0.978-0.979	<0.01
Anandaraja	0.961	0.947-0.970	<0.01
Chen	0.973	0.906-0.987	<0.01
Sampson	0.975	0.968-0.980	<0.01

Table 7: Correlation analysis between direct LDL-C and formula-based LDL-C with TG <100 mg/dl

Method	Intraclass correlation coefficient	95%CI	p-value
Friedewald	0.978	0.976-0.979	<0.01
Hattori	0.969	0881-0.986	<0.01
de Cordova	0.925	0.081-0.978	<0.01
Vujovic	0.978	0.974-0.981	<0.01
Anandaraja	0.967	0.965-0.969	<0.01

Chen	0.976	0.943-0.987	<0.01
Sampson	0.977	0.976-0.979	<0.01

Table 8: Correlation analysis between direct LDL-C and formula-based LDL-C with TG 100-200 mg/dl

Method	Intraclass correlation coefficient	95%CI	p-value
Friedewald	0.965	0.898-0.982	<0.01
Hattori	0.941	0.282-0.982	<0.01
de Cordova	0.930	0.141-0.979	<0.01
Vujovic	0.977	0.976-0.978	<0.01
Anandaraja	0.955	0.897-0.975	<0.01
Chen	0.969	0.857-0.987	<0.01
Sampson	0.972	0.952-0.982	<0.01

To show the degree of agreement between formula-based LDL-C and direct LDL-C Bland Altman plot was plotted. Bland Altman is a scatter plot that plots the difference between two measurements against the mean of the same.

Figure 9: Bland Altman plot – Direct LDL-C vs Friedewald LDL-C

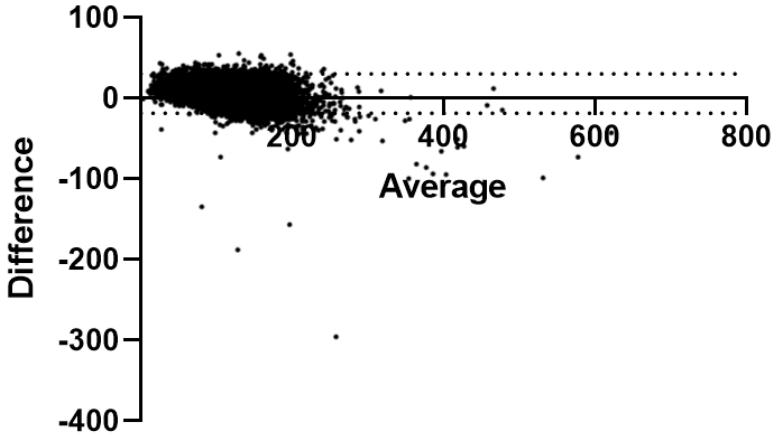


Figure 10: Bland Altman plot – Direct LDL-C vs Hattori LDL-C

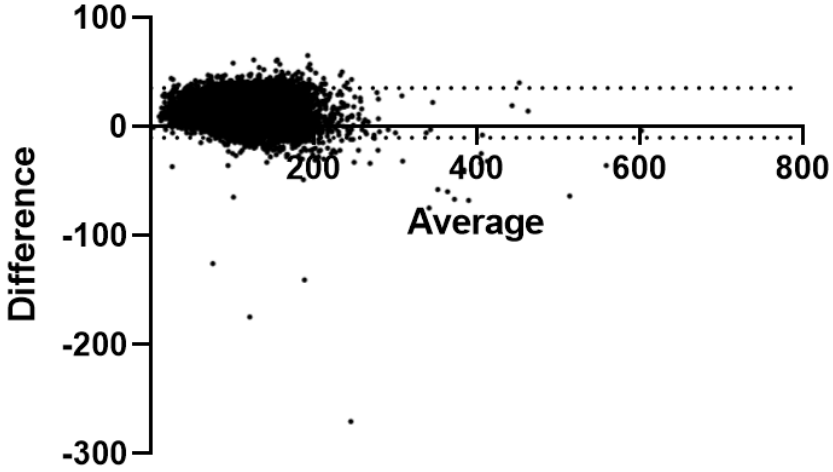


Figure 11: Bland Altman plot – Direct LDL-C vs de Cordova LDL-C

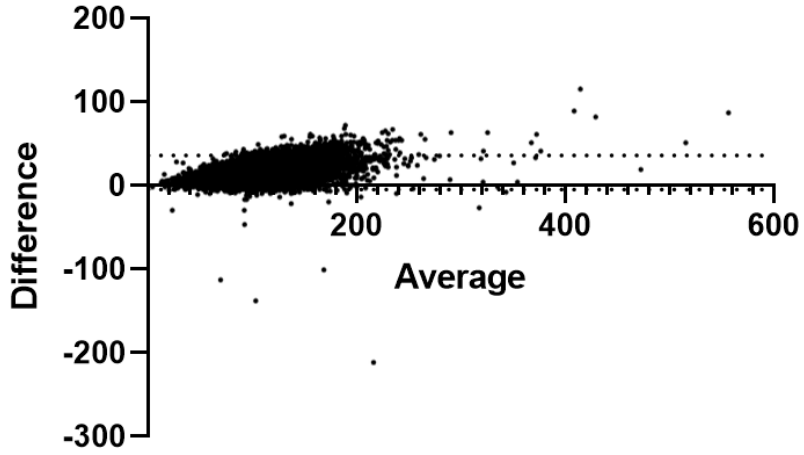


Figure 12: Bland Altman plot – Direct LDL-C vs VujovicLDL-C

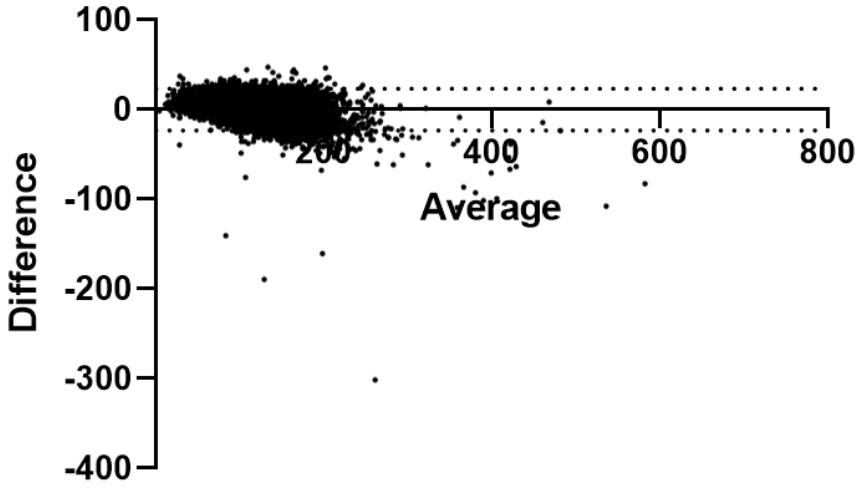


Figure 13: Bland Altman plot – Direct LDL-C vs Anandaraja LDL-C

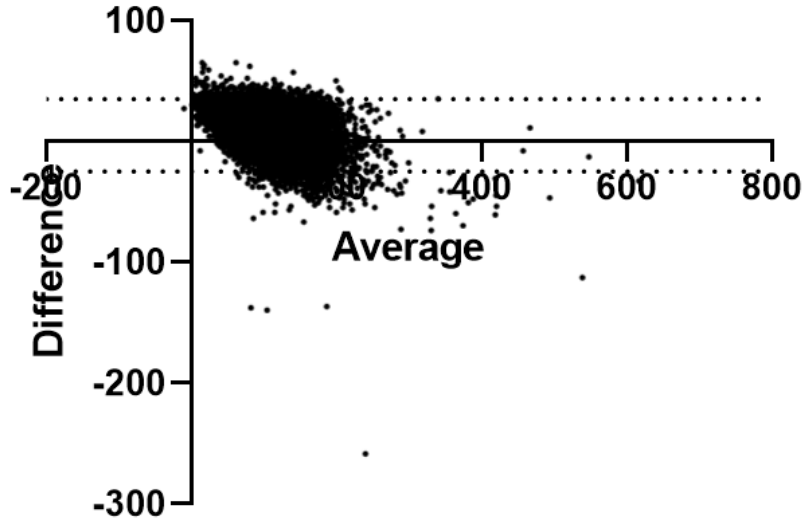


Table 14: Bland Altman plot – Direct LDL-C vs Chen LDL-C

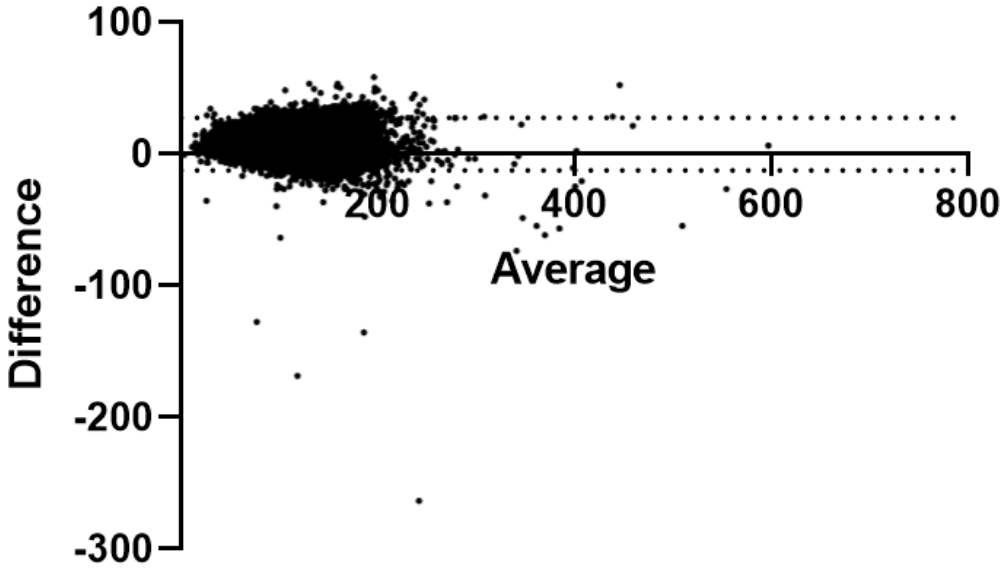
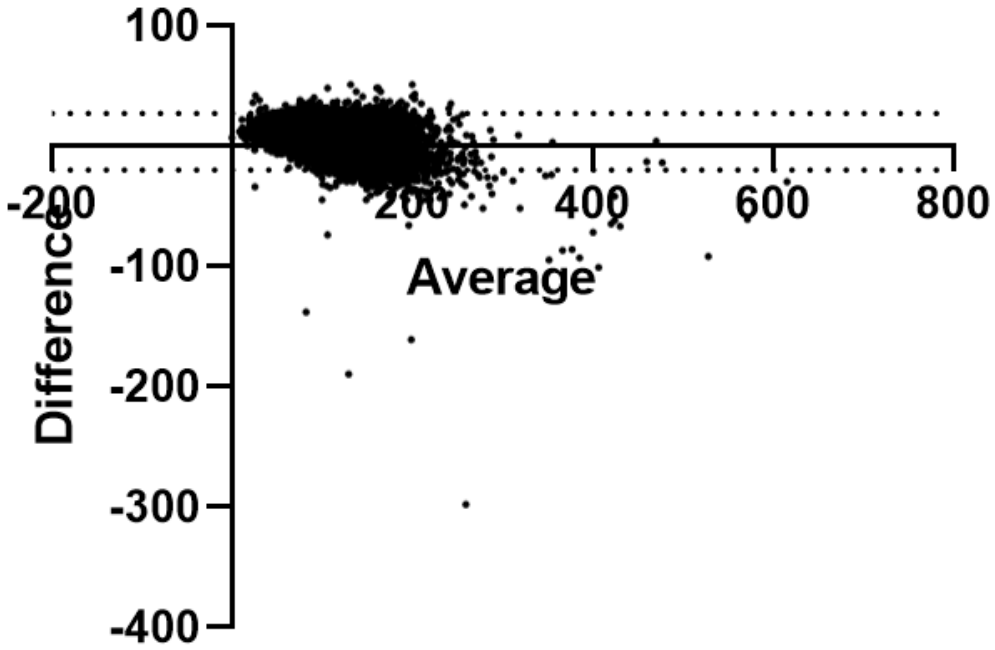


Figure 15: Bland Altman plot – Direct LDL-C vs Sampson LDL-C



By Bland Altman plot among seven formulae, the Vujovic formula shows -0.3853 bias, which is close to zero whereas other formulae show bias ≥ 3.8. Also, we can tell that LDL-C measured by the direct method may be 24 mg/dl below or 23 mg/dl above the LDL-C calculated by the Vujovic formula.

DISCUSSION

The estimation of LDL-C must be accurate and precise. This is because the level of serum LDL-C is a well-established atherogenic risk factor(2). Additionally, it serves as the foundation for risk assessment and categorizing those at risk for CVD and determines the treatment strategies(3).

Friedewald formula uses a constant TG to VLDL-C ratio of 5(6). Furthermore, the volatility in the TG: VLDL-C ratio will not be taken into account by any fixed factor. The majority of other equations, including the Chen formula, which calculates LDL-C as 90% non-HDL-C + 10% triglycerides(17), Vujovic formula, which uses a fixed factor of 6.85 instead of 5(15) share this problem. Thus, the fixed TG: VLDL-C ratio is the source of error majority of the equation. To over overcome this, equations with adjustable factors were developed by Rao et al and Martin et al (180-cell method). Rao employed an adjustable factor that takes only concentrations of triglycerides(24). In contrast, the 180-c method alterable the factor for the TG to VLDL-C ratio based on non–HDL-C and TG values(25).

In this study, we compared seven formulae that use fixed factor with direct LDL-C estimated by homogenous assay. However, a good correlation was observed between direct and formula-based LDL-C. All formula-based methods showed ICC >0.9 in overall both TG groups. In the group with TG >400 mg/dL. It is observed that the Vujovic formula shows a better correlation with the direct assay. A study done by Rim et al. showed that the 180-cell method (which was not included in our study) is the more accurate method followed by the Chen formula not in concordance with our finding(26). Oliveira et al compared Friedewald, Chen, Anandaraja, and Vujovic formula with β quantification and concluded that the Friedewald formula showed the best accuracy which is contrary to our findings(27). This might be because direct LDL-C is measured by β quantification which is the reference method for LDL-C estimation and also used fasting sample for LDL-C estimation.

Although LDL-C estimated by direct assay was used as a reference value in formula-based LDL-C comparisons, the application of a variety of equipment and reagents for measurements will cause variation in findings.

Limitations

In this study, LDL-C estimation was performed using direct homogenous methods which is not the reference method for LDL-C estimation

A flaw in the current study was since this is a retrospective study, we were not able to get the data on the study subject's co-morbidities and fasting state.

In this study, we didn't compare the formulae which use adjustable factors.

Conclusion

From this study, we conclude that the Vujovic formula is the best formula for LDL-C level estimation. This formula can be used as an alternative for direct LDL-C estimation and also can be used to calculate LDL-C in non-fasting samples.

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