



# Which LDL Value Should Clinicians Look at?

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## Abstract

**Objectives** LDL cholesterol is routinely estimated by the Friedewald formula to guide the treatment of dyslipidemia. However, Friedewald equation has certain limitations, especially with high triglyceride levels. Direct methods are available for LDL estimation but have received relatively little scrutiny in the Indian setting. This study was aimed at comparing the calculative and direct methods of LDL estimation in Indian hyperlipidemic patients.

**Materials and Methods** In this observational study, data from 380 consecutive lipid profiles of patients visiting a tertiary care hospital in Mumbai were analyzed retrospectively. CHOD PAP method was used to estimate total cholesterol. Enzymatic colorimetric method was used to estimate triglycerides. Enzyme selective protection method was used to estimate HDL. Direct LDL was estimated by homogenous enzymatic colorimetric assay and very low-density lipoprotein was calculated, whereas Friedewald's formula was used to derive calculated LDL.

**Results** Total cholesterol values correlated positively with the LDL values measured by both methods. However, a statistically significant difference was noted between the correlation coefficients of both the methods. Triglyceride values correlated weakly with the LDL levels measured by both the methods. A weak negative correlation was observed with LDL by the calculated method, whereas a weak positive correlation existed between TG and LDL by the direct method. The difference between the correlation coefficients was statistically significant.

**Conclusion** Both direct and calculated methods of LDL estimation have their limitations. A robust study with a larger sample size is needed to further investigate whether the differences in the different LDL estimation methods can translate to “clinical relevance” in the Indian setting.

## Keywords

- ▶ hyperlipidemia
- ▶ direct LDL estimation
- ▶ Friedewald's formula
- ▶ LDL cholesterol

## Introduction

Robust clinical evidence supports the fact that elevated level of low-density lipoprotein cholesterol (LDL-C) is an independent risk factor for coronary artery disease (CAD).<sup>1-3</sup> This has led to an understanding that lowering LDL-C is one of the key therapeutic targets in patients with CAD or those at a

risk of developing it. Dietary changes, lifestyle modification, and drug therapy to lower LDL-C can considerably reduce the morbidity and mortality associated with cardiovascular disorders, particularly CAD.<sup>4-6</sup> Given the crucial role played by LDL-C in etiopathogenesis and clinical management of CAD, laboratorial measurements of LDL-C have assumed paramount importance in its diagnosis and monitoring,

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particularly in patients presenting with hyperlipidemia or dyslipidemia.<sup>7</sup>

Different methods have been established for the measurement of LDL-C, each having their pros and cons. LDL-C measured by ultracentrifugation is recommended by Lipid Research Clinic.<sup>8</sup> Bioquantification low-density lipoprotein (BQ-LDL) has also been recommended as a standard technique for LDL-C estimation for measuring LDL-C. However, this method could not gain popularity at ground level due to several shortcomings. As a laboratory method, BQ-LDL is expensive, labor intensive, and is not freely available.<sup>9,10</sup> Therefore, most laboratories prefer to use the indirect method of LDL-C estimation, also called the Friedewald method.<sup>11,12</sup> Under this method, laboratory values for triglycerides (TGs) and total cholesterol (TC) are utilized to arrive at an indirect estimation of LDL-C. The TG and TC values are fed into the Friedewald formula (FF) to yield LDL-C values. This method is widely used for LDL-C estimation even today. However, several concerns have been expressed with the use of this method.<sup>11,12</sup>

To begin with, this method is based on the postulate that a constant nondynamic correlation exists between TG/TC and LDL-C. Hence, TG and TC values can be extrapolated for LDL-C calculations. However, evidence has shown that this may not hold true for all clinical situations and scenarios and might adversely impact LDL-C calculations.<sup>12-14</sup> Besides, combining TG, TC, and LDL-C values gives rise to significant analytical variability.<sup>12-14</sup> Clinically, the most noteworthy limitation of the indirect method is that FF cannot be applied to samples with TG levels above 400 mg/d. Also, FF cannot be used in patients with dysbetalipoproteinemia (type III hyperlipoproteinemia) and when chylomicrons are present.

Hence, if LDL-C is to be estimated by the indirect method, the clinician is left with no choice but to opt for a fasting sample. This limits the postprandial assessment and is also cumbersome for the patient.<sup>12-14</sup>

Given these limiting factors of the indirect method of LDL estimation, a need was felt to improvise the laboratory technique for LDL-C measurement. Hence, several commercially available assays have been developed for the direct measurement of LDL-C. Numerous such commercial assay kits are available and currently used. Direct estimation of LDL-C represents the third generation of laboratory techniques for LDL-C estimation.<sup>12</sup> However, discrepancies have been reported between LDL-C values calculated using the FF and those obtained by direct assays.<sup>15-18</sup> These discrepancies are of notable concern as some laboratories continue to use the FF method whereas others have shifted to the direct method. The discrepancy between LDL-C estimates obtained by the two methods is further augmented if the two methods are used interchangeably. This can trigger confusions and misinterpretations, particularly while stratifying patients into high- and low-risk groups during the process of therapy decision-making and therapeutic monitoring.<sup>19,20</sup>

There is very limited data comparing the direct method for LDL estimation with the FF method, particularly in Indian patients with hyperlipidemia. Hence, this study was conducted to compare the calculative (FF method) and direct

methods of LDL-C estimation at given TC and TG values in selected Indian population.

## Materials and Methods

This study uses observational data from 380 consecutive lipid profiles done at a laboratory in Mumbai, Maharashtra, which is certified by the International Organization for Standardization and accredited by the College of American Pathologists and the National Accreditation Board for Testing and Calibration Laboratories. The data was collected from October 2008 to January 2009. Institutional Ethics Committee's permission was obtained prior to the study.

Patients aged 18 to 65 years with hyperlipidemia attending the cardiology outpatient department at a tertiary care hospital in Mumbai were screened. During the screening, a general clinical examination was done and blood samples were collected for lipid profile after their informed consent was taken. The lipid profile included serum levels of LDL-C, high-density lipoprotein cholesterol (HDL-C), TC, TG, and very low-density lipoprotein cholesterol (VLDL-C). All investigations were done at an accredited laboratory. LDL-C was measured by the calculated method using FF and by the direct method.

Most parameters in lipid profile were estimated by photometric technology. CHOD PAP method (mode of reaction: end point; linearity: 600 mg/dL) was used to estimate TC.<sup>21</sup> Enzymatic colorimetric method GPO PAP was used to estimate TG.<sup>22</sup> Enzyme selective solubilization method (mode of reaction: end point; linearity: 150 mg/dL) was used to estimate HDL.<sup>23</sup> Homogenous enzymatic colorimetric assay with rapid reagent kit (mode of reaction: differential; linearity: 700 mg/dL) was used to estimate direct LDL.<sup>23</sup> Commercial kits from Agappe were used for testing TC, HDL-C, and direct LDL-C. Calibrators received with the testing kits were used for the assay. Stringent internal quality control checks were performed regularly.

VLDL was calculated as follows:  $VLDL = TG/5$ . LDL-C readings were derived by FF as follows:  $LDL-C = [TC] - [HDL-C] - [TG/5]$ .<sup>11</sup>

## Statistical Analysis

Descriptive statistics means, standard deviations (SDs), and covariance were calculated with Microsoft Excel. Data was reported as mean  $\pm$  SD. Linear regression and paired *t*-test were used. Mean values for LDL-C by the two methods were compared by paired students' *t*-tests. Linear relationships were determined from the standard Pearson correlation coefficients by linear regression analyses using SPSS (VER 10.0).

## Results

Mean age of patients was  $40.20 \pm 9.06$  years with a mean weight of  $62.76 \pm 11.63$  kgs and body mass index of  $25.12 \pm 3.24$ . The male to female ratio was 1:2.6.

For the purpose of data analysis, TG values of the study patients were stratified into three ranges: 1 to 100, 101 to 200, and 201 to 400 (mg/dL). Similarly, TC values were

also stratified into the following three ranges: 100 to 200, 201 to 250, and > 250 (mg/dL). The correlation of TC and TG values with LDL measured by both the methods was also analyzed without categorizing the TC and TG values into different ranges. In this case, the TC and TG values were considered as whole unstratified datasets. Correlation of TG levels with LDL values measured through the direct and calculated methods is mentioned in ►Table 1. Correlation of TC levels with LDL values measured through the direct and calculated methods is mentioned in ►Table 2. Correlation between TC and LDL values when LDL is measured by the direct as well as the calculated method is mentioned in ►Table 3. Correlation between TG and LDL values when LDL is measured by the direct as well as the calculated method is mentioned in ►Table 4. The mean LDL values obtained through both the methods are mentioned in ►Table 5.

The study data presented here explores how the dynamics of the clinical correlation between TG/TC and LDL is impacted with a change in the method of measurement of LDL (calculated or direct).

In the TG ranges of 1 to 100 and 101 to 200 mg/dL, a statistically significant difference was noted in the correlation of TG values with LDL values depending upon the method of LDL measurement. This difference was not seen in the TG value range above 201 mg/dL. Similarly, in the TC range of 100 to 200 mg/dL, a statistically significant difference was not noted in the correlation of TC with LDL-C and low-density lipoprotein-direct (LDL-D) values. However, TC values above 200 mg/dL show a statistically significant difference in their correlation with LDL-C and LDL-D. A statistically significant difference was also noted between the overall mean LDL values obtained through the direct and the calculated methods.

The discrepancy in LDL-C measurements between the

**Table 1** Correlation of TG levels with LDL values measured through the direct and calculated methods

TG range (mg/dL)	n	Mean ± SD LDL-C (mg/dL)	Mean ± SD LDL-D (mg/dL)	p-Value (95% CI)
1–100	123	143.90 ± 20.27	137.71 ± 19.16	0.0146 (1.22–11.13) <sup>a</sup>
101–200	195	148.77 ± 20.85	144.27 ± 17.26	0.0208 (0.68–8.31) <sup>a</sup>
201–400	62	142.47 ± 25.68	145.67 ± 19.80	0.3829 (10.42–0.43)

Abbreviations: CI, confidence interval; LDL-C, low-density lipoprotein calculated; LDL-D, low-density lipoprotein-direct; TG, triglyceride. Notes: Two-tailed *p*-values have been calculated. Both *p*-values marked with <sup>a</sup> are statistically significant as per conventional criteria.

**Table 2** Correlation of TC levels with LDL values measured through the direct and calculated methods

TC range (mg/dL)	n	Mean ± SD LDL-C (mg/dL)	Mean ± SD LDL-D (mg/dL)	p-Value (95% CI)
100–200	62	116.60 ± 12.61	118.52 ± 12.41	0.3933 (6.37–2.52)
201–250	270	147.45 ± 13.92	143.68 ± 12.57	0.0010 <sup>a</sup> (1.52–6.01)
> 250	42	177.15 ± 17.74	165.88 ± 18.60	0.0031 <sup>a</sup> (3.89–18.62)

Abbreviations: CI, confidence interval; LDL-C, low-density lipoprotein calculated; LDL-D, low-density lipoprotein-direct; TC, total cholesterol. Notes: Two-tailed *p*-values have been calculated. Both *p*-values marked with <sup>a</sup> are statistically significant as per conventional criteria.

**Table 3** Correlation between TC and LDL values when LDL is measured by the direct as well as calculated method

Type of LDL measurement	Correlation co-efficient (r)	p-Value
LDL-C	0.86074	0.0418
LDL-D	0.81708	

Abbreviations: LDL-C, low-density lipoprotein calculated; LDL-D, low-density lipoprotein-direct; r, co-efficient of correlation; TC, total cholesterol.

**Table 4** Correlation between TG and LDL values when LDL is measured by the direct as well as calculated method

Type of LDL measurement	Correlation co-efficient (r)	p-Value
LDL-C	0.0506 <sup>a</sup>	0.009424
LDL-D	0.13758 <sup>a</sup>	

Abbreviations: LDL-C, low-density lipoprotein-calculated; LDL-D, low-density lipoprotein-direct; r, co-efficient of correlation; TG, triglyceride. Note: Weak correlation marked with <sup>a</sup>.

**Table 5** The mean LDL values obtained through both methods

LDL type	n	Mean ± SD LDL-C (mg/dL)	p-Value (95% CI)
LDL-C	380	146.17 ± 21.64	0.0098 (0.92–6.66)
LDL-D	380	142.38 ± 18.56	

Abbreviations: LDL-C, low-density lipoprotein-calculated; LDL-D, low-density lipoprotein-direct; n, number of observations.

two methods was also statistically significant (*p* = 0.0098) when the entire study data was analyzed as a single unstratified dataset. TC values correlated positively with LDL values measured by both the methods. However, a statistically significant difference (*p* = 0.0418) was noted between the correlation coefficients of both the methods. TG values correlated weakly with LDL levels measured by both the methods. A weak negative correlation was observed with LDL-C, whereas a weak positive correlation existed between TG and

LDL-D values. The difference between the correlation coefficients was statistically significant.

## Discussion

In 2002, Nauck and colleagues published a review which analyzed various studies comparing the calculated and direct methods of LDL estimation. They concluded that the direct method of LDL estimation should be recommended to supplement the FF, particularly in cases where the calculation is known to be unreliable, for example, where TGs > 4,000 mg/L.<sup>24</sup> Miller et al have mentioned seven direct methods for measuring HDL and LDL cholesterol. However, comparative studies between them are not available.<sup>25</sup>

Recently, Warade and colleagues found that the calculated method of LDL estimation underestimates values at lower levels of LDL and higher levels of TG as compared with the direct method. Our study substantiates the same.<sup>26</sup> Sahu and colleagues have compared these two methods of LDL estimation earlier, which was published in 2005. They showed that a significant difference exists in the mean LDL-C levels obtained by the two methods at TG levels < 200 mg/dL ( $p < 0.02$ ) and TC levels > 150 mg%.<sup>27</sup> These findings are consistent with the results obtained in the present study. Kannan and colleagues compared the findings from the FF and direct methods from an Indian laboratory database. They suggested repeating the LDL by direct assay techniques, particularly in patients with TG > 200 mg/dL and when LDL < 70 or > 130 mg/dL.<sup>28</sup> However, it may not be a cost-effective option.<sup>29</sup>

Nevertheless, the present study has its own limitations. The study data tested the statistical significance of the difference between the direct and indirect methods of LDL-C estimation. However, the study did not investigate further whether this “statistical significance” translated into “clinical relevance.” This leaves us with a couple of unanswered questions: Is the statistically significant difference between the two methods of LDL-C estimation a clinically meaningful or relevant difference? Does a statistically significant difference between the two methods also imply that this difference could have a cognizable impact on therapy decision-making, monitoring, and prognostication? Perhaps the statistically significant differences between the two arms of a clinical study should be investigated further to understand their clinical impact, to make a clinical recommendation in favor of any one of the study arms. With the recent controversy surrounding lipid levels and its clinical significance, we need to be sure of using the right technique without spending excess money from the patients’ pocket.<sup>30</sup>

With respect to study design, the sample size of the study was limited to arrive at any robust conclusion as to which of the two methods is superior for LDL-C estimation. Besides, to ascertain which of these two methods is more robust, it is imperative to compare both with an accepted standard method. The current study involves a comparison between the two methods only and does not compare the two methods with a third standard reference method; thus, a comment cannot be made vis-à-vis the accuracy of the rate of detection, sensitivity, and specificity of the two methods being

compared. This study does not have LDL-C estimations made by the modified FF equation, Martin/Hopkins estimation, or Anandraja’s formula. All these new methods can be tested together to get robust results.<sup>31-33</sup> These limitations need to be taken into account while designing future clinical studies for such a comparison. Future clinical studies need to involve a larger sample size and be adequately powered to test the difference between multiple methods. A reference standard needs to be incorporated into the study design so that the different methods of LDL-C estimation can be compared against this standard technique. The study population should perhaps involve more heterogeneous subgroups of dyslipidemic patients, for example, those with mild, moderate, and severe hypertriglyceridemia and hypercholesterolemia. Perhaps a prospective study comparing all the methods mentioned so far, with a larger sample size and heterogeneous patient subgroups, may yield more robust information. Moreover, we need to find out which is the more cost-effective and accurate method for estimating LDL in the Indian setting.

## Permissions

All relevant permissions were taken. Institutional Ethics Committee’s approval was taken.

## Authors’ Contribution

H.M. searched literature and conceived the study. H.M. was involved in protocol development, gaining ethical approval, patient recruitment, and data analysis. H.M. wrote the first draft of the manuscript. A.A. gave inputs on results and expert opinion on discussion. A.A. provided valuable suggestions on answering queries from the journal and creating a revised version. Both H.M. and A.A. reviewed and edited the manuscript and approved the final version of the manuscript. The guarantor for this document is H.M.

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## Conflict of Interest

None declared.

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