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*Original Article*

# Evaluation of the Novel Method and the Regression Equation for Calculation of Low-Density Lipoprotein Cholesterol

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## *Abstract*

***Background**: Friedewald's formula (FF) is used worldwide to calculate low-density lipoprotein cholesterol (LDL-chol). But it has several shortcomings: overestimation at lower triglyceride (TG) concentrations and underestimation at higher concentrations. In FF, TG to very low-density lipoprotein cholesterol (VLDL-chol) ratio (TG/VLDL-chol) is considered as constant, but practically it is not a fixed value. Recently, by analyzing lipid profiles in a large population, continuously adjustable values of TG/VLDL-chol were used to derive a novel method (NM) for the calculation of LDL-chol. **Objective**: The aim of this study was to evaluate the performance of the novel method compared with direct measurement and regression equation (RE) developed for Bangladeshi population. **Materials and Methods**: In this cross-sectional comparative study we used lipid profiles of 955 adult Bangladeshi subjects. Total cholesterol (TC), TG, HDL-chol and LDL-chol were measured by direct methods using automation. LDL-chol was also calculated by NM and RE. LDL-chol calculated by NM and RE were compared with measured LDL-chol by two-tailed paired t test, Pearson's correlation test, bias against measured LDL-chol by Bland-Altman test, accuracy within ±5% and ±12% of measured LDL-chol and by inter-rater agreements with measured LDL-chol at different cut-off values. **Results**: The mean values of LDL-chol were 110.7 ± 32.0 mg/dL for direct measurement, 111.9 ± 34.8 mg/dL for NM and 113.2 ± 31.7 mg/dL for RE. Mean values of calculated LDL-chol by both NM and RE differed from that of measured LDL-chol ($p<0.01$ for NM and $p<0.0001$ for RE). The correlation coefficients of calculated LDL-chol values with measured LDL-chol were 0.944 ($p<0.0001$) for NM and 0.945 ($p<0.0001$) for RE. Bland-Altman plots showed good agreement between calculated and measured LDL-chol. Accuracy within ±5% of measured LDL-chol was 49% for NM, 46% for RE and within ±12% of measured LDL-chol was 79% for both NM and RE. Inter-rater agreements (κ) between calculated and measured LDL-chol at LDL-chol <100 mg/dL, 100–130 mg/dL and >130 mg/dL were 0.816 vs 0.815, 0.637 vs 0.649 and 0.791 vs 0.791 for NM and RE respectively. **Conclusion**: This study reveals that NM and RE developed for Bangladeshi population have similar performance and can be used for the calculation of LDL-chol.*

***Key words**: Friedewald's formula; Novel method; Regression equation (RE); Bangladeshi population; Low-density lipoprotein cholesterol (LDL-chol)*

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## Introduction

Measurement and evaluation of circulating low-density lipoprotein cholesterol (LDL-chol) is crucial for the prevention and management of cardiovascular diseases (CVDs) since it is the recommended primary basis for the correct classification in risk categories[1] and is one of the important independent risk factors for the development of coronary heart disease.[2] The reference method for the measurement of serum LDL-chol is the

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preparative ultracentrifugation, i.e., β-quantification.[3] Use of this reference method is limited for routine clinical practice due to the technical difficulties. To resolve these problems direct homogeneous methods have been developed and recommended for the measurement of LDL-chol as alternatives to the reference method.[4,5] The direct methods are costly and require expensive automation and are not affordable by most of the laboratories in the developing countries. As a result Friedewald's formula[6], the worldwide used formula, is generally used for the estimation of LDL-chol by most of the laboratories in Bangladesh. In 1972, Friedewald et al[6] published the landmark formula by analyzing data of 448 US subjects that allows rapid, inexpensive and suitable approach for the estimation of LDL-chol from three other lipid parameters: serum total cholesterol (TC), serum triglycerides (TG) and serum high-density lipoprotein cholesterol (HDL-chol), based on the observation that the ratio of the mass of TG to mass of cholesterol in very low-density lipoprotein cholesterol (VLDL) is apparently constant and it is about 5:1 (in conventional unit) in normal subjects and in all patients with all types of hyperlipoproteinemia, except the rare type III.[6-11] But there are several limitations in using this formula. There are underestimation in the measurement of LDL-chol at higher TG levels[12,13] and overestimation at low TG levels.[14] Recently by analyzing lipid profiles from 1.34 million consecutive adult subjects referred for direct measurement of cholesterol subfraction by the Vertical Auto Profile (VAP, density gradient ultracentrifugation or vertical spin density gradient ultracentrifugation) Martin et al[15,16] also reported a meaningful underestimation of LDL-chol in US adults. These are related to the use of a fixed value of TG to VLDL-cholesterol.

The underestimation of LDL-chol calculated by FF is also common in Bangladeshi population[17-23] and there is no evidence of systematic overestimation of LDL-chol by FF in this population.[17-23] Till now only one regression equation (RE) has been developed and validated externally in our population.[23] Recently, a novel method[24] has been developed using continuously adjustable ratio of TG/VLDL-cholesterol rather than a fixed factor as in Friedewald formula[6], DeLong modification[25] or other formulas[26-30] by analyzing lipid profiles of 1.3 million US adults. They have generated a two dimensional table of different median values of TG/VLDL-chol against different combination of TG range and non-HDL-chol range. The 180-cell table produces an overall improvement of LDL-chol calculation. So, it is urgently needed to evaluate and also to compare the RE for Bangladeshi population and recently developed NM against measured LDL-chol simultaneously. In this context, this cross-sectional comparative study was designed to evaluate the performance of the NM and RE against measured LDL-chol in this population.

## Materials and Methods

This cross-sectional comparative study was conducted in the Department of Biochemistry, Chevron Clinical Laboratory, Chittagong, Bangladesh during the period of July to December 2013. In this study, 1016 adult subjects, both male and female, from the outpatient department of Chevron Clinical Laboratory were included. Venous blood specimens were collected in tubes without anticoagulant for analysis of lipids from all the selected subjects after 12-hour fast. The specimens were allowed to clot at room temperature, and serum was obtained by centrifugation at 3000 rpm for 15 minutes. All blood lipid analyses were performed within 24 hours of specimen collection. Serum TG and TC were measured by enzymatic end-point method and HDL-chol and LDL-chol were measured by direct automated method using Olympus AU400 clinical chemistry analyzer (Japan). All kits, calibrators and quality control materials were purchased from Beckman, Ireland through local distributor. Lipid profiles with TG concentration above 400 mg/dL were excluded and 955 lipid profiles with TG ⩽400 mg/dL were included.

Statistical analyses were done by two-tailed paired *t* test, Pearson's correlation test, Bland-Altman plots for bias, accuracy within ±5% and ±12% of measured LDL-chol and inter-rater agreements (κ) at cut-off values of LDL-chol. The cut-off values were 100 mg/dL and 130 mg/dL of LDL-chol. For statistical analyses we used MedCalc® version 11.4 for Windows. A $p$ value $<0.05$ was considered as statistically significant.

## Results

A total of 1016 adult subjects were included in this study. Among them 61 (6%) subjects had serum TG above 400 mg/dL. The remaining 955 (94%) subjects

had serum TG levels up to 400 mg/dL and we considered them as study subjects. The mean age of the study subjects was 47 ± 12 years. Among them 566 (59%) were male and 389 (41%) were female. The means of TC, TG, HDL-chol and measured LDL-chol were 183 ± 42 mg/dL, 191 ± 80 mg/dL, 40.0 ± 7.3 mg/dL and 110.7 ± 32.0 mg/dL respectively. Thirty eight percent (38%) of the study subjects had LDL-chol up to 100 mg/dL, 39% had LDL-chol 101–130 mg/dL and 23% had LDL-chol >130 mg/dL.

The mean values of calculated LDL-chol were 111.9 ± 34.8 mg/dL for NM and 113.2 ± 31.7 mg/dL for RE. Compared with measured LDL-chol, NM LDL-chol was 1.2 ± 11.5 mg/dL higher ($p$<0.01) and RE LDL-chol was 2.5 ± 10.6 mg/dL higher (p<0.001). The correlation coefficients of calculated LDL-chol values with measured LDL-chol were 0.944 (p<0.0001) for NM and 0.945 (p<0.0001) for RE. Fig. 1 shows the Bland-Altman plots of LDL-chol calculated by NM and RE against measured LDL-chol. The bias was 1.1% for NM (Fig 1A) and 3.4% for RE (Fig 1B).

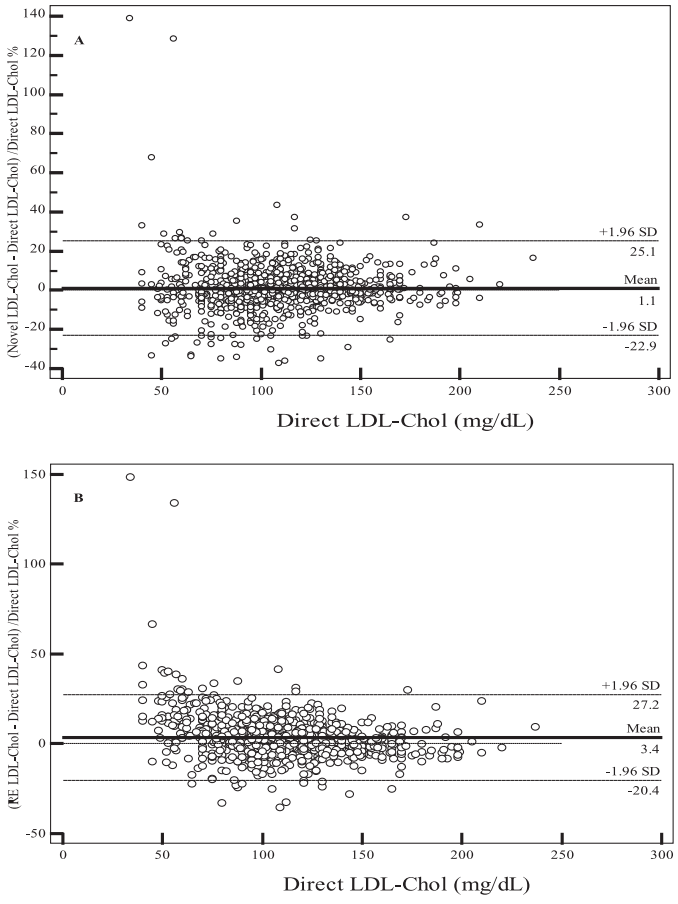


Fig 1. Bland-Altman plots of NM LDL-chol (A) and RE LDL-chol (B) against measured LDL-chol

Forty nine percent (49%) of NM LDL-chol and 46% of RE LDL-chol fall within ±5% of measured LDL-chol. Seventy nine percent (79%) of calculated LDL-chol values by NM and RE fall within ±12% of measured LDL-chol. Inter-rater agreements (κ) between calculated and measured LDL-chol at <100 mg/dL, at 100–130 mg/dL and at >130 mg/dL were 0.816 (95% CI: 0.778–0.854) *vs* 0.815(95% CI: 0.777–0.853), 0.637 (95% CI: 0.586–0.688) *vs* 0.649 (95% CI: 0.600–0.699) and 0.791 (95% CI: 0.746–0.835) *vs* 0.791 (95% CI: 0.746–0.835) for NM and RE respectively.

## Discussion

Friedewald's formula is frequently used in clinical practice and population-based epidemiological studies. Underestimation of LDL-chol by Friedewald's formula is common.[12,13,15,16] In our population, some comparative studies are available.[17-23] All studies reported remarkable underestimation of LDL-chol by Friedewald's formula. Recently, Martin et al[24] derived a novel method (NM) for the calculation of LDL-chol. In this study, we compared LDL-chol calculated by NM and LDL-chol calculated by a regression equation (RE) developed for Bangladeshi population with measured LDL-chol.

Differences of mean values of calculated LDL-chol using NM and RE with measured LDL-chol were statistically significant (1.2 mg/dL for NM and 2.5 mg/dL for RE developed for Bangladeshi population), but possibly insignificant clinically. On the other hand it was meaningful and large for Friedewald's formula in US population[15,16] and >11 mg/dL in Bangladeshi population.[17,20,24] Strong and similar correlation coefficients were observed for NM and RE (0.944 and 0.945). Bias of calculated LDL-chol was lower for NM compared with RE (1.1% vs 3.4%), but both are within good agreement whereas this is higher for Friedewald's formula.[23] Accuracy of NM and RE within 5% and 12% of measured LDL-chol was similar and improved compared to Friedewald's formula.[23] Furthermore, we observed good agreements (κ) between measured and calculated LDL-chol at LDL-chol values <100 mg/dL, 100–130 mg/dL and >130 mg/dL. Thus, underestimation of LDL-cholesterol by Friedewald's formula is significantly reduced

using these methods (novel method and regression equation).

From the findings of our study we can conclude that good agreements exist between direct measurement and novel method and also between direct measurement and regression equation developed for Bangladeshi population. Therefore, both these formulas can be used for the calculation of LDL-cholesterol.

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