Comparison of Regression Equation and Friedewald's Formula with Direct Measurement of Low-density Lipoprotein Cholesterol in Bangladeshi Population

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Abstract

Friedewald's formula (FF) is the most widely used formula in clinical practice to calculate low-density lipoprotein cholesterol (LDLC) from total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDLC). But this formula frequently underestimates LDLC. The aim of this study was to derive a regression equation (RE) to abolish the underestimation and to compare the performance of RE and FF in Bangladeshi population. RE was derived from 531 lipid profiles (equation derivation group) for the calculation of LDLC by multiple linear regression analysis. The RE was then used to calculate LDLC in another 952 subjects (equation validation group). LDLC calculated by RE and FF were compared with measured LDLC by appropriate statistical analyses. In equation validation group, measured LDLC, LDLC calculated by RE and FF were 2.97 ± 0.81, 2.91 ± 0.80 and 2.72 ± 0.93 mmol/L respectively. Precision (r) was 0.9525 for RE and 0.9193 for FF. Passing & Bablok linear regression equations against measured LDLC were $y = 0.9792x + 0.007$ for RE and $y = 1.1412x - 0.6781$ for FF. Accuracy within $\pm12\%$ of measured LDLC was 79% and 57% for RE and FF, respectively. The derived RE is more accurate than FF for the calculation of LDLC in Bangladeshi population.

Keywords: Lipoprotein cholesterol; Friedewald's formula; Bangladeshi population.

1. Introduction

Among the noncommunicable diseases cardiovascular diseases (CVD) remain the biggest cause of death worldwide. Over the last two decades cardiovascular mortality rates have

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declined in many high-income countries. At the same time cardiovascular disease and deaths have increased at an astonishingly fast rate in low- and middle-income countries [1]. Elevated serum low-density lipoprotein cholesterol (LDLC) is one of the important independent risk factors for the development of coronary heart disease [2]. It is the recommended primary basis for the correct classification in risk categories and management of CVDs [3]. The reference method for the measurement of serum LDLC is the β-quantification [4]. This reference method is not suitable for routine clinical practice due to technical difficulties. The new generation direct homogeneous methods have been developed and recommended for the measurement of LDLC as alternatives to the reference method [5,6]. 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 [7], the worldwide used formula is generally used for the estimation of LDLC by most of the laboratories in Bangladesh. In 1972 Friedewald *et. al.* [7] published the landmark formula (Eq. 1) that allows rapid, inexpensive and suitable approach for the estimation of LDLC from three other lipid parameters, *i.e.*, serum total cholesterol (TC), serum triglycerides (TG) and serum high-density lipoprotein cholesterol (HDLC) by analyzing data of 448 US subjects [7] based on the observation that the ratio of the mass of TG to mass of cholesterol in very low-density lipoprotein cholesterol (VLDLC) is apparently relatively constant and it is about 2.2:1 (in SI unit) in normal subjects and in all patients with all types of hyperlipoproteinemia, except the rare type III [8–13]. One of the most important limitations of the Friedewald's formula (FF) is that it cannot be used when fasting serum TG concentration is above 4.52 mmol/L (400 mg/dL) [7] due to underestimation and lack of concordance with measured LDLC. But in practice, the underestimation of LDLC starts when TG concentration is above 3.39 mmol/L (300 mg/dL) [14,15]. Recently Eljamil *et. al.* [16] reported that the underestimation begins when level of serum TG is above 1.13 mmol/L (100 mg/dL) while vander Heul-Nieuwenhuijsen *et. al.* [17] described that FF underestimates LDLC at TG levels above 2.0 mmol/L. Martin *et. al*. [18,19] also reported a meaningful underestimation of LDLC in US adults 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). To reduce the underestimation, in 1986, DeLong *et. al.* [20] have modified the FF (Eq. 1) by analyzing data of 10,000 subjects using multivariate regression model, though it is rarely used in clinical practice. TG to TC ratios in different lipoproteins were also used for the derivation of LDLC calculation formula [21]. Most of the other equations have been developed by using multivariate linear regression analysis considering LDLC as a dependent variable and TC, TG and HDLC as independent variables [21–25]. Of these only Anandaraja's formula [23] has been developed without the use of HDLC. However, several studies validated and recommended the use of FF in different populations [26,27], and in some studies overestimation was found at lower TG levels [28] and also in a particular population [29].

In Bangladeshi population, underestimation of LDLC by FF has been reported [30–32] and there is no evidence of systematic over estimation of LDLC by FF in this population [30–32]. No study has yet been carried out for the modification or derivation of equation to calculate LDLC in Bangladeshi population. The aim of this study was to derive a regression equation (RE) to estimate LDLC from TC, TG and HDLC more accurately than FF by multivariate linear regression analysis using data of lipid profiles in one tertiary healthcare center and to validate the equation using lipid profiles in another tertiary healthcare center of Bangladesh.

2. Experimental

2.1. *Study subjects and specimen collection*

The initial study population (equation derivation group) consisted of 531 adult subjects of both sexes who underwent lipid profile estimation in a tertiary healthcare center, Dhaka, Bangladesh during the period of January 2009 to December 2009. The second study population (equation validation group) consisted of 952 adult subjects who attended another tertiary healthcare centre, Chittagong, Bangladesh during the period of January 2011 to July 2011. Verbal consent was taken from each subject before data and specimen collection. Four milliliters of blood specimens were collected in fasting state in plain blood collection tube without anticoagulant from each subject with all aseptic precautions.

2.2. *Biochemical analysis*

After centrifugation serum was separated in microtubes for lipid profile analysis.

2.2.1. *Equation derivation group*

All biochemical analyses were done on the day of specimen collection with Dimension® *RxL* Max automation (Siemens Healthcare Diagnostics Ltd. UK) using reagents manufactured by Siemens Healthcare Diagnostics Ltd. UK. Serum TG and TC levels were measured by enzymatic endpoint technique and HDLC, LDLC concentrations were measured by direct automated method using AHDLC Flex® and ALDLC Flex® reagents. The homogeneous methods for HDLC and LDLC measurements have been certified by the Cholesterol Reference Method Laboratory Network (CRMLN) at Northwest Lipid Research Laboratories, University of Washington, Seattle, Washington, 98103. Serum LDLC was also calculated by using FF and by the derived RE. All test kits, calibrators, quality control materials were from Siemens Healthcare Diagnostics Ltd., Sir William Siemens Sq. and Frimley, Camberly, UK GU16 8QD.

2.2.2. *Equation validation group*

All specimens in the equation validation group (external) were collected and analyzed in the Department of Biochemistry, Chevron Clinical Laboratory, Chittagong, Bangladesh during the period of January to July, 2011. Serum TG and TC were measured by enzymatic end point method and HDLC and LDLC 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.

2.3. *Equations*

Friedewald's formula (Eq. 1) and derived regression equations (Eqs. 2 and 3) are listed below:

$$LDLC = TC - \frac{TG}{2.2} - HDLC \tag{1}$$

$$LDLC = 0.83 \times TC - 0.23 \times TG - 0.62 \times HDLC + 0.15 \tag{2}$$

$$LDLC = 0.83 \times TC - 0.10 \times TG - 0.62 \times HDLC + 5.6 \tag{3}$$

2.4. *Statistical analysis*

Two-tailed paired *t* test, Bland-Altman plots for bias [33-35], Pearson's correlation coefficient for precision, Passing & Bablok regression equation [36] of calculated LDLC against measured LDLC, concordance correlation coefficient (P_c), accuracy within $\pm$ 12% of measured LDL cholesterol were done by MedCalc® version 11.4 for Windows and multivariate linear regression analysis was done by Statsoft *STATISTICA* version 8.0 for Windows. A *p* value < 0.05 was considered as statistically significant.

3. Results and Discussions

3.1. *Characteristics of equation derivation and equation validation groups*

Characteristics and lipid parameters of the equation derivation group and equation validation group are shown in Table 1. In equation derivation group, the absolute difference between measured LDLC and LDLC calculated by FF was 0.20 ± 0.50 mmol/L and it was significantly different ($t = 9.067$, $p<0.0001$, Fig. 1A) and in the equation validation group, the absolute difference between measured LDLC and LDLC calculated by FF was 0.30 ± 0.37 mmol/L and it was also significant ($t = 24.67$, $p<0.0001$, Fig. 1B). This observation is consistent with the findings of previous studies as reported elsewhere [14–19, 30-32].

3.2. *Equation derivation*

In the equation derivation group, Passing & Bablok linear regression analyses of measured LDLC (x) versus Friedewald's LDLC (y) yielded the regression equation $y = 1.1181x – 0.6039$ (Fig. 2A) and mountain plot of the absolute differences between measured LDLC and Friedewald's LDLC showed that the whole distribution was shifted to positive (underestimation) direction (Fig. 2B). This result led to the derivation of a regression equation to calculate LDLC from TC, TG and HDLC by multiple linear regression analysis.

Table 1. Characteristics and lipid parameters in equation derivation and equation validation groups.

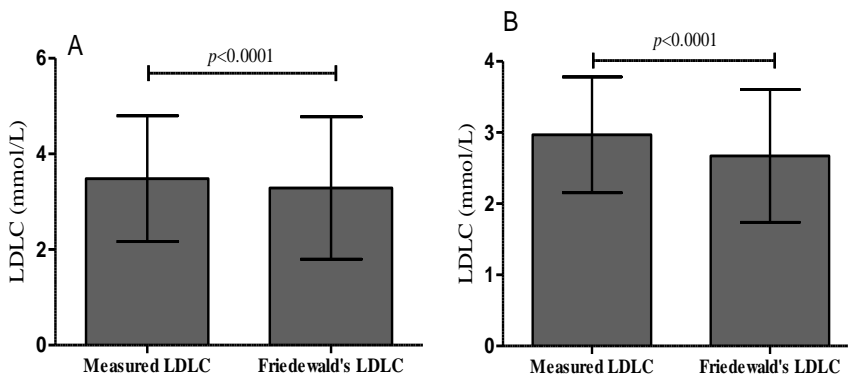|	Equation derivation group ($n = 531$)	Equation validation group ($n = 952$)
Age (yrs)	50.2±11	46.9±13
Sex (Male/Female, %)	60/40	58/42
TC (mmol/L)	5.46±1.60	4.71±1.14
TG (mmol/L)	2.68±1.00	2.16±0.94
HDLC (mmol/L)	0.94±0.22	1.05±0.18
Measured LDLC (mmol/L)	3.48±1.32	2.97±0.81
Friedewald's LDLC (mmol/L)	3.29±1.49	2.72±0.93

Fig. 1. Comparison of LDLC calculated by Friedewald's formula and measured LDLC in equation derivation (A) and equation validation group (B).
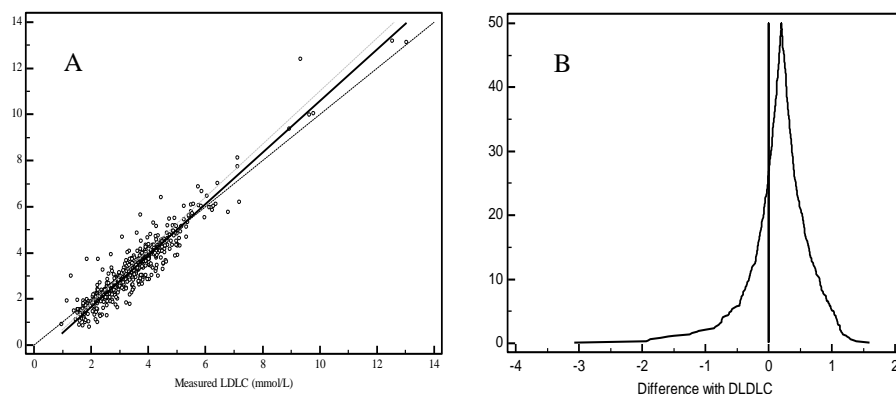
Fig. 2. Passing and Bablok linear regression (A) of LDLC calculated by Friedewald's formula against measured LDLC and mountain plot (B) of LDLC by Friedewald's formula against measured LDLC (measured LDLC − calculated LDLC) in the equation derivation group.

In the equation derivation group, considering measured LDLC as a dependent variable and TC, TG and HDLC as independent variables, multiple linear regression analysis showed that the ß values of LDLC were 1.0094 ($p<0.0001$) for TC, − 0.1717 ($p<0.0001$) for TG and − 0.1037 ($p<0.0001$) for HDLC and the coefficients of TC, TG and HDLC were 0.83, − 0.23 and − 0.62, respectively with 0.15 as constant in SI unit and thus yielded Eq. 2. Similarly, when all concentrations were expressed in conventional unit (mg/dL) multivariate linear regression analyses yielded Eq. 3.

3.3. Equation validation

In the equation validation group, LDLC calculated by derived RE and FF were compared with the measured LDLC. The mean values of measured LDLC, LDLC calculated by derived RE and by FF were 2.97 ± 0.81, 2.91 ± 0.80 and 2.72 ± 0.93 mmol/L respectively. The mean differences between measured LDLC and calculated LDLC were − 0.06 ± 0.27 mmol/L ($p<0.0001$) and − 0.30 ± 0.37 mmol/L ($p<0.0001$) for derived RE and FF respectively (Fig. 3). Both RE and FF underestimated LDLC compared to measured LDLC, but absolute bias was lower for derived equation than Friedewald's formula (−0.06 ± 0.27 mmol/L *vs* − 0.30 ± 0.37 mmol/L). The correlation coefficients of calculated LDLC with measured LDLC were 0.9525 ($p<0.0001$) for derived RE and 0.9193 ($p<0.0001$) for FF (Fig. 4). The correlation coefficient was stronger for RE than FF (0.9525 *vs* 0.9193). Comparison of measured LDLC (x) versus LDLC by derived FF (y) and measured LDLC (x) versus LDLC by the RE (y) yielded the following regression equations: $y = 1.1412 x − 0.6781$ (Fig. 4A) and $y = 0.9792 x + 0.007$ (Fig. 4B), respectively. These Passing & Bablok linear regression equations (Fig. 4) showed that deviation from the $y = x$ line was lower for derived RE than FF. Mountain plots of the absolute differences between measured LDLC and calculated LDLC (measured LDLC − calculated LDLC) showed that

calculated LDLC values were shifted from the positive (underestimation) direction to the zero point and symmetrical around the zero point for the derived RE (Fig. 5). But LDLC calculated by the FF shifted to the positive (underestimation) direction (Fig. 5).
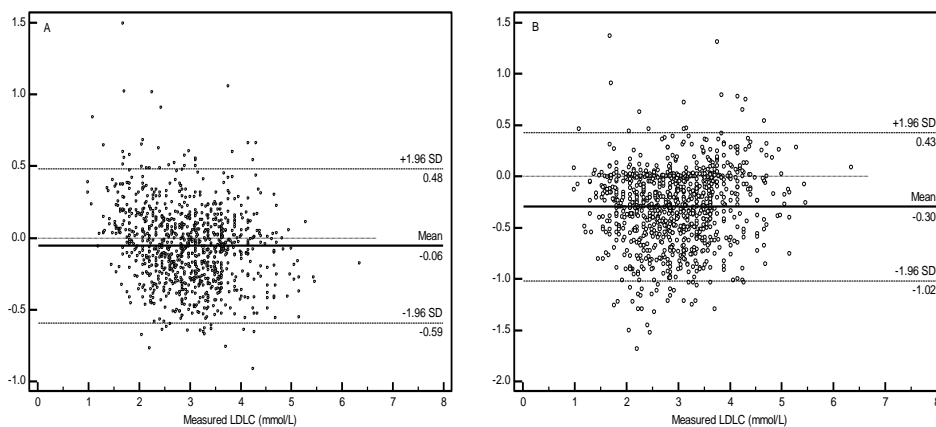

Fig. 3. Bland-Altman plot for bias of calculated LDLC against measured LDLC, (A) for derived equation and (B) for Friedewald's formula.
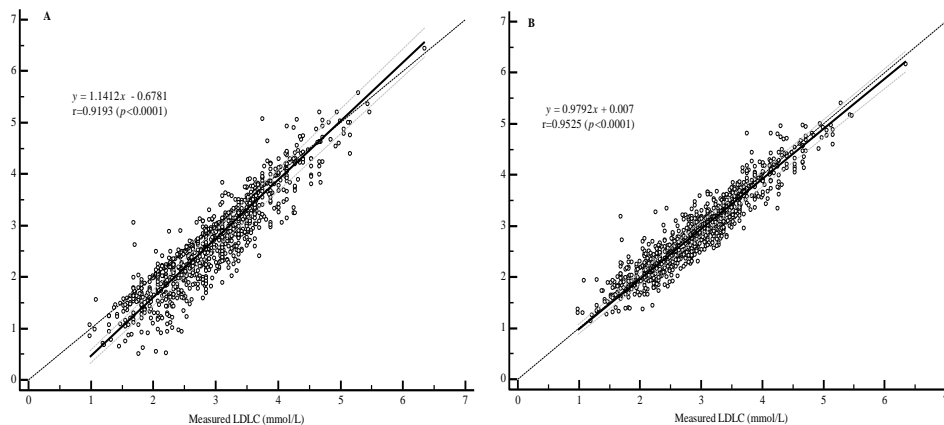

Fig. 4. Passing & Bablok linear regression of calculated LDLC and measured LDLC. Solid line indicates the regression line of Friedewald's LDLC (A) and RE (B) and broken lines indicate the 45º line passing through the origin.

Comparison of calculated LDLC with measured LDLC at various increasing TG levels are presented in Fig. 6 which clearly demonstrates that the underestimation of LDLC was almost abolished by RE.

The Pearson's concordance correlation coefficients (P_c) of calculated LDLC with measured LDLC were 0.9400 (95% CI: 0.9322 to 0.9469) for the derived regression equation and 0.8613 (95% CI: 0.8459 to 0.8752) for Friedewald's formula. The bias correction factor (C_b), a measure of accuracy was 0.9974 for the derived equation and 0.9369 for the Friedewald's formula. The accuracy (C_b) of the derived equation was higher and closer to perfect ($C_b = 1.0$) compared to Friedewald's formula (0.9974 vs 0.9369).
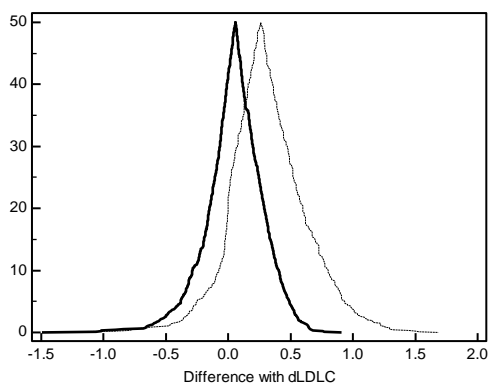

Fig. 5. Mountain plot of calculated LDLC against measured LDLC (measured LDLC − calculated LDLC). The solid line indicates the derived regression equation and the broken line indicates the Friedewald's formula.
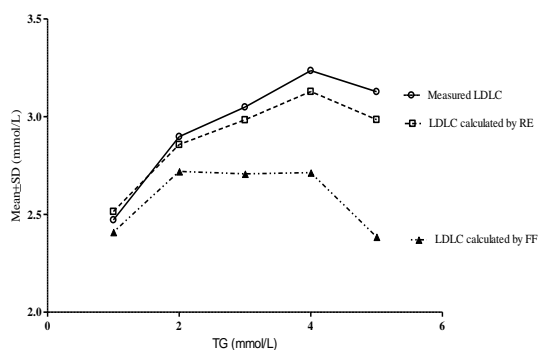

Fig. 6. Mean ±SD of LDLC at increasing TG levels

Comparison of percentage differences between measured LDLC and calculated LDLC showed that 79% of the calculated LDLC by the derived regression equation fall within ±12% of measured LDLC, whereas 57% of the calculated LDLC by Friedewald's formula fall within ±12% of measured LDLC. After adjustment of Friedewald's formula for bias, 72% of the calculated values fall within this limit of measured LDLC (data not shown).

   Though the original Friedewald's formula is frequently used in clinical practice, population-based and epidemiological study, investigators have modified/derived new equation to eliminate underestimation. In our population, few comparative studies are available [30–32]. All studies reported remarkable underestimation of LDLC by Friedewald's formula. In this study, we have derived a regression equation based on the lipid profiles in one tertiary healthcare center and validated this equation externally. The external validation using lipid profiles in another tertiary healthcare center strengthens the effectiveness of the derived regression equation for the estimation of LDLC in Bangladeshi population.

4. Conclusion

This study revealed that the derived regression equation is more accurate than original Friedewald's formula for the calculation of serum low-density lipoprotein cholesterol in Bangladeshi population.

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