OriginalArticle

Journal of Enam Medical College
Vol 5 No 2 May 2015

Evaluation of Recently Developed Regression Equation with Direct Measurement of Low-density Lipoprotein Cholesterol in a Bangladeshi Population

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Received: October 9, 2014 Accepted: February 10, 2015
doi: http://dx.doi.org/10.3329/jemc.v5i2.23376

Abstract

Background*: Meaningful underestimation of low-density lipoprotein (LDL) cholesterol is an important shortcoming of Friedewald's formula (FF) at higher triglyceride (TG) levels. Recently a regression equation (RE) has been developed using lipid profiles in one setting and validated externally for the calculation of LDL cholesterol. This newly developed RE requires more studies in different settings.* ***Objective****: The aim of this study was to evaluate the performance of the regression equation against direct measurement.* ***Materials and Methods****: Lipid profiles of 600 subjects attending a tertiary healthcare center were included in this study. Specimens were collected and lipid profiles were measured by standard methods. Sixty two lipid profiles with TG above 400 mg/dL were excluded. Calculated LDL cholesterol values using FF and RE were compared with measured LDL cholesterol by Pearson's correlation test, Passing & Bablok regression, accuracy within ±5% and ±12% of measured LDL cholesterol and two-tailed paired t test at various TG ranges.* ***Results****: The mean value of LDL cholesterol was 148.6 ± 37.2 mg/dL for direct measurement, 146.9 ± 42.4 mg/dL for FF and 148.6 ± 34.7 mg/dL for RE. The correlation coefficients of calculated LDL cholesterol values with measured LDL cholesterol were 0.949 ($p<0.001$) for FF and 0.959 ($p<0.001$) for RE. Passing & Bablok regression equation against measured LDL cholesterol was $y = 0.897x + 16.2$ for FF and $y = 1.0842x – 13.1$ for RE. Accuracy within ±5% of measured LDL cholesterol was 45% for FF, 57% for RE and within ±12% of measured LDL cholesterol was 84% for FF, 93% for RE. When calculated LDL cholesterol was compared with measured LDL cholesterol at different TG ranges, FF significantly underestimated LDL cholesterol at TG concentrations above 200 mg/dL whereas no significant difference was observed for RE.* ***Conclusion****: This study reveals that RE equation has similar performance to direct measurement for calculation of LDL cholesterol.*

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

J Enam Med Col 2015; 5(2): 75–79

Introduction

Cardiovascular diseases, the leading cause of death worldwide, increase the global health burden. Circulating low-density lipoprotein (LDL) cholesterol is thought to be critically involved in the development of coronary heart disease (CHD)[1] and it is considered as the primary basis for accurate

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classification in risk categories.[2] Ultracentrifugation, i.e., b-quantification[3] is the reference method for the quantitative determination of LDL cholesterol in circulation. Use of this reference method has limitations for routine clinical practice due to technical difficulties. The other recommended methods include homogeneous direct measurement.[4,5] The direct methods are costly and require expensive automation and are not affordable by most of the laboratories in the developing countries. Because of these limitations clinical laboratories throughout the world use a less expensive and easy approach for the estimation of LDL cholesterol, i.e., Friedewald's formula.[6] Nearly all laboratories in Bangladesh use this formula for the estimation of LDL cholesterol. In 1972, Friedewald et al[6] published the landmark formula by analyzing data of 448 US subjects. This allows rapid, inexpensive and suitable approach for the estimation of LDL cholesterol from three other lipid parameters: serum total cholesterol (TC), serum triglycerides (TG) and serum high-density lipoprotein (HDL) cholesterol. They developed this formula 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). But there are several shortcomings while using this formula — underestimation of LDL cholesterol at higher TG levels[7-13] and overestimation at low TG levels.[14] Recently by analyzing lipid profiles from 1.3 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[15-17] also reported a meaningful underestimation of LDL cholesterol in US adults. These are related to the use of fixed value of TG to VLDL cholesterol. Like the underestimation reported in different population, the underestimation of LDL cholesterol calculated by FF is also common in Bangladeshi population[18-25] and there is no evidence of systematic overestimation of LDL cholesterol by FF in this population.[18-24] In 2014 Saiedullah et al published a regression equation by analyzing lipid profiles of 531 adult Bangladeshi subjects which was validated externally using lipid profiles of 952 Bangladeshi subjects using linear regression.[24] Subsequently it has been evaluated using another set of lipid profiles collected from the setting of external validation group of the previous study.[25] However, it requires external validation using lipid profiles collected from a setting other than equation development and equation validation. In this context, this comparative study was designed to evaluate the performance of the RE against measured LDL cholesterol in this population in a different setting.

Materials and Methods

This cross-sectional study was conducted in the department of Applied Laboratory Sciences, Bangladesh University of Health Sciences (BUHS) during the period of April to June 2014. Venous blood specimens were collected in tubes without anticoagulant from 600 subjects (after ~12 hour fast) attending the outpatient department of a tertiary healthcare center for lipid analysis. The specimens were allowed to clot at room temperature, and serum was obtained by centrifugation at 3000 rpm for 15 minutes. Biochemical analyses were done within 12 hours of specimen collection. Serum TG and total cholesterol (TC) were measured by enzymatic end-point method and high-density lipoprotein (HDL) cholesterol and LDL cholesterol were measured by direct automated method using Dimension RxL Max (Siemens, USA) clinical chemistry analyzer. All kits, calibrators and quality control materials were purchased from Siemens, USA through local distributor. Sixty two lipid profiles with TG concentration above 400 mg/dL were excluded and 538 lipid profiles with TG <400 mg/dL were included in the study.

LDL cholesterol concentrations were also calculated by Friedewald's formula[6] and by regression equation.[24] Calculated LDL cholesterol values were compared with measured LDL cholesterol by Pearson's correlation test, Passing & Bablok regression, accuracy within ±5% and ±12% of measured LDL cholesterol and two-tailed paired *t* test at various TG ranges. Statistical analyses were performed by MedCalc® version 11.4 for Windows. *p* value <0.05 was considered as statistically significant.

Results

A total of 538 lipid profiles from 538 adult study subjects were included in this study. Among them

273 were males and 265 were females. The mean age of the study subjects was 48 ± 13 years. Lipidemic status of the study subjects is presented in Table I.

Table I: Lipidemic status of the study subjects

Lipid parameters	Mean ± SD
Total cholesterol (mg/dL)	226.5 ± 42.6
Triglyceride (mg/dL)	180.5 ± 75.7
HDL cholesterol (mg/dL)	43.4 ± 12.4
Measured LDL cholesterol (mg/dL)	148.6 ± 37.2
Calculated LDL cholesterol by FF (mg/dL)	146.9 ± 42.4
Calculated LDL cholesterol by RE (mg/dL)	148.6 ± 34.7

The mean values of LDL cholesterol was 148.6 ± 37.2 mg/dL for direct measurement, 146.9 ± 42.4 mg/dL for FF and 148.6 ± 34.7 mg/dL for RE. Mean value of calculated LDL cholesterol by FF differed from measured value (p=0.004) whereas mean value of calculated LDL cholesterol by RE did not (p=0.899). Comparison of calculated LDL cholesterol with measured LDL cholesterol at different TG ranges is presented in Table II. The correlation coefficients of calculated LDL cholesterol values with measured LDL cholesterol were 0.949 (p<0.001) for FF and 0.959 (p<0.001) for RE. Passing & Bablok regression equation against measured LDL cholesterol was $y = 0.897x + 16.2$ for FF (Fig 1A) and $y = 1.0842x - 13.1$ for RE (Fig 1B). Accuracy within ±5% of measured LDL cholesterol was 45% for FF, 57% for RE and within ±12% of measured LDL cholesterol was 84% for FF, 93% for RE.

Table II: Comparison of calculated LDL cholesterol at various TG ranges with measured LDL cholesterol

TG range	Measured LDL cholesterol	FF LDL cholesterol		RE LDL cholesterol	
		Mean ± SD	*p values*	Mean ± SD	*p values*
Up to 100 mg/dL (n=72)	135.6 ± 33.9	137.6 ± 38.2	0.145	136.1 ± 31.3	0.620
101–200 mg/dL (n=279)	154.0 ± 29.9	155.1 ± 31.8	0.102	153.6 ± 26.5	0.528
201–300 mg/dL (n=141)	154.7 ± 38.7	150.8 ± 44.7	<0.001	154.8 ± 37.3	0.912
301–400 mg/dL (n=46)	117.9 ± 55.1	100.0 ± 61.9	<0.001	118.4 ± 51.8	0.848

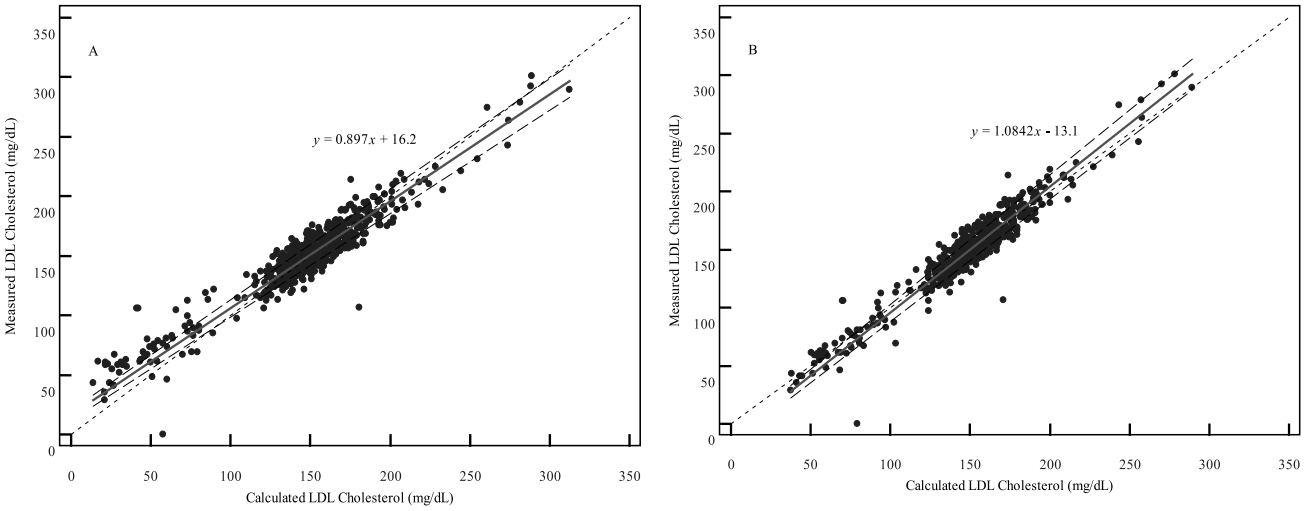

Fig 1. Passing & Bablok regression equation of calculated LDL cholesterol against measured LDL cholesterol (A for FF and B for RE)

Discussion

In this study LDL cholesterol calculated by both FF and RE correlated well with measured LDL cholesterol. The correlation coefficient was stronger for RE compared with FF (0.959 vs 0.949). This is consistent with a previous study done in this population.[24] Passing & Bablok regression of calculated LDL cholesterol with measured LDL cholesterol also revealed better agreement of RE with direct measurement compared with that of FF. In this study, though accuracy within ±12% of measured LDL cholesterol was higher compared to previous study[24] (84% vs 57%) for FF, better accuracy within ±5% (57% vs 45%) and ±12% (93% vs 84%) of measured LDL cholesterol was observed for RE compared to FF. Differences of mean values of calculated LDL cholesterol using FF with measured LDL cholesterol in the total study subjects were minimal; but it was high and statistically significant at TG concentrations above 200 mg/dL. On the other hand, mean differences were similar and statistically insignificant for RE in the total study subjects as well as at different TG ranges. The underestimation of LDL cholesterol by FF was meaningful and large for Friedewald's formula in US population[15,16] and it was >11 mg/dL in Bangladeshi population.[24] However, this study revealed that FF underestimated LDL cholesterol by >17 mg/dL when TG concentration was above 300 mg/dL. Thus, considering better correlation and accuracy, minimal mean difference of LDL cholesterol by using RE and similar performance to direct measurement, RE can be used in clinical evaluation and in epidemiological studies. However, more studies in different settings are recommended.

Finally, this study reveals that the newly developed regression equation has similar performance to direct measurement for the estimation of LDL cholesterol in this population.

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