

Original Article**Comparative Analysis between Martin's Formula and Friedewald's Formula with Direct Homogenous Assay for Estimating Low Density Lipoprotein Cholesterol Level in Nepalese Population****Bishal Raj Joshi*, Shikha Rizal**

Department of Biochemistry, Nobel Medical College Teaching Hospital, Biratnagar, Nepal

Article Received: 17th April, 2021; Accepted: 25th June, 2021; Published: 30th June, 2021DOI: <http://dx.doi.org/10.3126/jonmc.v10i1.37944>**Abstract****Background**

Low density lipoprotein forms a basis of decision making in treatment of hypercholesterolemic patients and primary target of intervention. Its cost effective and accurate measurement is a need for every clinical laboratories and different calculation methods has been adopted as a replacement to direct assays. This study aims to evaluate the Martin's formula and Friedewald's formula in a sample of Nepalese population compared against direct homogenous assay.

Materials and Methods

This is a cross-sectional study conducted in Department of Biochemistry from Feb 2020 to January 2021. Serum samples of the participants were analysed for total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein. Martin's and Friedewald's formula were applied to get calculated value of low density lipoprotein from both methods. Passing and Bablok regression analysis was used for methods comparison.


Results

The mean age of participants was 54.2 ± 8.9 years. Passing-Bablok regression analysis showed Friedewald's formula performed better than Martin's formula as per systematic and proportional bias when compared with direct assay. However at lower serum low density lipoprotein level, underestimation of low density lipoprotein compared to direct assay was more common in Friedewald's formula. At high triglyceride level more percentage error of difference of mean from direct assay was found for Friedewald's formula.

Conclusion

When compared to direct assay, Friedewald's formula was found to be in better agreement than Martin's formula. Martin's formula had advantage over Friedewald's formula at lower serum low density lipoprotein level and higher triglyceride level where Friedewald's formula mostly underestimated low density lipoprotein.

Keywords: Cholesterol, Low-density lipoprotein, Triglycerides

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Introduction

Serum low density lipoprotein (LDL) cholesterol is a well-recognized risk factor of atherosclerotic vascular disease [1]. Based on the serum LDL levels, the National Cholesterol Education program (NCEP) suggests different criteria for decision making in treatment of hypercholesterolemic patients who have coronary heart disease or other risk factors [2].

LDL measurement in laboratory can be done by ultracentrifugation, lipoprotein electrophoresis, precipitation [3], direct homogenous assays [4], as well as Friedewald's equation [5]. Friedewald's equation being an easy calculation method is commonly used in most of the laboratory. The Friedewald's formula is based on cholesterol determinations (Total and HDL) and triglyceride determination and presumes that a direct relationship exists between VLDL-cholesterol and triglycerides in fasting blood samples ($VLDL = TG/5$) [5].

Despite being an easy calculation method and preferred method in most laboratories, Friedewald's formula might lead to significant amount of error in calculation of LDL. Martin proposed a formula addressing inter-individual variance in the TG:VLDL-C ratio. In his study, he proposed an adjustable factor for the TG:VLDL-C ratio based on TG and non-HDL-C concentrations in contrast to fixed factor of 5 as proposed by Friedewald [6].

Various studies have shown the poor applicability of the Friedewald formula in the assessment of serum LDL [7-9]. Very high level and low level of TG affects the calculation. Thus this study aims to evaluate the Martin's formula in a sample of Nepalese population and comprehend its performance with Friedewald's formula when both are compared against direct homogenous assay.

Materials and Methods

This comparative cross-sectional study was conducted in Department of Biochemistry from Feb 2020 to January 2021 after taking ethical approval from Institutional Review Committee, Nobel Medical College and Teaching Hospital, Biratnagar, Nepal. Informed consent from the patients was taken. Samples from patients advised for lipid profile test from regular OPD were included in the study. Samples having triglyceride level greater than 400 mg/dl and hemolytic samples were excluded from the study. Sample size for the study was taken as 502, which is based on guideline for sample size in comparison of measurement procedures published by clinical and laboratory standards

institute which states minimum of 40 samples are required for method comparison studies [10].

Fasting blood sample from patients sent for lipid profile were collected in plain vial. Sample was centrifuged at 3000 rpm for 10 minutes, serum separated and analysed for lipid parameters in routine biochemistry automated analyzer. Total cholesterol was measured by enzymatic endpoint CHOD-PAP method, triglyceride was measured by enzymatic glycerol phosphate oxidase/peroxidase method, and LDL and HDL was measured by homogenous enzymatic direct assay. Non-HDL cholesterol was calculated as Total cholesterol (TC) – HDL cholesterol. LDL by the Friedewald's formula was calculated as, $LDL = TC - HDL - (TG/5)$ [LDL = low density lipoprotein, TC = Total Cholesterol, HDL = High Density Lipoprotein, TG = Triglyceride]. LDL by Martin/Hopkin's formula was calculated as, $LDL = Non\ HDL-C - (TG/adjustable\ factor)$. Adjustable factor depends upon Triglyceride level and non-HDL cholesterol level and obtained from 180-Cell table proposed by Martin et al. [6] and ranged from 3.1 to 9.5.

The data was collected and entered in MS-excel 2013 and analyzed using the Statistical Package for Social Sciences (SPSS) version 16 software. Descriptive data were presented as mean \pm SD. Kolmogorov-Smirnov test was done to check the normality of the data. Passing and Bablok regression [11] was used to compare direct LDL with Friedewald formula and Martin formula to estimate the systematic bias and proportional bias. The Cusum test for linearity obtained the linear relationship between the methods and thus justifies the applicability of Passing and Bablok regression to compare these methods. MedCalcver 20 software was used for Passing and Bablok regression analysis [12].

Result were interpret as regression equation $y = a + bx$, where $a = y$ intercept and defines the systematic differences present between two methods. The 95% confidence interval for the intercept 'a' was used to test the hypothesis that $a = 0$. This hypothesis was accepted if the confidence interval for 'a' contains the value 0. Similarly $b =$ slope and measures the proportional differences between two methods. The 95% confidence interval for the slope 'b' was used to test the hypothesis that $b = 1$. The hypothesis was accepted if the confidence interval for slope 'b' contains the value 1.

Results

A total of 502 participants were included in the study and mean age of participants was 54.2 ± 8.9 years. Biochemical characteristics of the



study population are as shown in Table 1.

Table 1: Biochemical characteristics of study participants expressed as mean SD

Variables	Mean \pm SD
Age (years)	54.2 \pm 8.9
Total Cholesterol (mg/dl)	189.13 \pm 48.50
Triglyceride (mg/dl)	163.85 \pm 72.70
HDL cholesterol (mg/dl)	45.34 \pm 12.12
Non-HDL cholesterol (mg/dl)	143.8 \pm 45.2
LDL cholesterol (Direct assay) (mg/dl)	112.62 \pm 40.66
LDL cholesterol (Friedewald's formula) (mg/dl)	111.02 \pm 40.34
LDL cholesterol (Martin's formula) (mg/dl)	115.89 \pm 39.52

Pearson correlation was done between Direct LDL cholesterol and Friedewald LDL cholesterol and strong correlation was found between the LDL calculated by these methods ($r=0.963$, $p<0.001$). Similarly, strong correlation was found between the LDL calculated by direct method and Martin formula ($r=0.971$, $p<0.001$). Comparison of correlation coefficients done by Fisher r-to-z transformation [13] showed correlation coefficient with direct LDL was higher for Martin formula than Friedewald formula ($p=0.05$). (Figure 1)

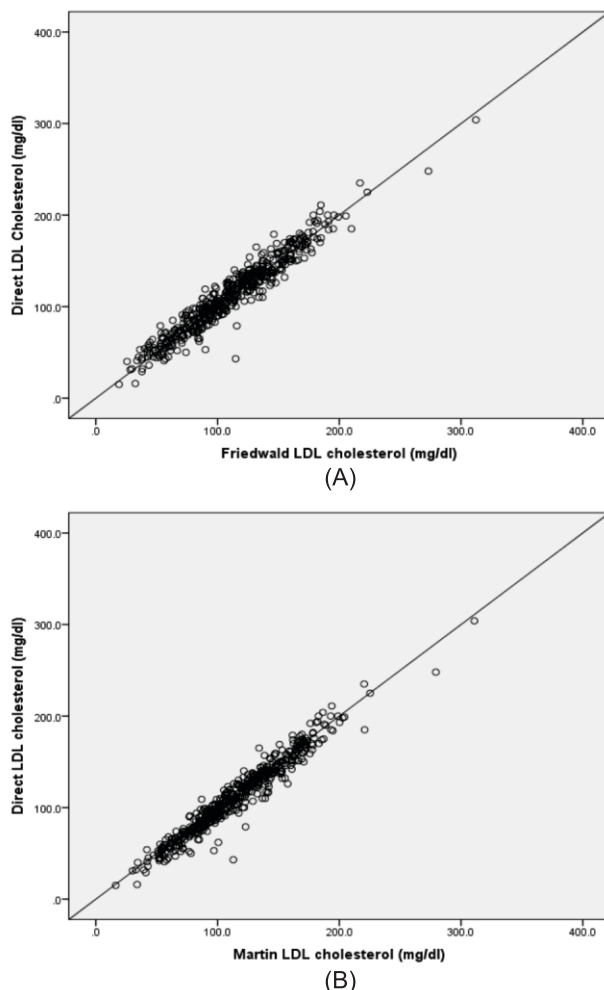


Figure 1: Correlation between direct LDL and LDL calculated by Friedewald formula (A) and by Martin formula (B).

Passing-Bablok regression done to compare the agreement between two methods, direct LDL vs Friedewald formula and direct LDL vs Martin formula. The regression equation $y=5.55 + 0.97 x$, CI 95% for y: 3.40-7.92, CI 95% for x: 0.95-0.99 was obtained for Martin formula and $y = -1.53 + 0.99 x$, CI 95% for y: -3.76-0.81, CI 95% for x: 0.96-1.01 was obtained for Friedewald formula. LDL calculated by Martin formula showed systematic bias of 5.55 (CI 3.4-7.92) and proportional bias of 0.97 (CI 0.95-0.99) and both were significant (Null hypothesis was rejected). LDL calculated by Friedewald formula showed systematic bias of -1.53 (CI -3.76-0.81) and proportional bias of 0.99 (CI 0.96-1.01) and both were not significant (Null hypothesis accepted). (Figure 2)

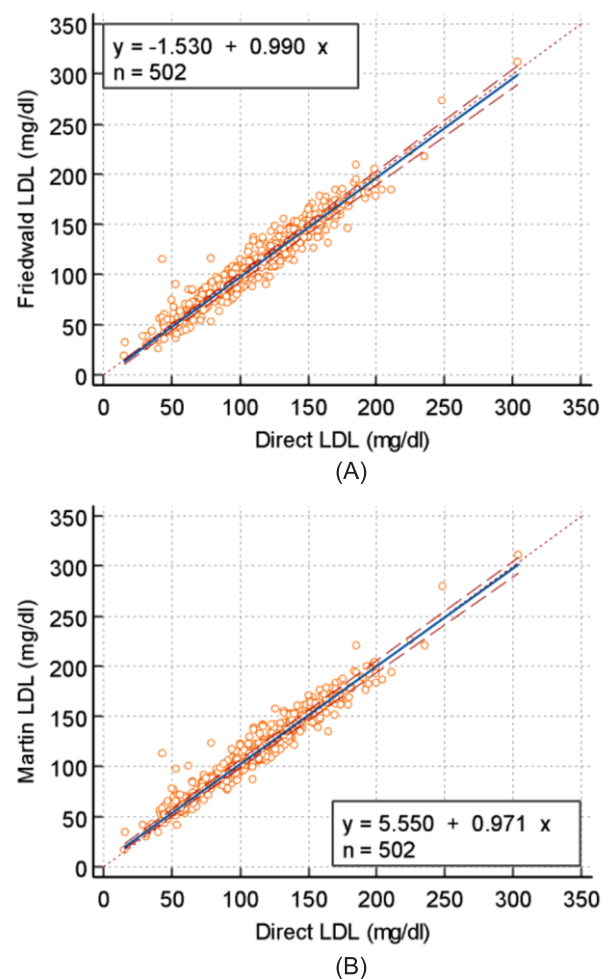


Figure 2: Passing and Bablok regression to compare agreement between two methods.

- (A) Between Direct LDL and Friedewald LDL
 (B) Between Direct LDL and Martin LDL Solid line = regression line, dashed lines = Confidence interval for regression line

Scatter plot between absolute difference of test methods and Friedewald (figure 3 A) and Martin (figure 3 B) to see whether Friedewald formula



and Martin formula underestimates the LDL at lower LDL level. Friedewald formula showed more underestimation than Martin formula.

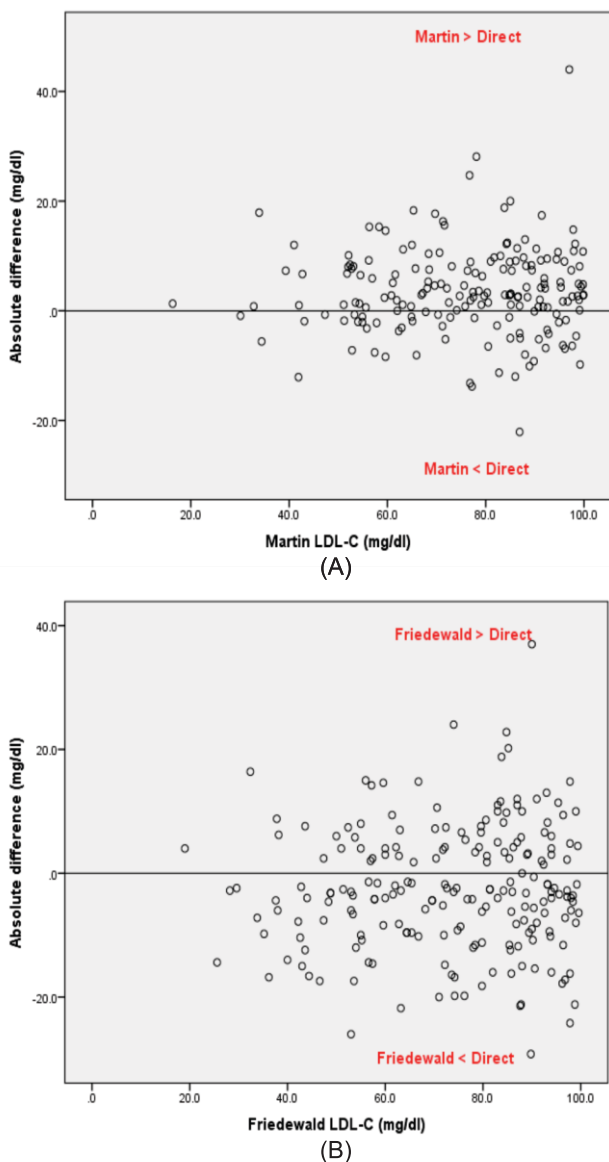


Figure 3: Scatter plot to show agreement of Martin formula (A) and Friedewald formula (B) with direct LDL at lower LDL level. Absolute difference (Martin LDL or Friedewald LDL – Direct LDL).

Table 2 shows the mean LDL by direct assay, Friedewald and Martin formula across different

Table 2: Percentage error of means between direct LDL and calculated LDL.

TG classification (NCEP ATP III)	TG range (mg/dl)	N	Mean LDL (Direct assay) (A)	Mean LDL (Friedewald's formula) (B)	Mean LDL (Martin's formula) (C)	% error of mean between (A) and (B)	% error of mean between (A) and (C)
Normal	<100	108	90.1	94.2	93.2	4.55	3.44
	100-149	139	111.3	113.2	114.9	1.70	3.23
Borderline high	150-199	113	119.3	116	121.2	-2.76	1.59
High	200-300	120	125.9	118.9	129.7	-5.55	3.01
	>300	22	126.5	113.6	132	-10.19	4.34

strata of TG level. LDL calculated by Friedewald formula showed maximum percentage error of -(-10.19%) 10.19% with direct LDL at TG level > 300 mg/dl. Martin formula showed maximum percentage error of only 4.34% with direct LDL at TG level > 300 mg/dl.

Discussion

Low density lipoprotein (LDL) is one of the primary target of therapy as elevated LDL is one of the major cause of CHD and various national and international guidelines have proposed LDL attainment goals [1,2,14]. Accurate LDL-C estimation is one of the key factor for interventional treatment. Calculation methods provides easy and no-cost mode for LDL estimation. Estimation of LDL by calculation method is affected by methodological errors since formula requires three separate analysis of TC, TG and HDL. Friedewald formula has been found to underestimate LDL at very low LDL level and in patients with high TG. New formula as proposed by Martin addresses interindividual variance in TG:VLDL ratio and has been found better in estimating accurate LDL in different studies in other parts of world [6, 15, 16]

Our study compares the performance of martin formula and friedewald formula with that of direct homogenous assay for estimation of LDL in 502 subjects of Nepalese population. The mean age of our study population was 54.2 ± 8.9 years. Strong correlation was found between direct estimation of LDL cholesterol and Friedewald calculation, r = 0.963 (p<0.001) and also between direct estimation of LDL cholesterol and Martin calculation, r = 0.971 (p<0.001). Studies done in Nepal and other countries also showed similar correlation between direct LDL and Friedewald calculation [17-19]. Comparison of correlation coefficients was done by Fisher r-to-z transformation, and it was found that Pearson correlation coefficient with direct LDL was higher for Martin formula than Friedewald formula (p=0.05). Similar finding was obtained by Martin et al [20]. Strong correlation found in these studies is the reason that these studies using formulas are conducted with excluding TG > 400 mg/dl. LDL estimation by formulas are near to accurate up to TG < 400 mg/dl.

Passing-Bablok regression was used to compare the agreement between two methods, direct LDL vs Friedewald formula and direct LDL vs Martin formula, and interpret systematic bias and proportional bias between methods. In our study Martin formula for LDL calculation showed both systematic bias and proportional bias from direct LDL assay (y=5.55 + 0.97 x, CI 95% for y: 3.40-



7.92, CI 95% for x: 0.95-0.99). Friedewald formula was found to be with minimal of systematic bias and proportional bias and obtained equivalent measurement as direct LDL assay ($y = -1.53 + 0.99x$, CI 95% for y: -3.76-0.81, CI 95% for x: 0.96-1.01). Martin formula showed the constant error of 5.55 (3.4-7.92) showing high and entirely positive deviation compared to -1.53 (-3.6-0.81) by Friedewald formula. This finding is in contrast to study done by Martin et al. who found least bias in Martin formula compared to Friedewald formula [20]. Presence of bias in Martin formula lead to overestimation of LDL by Martin formula compared to direct method. De Cardova et al however in their study of comparison of direct method and Friedewald formula in large population discouraged the use of Friedewald formula in diabetes and type III dyslipidemia [21]. Some author claimed no obvious advantage of using Martin formula over already using Friedewald formula in low cardiovascular risk patients [22].

Friedewald formula has been found to underestimate LDL at low LDL levels and higher TG level. Scatter plot between absolute difference (Friedewald LDL – Direct LDL) and Friedewald LDL at LDL level less than 100 mg/dl showed underestimation of LDL (Friedewald LDL being lower than actual LDL). In contrast Martin formula showed lesser underestimation in similar scatter plot at LDL level less than 100 mg/dl. Underestimating actual LDL value at low cholesterol level can lead to deferral or withdrawal of intervention in high risk patients. LDL is used for interventional strategies based on total cardiovascular risk. ECS/EAS guideline 2016 suggests optimal level of LDL cholesterol < 70 for high risk patients [14]. Martin formula thus may be a better option than Friedewald formula in cases of low LDL. Several studies have found the underestimation of LDL by Friedewald formula at low LDL level [6-8]. Egbaria et al. in their study noted 10.8% of their samples could be reclassified to an upper risk category when Martin formula was used instead of Friedewald formula [23].

Percentage error of difference of mean of both Friedewald and Martin formula with direct assay at stratified TG level was calculated. Friedewald's formula showed greater error at high TG level (-5.5 % at TG 200-300 mg/dl, -10.19 % at TG >300 mg/dl). Friedewald formula was found to underestimate LDL at higher TG level. Martin formula showed constant positive deviation at all strata of TG levels. Percentage error of difference of mean was found to be 3.01 % at TG 200-300 mg/dl and 4.34 % at TG > 300 mg/dl. This suggests advantage of Martin formula over

Friedewald formula for measuring LDL when TG is at higher level. The reason may be due to determination of different TG: VLDL-C ratio at different level of TG and non HDL-C rather than fixed ratio used by Friedewald.

Our study showed Martin formula was positively deviated from direct LDL producing overestimation. Method comparison showed Friedewald formula to have less bias and superior to Martin formula. We still suggest the study to be done at larger population to see the applicability of Martin formula in Nepalese population as in our study it was found to be better at identifying people at risk at low LDL level which was mostly underestimated by Friedewald formula. The reason behind the suggestion is Martin formula has now been widely adopted by large laboratories worldwide and recent multi society guideline has provided a class IIa recommendation for using this formula in patients with LDL-C < 70 mg/dl [24,25]. Our study has limitation of not using beta quantification method for LDL estimation. Martin formula was validated comparing with beta quantification method so it may be the reason it showed substantial bias from direct LDL assay in our study.

Conclusion

In our study, method comparison showed Martin's formula for calculation of LDL have more bias than Friedewald's formula when both were compared to direct homogenous assay and mostly directing towards positive deviation. Nevertheless, Martin's formula was found superior to Friedewald's formula for estimating LDL at low LDL level or high TG level. Since both formulas have some sorts of shortcomings direct LDL would be better option for proper cardiac risk classification in high risk patients.

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Conflicts of interests: None

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