Original Article

Comparison between direct assay and popular equations for Low Density Lipoprotein-cholesterol estimation in Nepalese population

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ABSTRACT

Background: The aim of this study was to compare LDL-C estimations using various equations with directly measured LDL-C and to find the most accurate and reliable equation for measuring serum LDL-C at different triglycerides level.

Materials and Methods: In this study, we performed a retrospective analysis on the database of our Laboratory Information System to retrieve results of lipid profile in patients visiting Dhulikhel Hospital during the period of 6 months. A total of 1420 participants were classified into three groups according to triglyceride (TG) concentrations as follows: <150, 150–199 and >199 mg/dL. LDL-C was calculated using the Friedewald, Chen, Vujovic, Hattori, Anandaraja and modified Friedewald equations and compared with directly measured LDL-C concentration (enzymatic method on Biosystems, BA-400).

Results: In most of the instances, calculated LDL-C value was higher than the directly measured LDL-C values with negative mean difference with the exception of Hattori equation. The intraclass correlation coefficient (ICC) between the estimated and directly-measured LDL-C was higher with the Friedewald equation (ICC=0.917; 95% CI: 0.904-0.927) for all serum TG ranges compared with other equations. The reliability of all the equations was good with ICC being above 0.75 while that of the Friedewald equation was excellent in all the TG groups with ICC being above 0.9. Hattori equation was better in estimating LDL-C at normal TG range (ICC=0.927; 95% CI: 0.917-0.937) and borderline high TG (ICC= 0.933; 95% CI: 0.908-0.951).

Conclusion: Calculated LDL-C using appropriate equations can be an alternative cost-effective tool to measure LDL-C when the direct measurement cannot be afforded.

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INTRODUCTION

Dyslipidemia is one of the major modifiable risk factor for cardiovascular disease (CVD). Among the various component of traditional lipid profile, Low density Lipoprotein Cholesterol (LDL-C) is considered as the most appropriate factor for patient classification in risk management of CVD. Elevated LDL-C is a well-known atherogenic risk factor with high predictive value for coronary heart disease.[1]

The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) recommends a goal of maintaining serum LDL-C concentration<100mg/dl

as optimal. It is also the basis for initiating appropriate treatment and patient's risk stratification.[2] This highlights the importance of comprehensive understanding of the need for accurate and precise LDL-C estimation. Various methods are available for measuring serum LDL-C concentration. The accepted gold standard or reference method for LDL-C estimation is ultracentrifugation followed by beta quantification. Beta quantification is not suited for routine use, as it requires ultracentrifugation, large volume of samples, expensive instruments and is time consuming.[3] Direct homogeneous assays for measurement of LDL-C have been developed and have shown reasonable accuracy and precision when compared with the reference method. Measurement of LDL-C by direct method is expensive compared with other traditional lipid profile parameters.

In routine practice, most clinical laboratories in Nepal report LDL-C by indirect method using different equations. Several equations have been developed to estimate LDL-C.[4-9] It is very important to use suitable laboratory methods and achieve accurate results. It is necessary to know about the agreement of results obtained by these different methods. However, studies done in many parts of the world to compare the agreement of different equations with direct LDL-C estimation have shown conflicting results.[10-16] There is no any study published till date to guide the laboratory personals about the best equation at different triglyceride level in our setting. Thus the aim of this study was to assess the performance of the common equations and compare these formulas with direct measurement method and to find the most accurate and reliable equation for measuring serum LDL-C at different triglycerides level.

MATERIAL AND METHODS

In this study, we performed a retrospective analysis on the database of our Laboratory Information System (LIS) to retrieve results of lipid profile in patients visiting Dhulikhel Hospital during the period of 6 months (1st January 2019 to 30th June 2019). The lipid profile test included triacylglycerol (TG), total cholesterol (TC), High density lipoprotein cholesterol (HDL-C), and Low density lipoprotein cholesterol (LDL-C). A total of 1420 participants were classified into three groups according to triglyceride concentrations as follows: <150 mg/dl, 150–199 mg/dl, and >199 mg/dl. The basis of classification into three different TG groups was per according to ATP III levels of normal TG, borderline high TG and high TG.[2] We excluded all the cases with very high TG i.e>500 mg/dl as almost all studies done till date have discouraged the use of equations to calculate LDL-C above this range.[3, 4, 12, 16] The laboratory method for measurement of LDL-C, HDL-C, TC, and TG was enzymatic spectrophotometric method using commercial kits by BioSystems (BA-400, BioSystems S.A. Spain). In addition to direct measurement, LDL-C was calculated according to the following equations:

1) Friedwald Equation[4]: $LDL = TC - HDL - (TG / 5)$

2) Anandraja Equation[5]: $LDL = (0.9\ TC) - (0.9\ TG/5) - 28$

3) Chen Equation[6]: $LDL = (TC - HDL) \times 0.9 - (TG \times 0.1)$

4) Hattori Equation[8]: $LDL\text{-}C = (0.94 \times TC) - (0.94 \times HDL\text{-}C) - (0.19 \times TG)$

5) Vujovic Equation[9]: $LDL\text{-}C = TC - HDL\text{-}C - (TG/6.58)$

6) Modified Friedwald Equation[17]: $LDL\text{-}C = TC - (TG/6 + HDLC)$

Data was entered in MS Excel 2010, and analyzed with Statistical Package for Social Sciences (SPSS Inc., Chicago, USA) version 21.0. Data was classified according to the TG level into three groups as described previously. The performance of all estimated formulas was compared at different concentrations of TG. Continuous variables were described as means with standard deviations or as a median with an interquartile range depending on their distribution. Data were compared using an independent t-test, one way ANOVA and Wilcoxon rank sum test. Correlations between LDL-C by estimated formulas and by direct measurement was calculated using the Pearson's correlation. The intraclass correlation coefficient (ICC) analysis was performed in order to evaluate the reliability across two measurements. ICC estimates and their 95% confident interval (CI) were calculated. Bland–Altman plots were used to evaluate the agreement and absolute difference between the formulas and the directly measured LDL-C, respectively. Statistical significance was defined as a two-sided p-value of less than 0.05.

RESULTS

The total number of participants was 1420 with mean age of 48.4±14.7 years. Among the total participants 804 (56.6%) were male and 616 (43.4%) were female. The mean serum total cholesterol, HDL-C and direct LDL-C concentration was 173.5±40.7, 41.8±11.4 and 95.9±32 mg/dl respectively. The concentration of triacylglycerol (TG) ranged from 33 mg/dl to 498 mg/dl with median of 141 (95, 211) mg/dl. Among the total participants, 766 (53.9%) had normal TG (TG<150mg/dl), 254 (17.9%) had borderline high TG (TG=150-199 mg/dl) and 400 (28.2%) had high TG (TG ≥200 mg/dl). The distribution of age and lipid profile including calculated LDL-C values in both genders is shown in Table 1. The mean serum HDL-C was significantly higher in females whereas the mean serum TG was significantly higher in male patients.

The mean concentration along with standard deviations of different lipid profile parameters including direct LDL and calculated LDL across different triacylglycerol concentration

Table 1: Age and lipid profile differences according to gender

	Female	Male	p-value
Age	48.3±14.8	48.4±14.6	0.94
Total cholesterol	173.5±39	173.6±42	0.95
HDL-C	44.3±11.5	39.9±11	<0.001
Triacylglycerol	134 (93, 193)	156 (101, 236)	<0.001
Direct LDL-C	95.8 ±31.4	96.1±32.45	0.87
Friedewald LDL	98.6±34	98.6±35	0.98
Anandaraja LDL	100.7±33.1	96.6±33.5	0.02
Chen LDL	101±31.4	102.8±33.3	0.3
Hattori LDL	92.4±31.9	92.3±32.9	0.96
Vujovic LDL	106±34.3	107±35.8	0.57
Modified Friedewald LDL	103.7±34.1	104.4±35.5	0.7

Data expressed as mean ± SD, Median (25th percentile, 75th percentile)

Table 2: Lipid profile differences according to different TG concentration

	TG <150 mg/dl	TG 150-199 mg/dl	TG>199 mg/dl	p-value
HDL-C	45±11	40±11	36±11	<0.001
Total Cholesterol	159±35	182±40	195±40	<0.001
Direct LDL	89±29	104±33	103±34	<0.001
Friedewald LDL	94.1±31.6	107.6±35.8	101.8±37.8	<0.001
Anandaraja LDL	97.4±30.6	105.1±35.5	96.1±36.6	0.001
Chen LDL	92.6±29	110.6±32.3	114.6±33.4	<0.001
Hattori LDL	88.2±29.7	100.8±33.6	95.1±35.5	<0.001
Vujovic LDL	98.9±31.9	115.8±35.8	115.6±37.3	<0.001
Modified Friedewald LDL	97.4±31.8	113.3±35.8	111.4±37.4	<0.001

is shown in Table 2. Mean HDL-C concentration was lower in borderline high and high TG groups compared to normal TG group.

The comparison between estimated LDL-C using six formulas to directly measured LDL-C according to the TG concentration is shown in table 3. The mean value of LDL-C along with the mean difference in all groups classified according to TG level is also shown in Table 3. In most of the instances, calculated LDL-C value was higher than the directly measured LDL-C values with negative mean difference with the exception of Hattori equation. The ICC between the estimated and directly-measured LDL-C was significantly higher with the Friedewald equation (ICC=0.917; 95% CI: 0.904-0.927) for all serum TG ranges compared with other equations as shown in table 3. Except for the calculated LDL-C using Hattori equation (p value=0.923), there was significant difference between mean of directly measured and all other calculated LDL-C. The reliability of all the equations was good with ICC being above 0.75 while that of the Friedewald equation was excellent in all the TG groups with ICC being above 0.9.

To find the agreement between the direct and calculated LDL methods, Bland–Altman Plot was prepared [figure

1].The mean bias for the Friedewald formula was -2.66 ± 13.37 mg/dl, -2.44 ± 17.66 mg/dl for the Anandaraja formula, -6.04 ± 12.38 mg/dl for the Chen formula, 3.58 ± 12.79 mg/dl for the Hattori formula, -10.61 ± 12.89 mg/dl for the Vujovic formula and -8.18 ± 12.87 mg/dl for the modified Friedewald formula.

DISCUSSION

LDL-C is the primary target for diagnosis and treatment of patients with hyperlipidemia.[2,18] It has important implications in cardiovascular risk stratification and has been focused on therapeutic decision-making.[19] It is essential to accurately estimate LDL-C concentration, inability of which can adversely influence therapy and outcomes in patients. Currently, there are several methods for the estimation of LDL-C. In the present study we compared calculated LDL-C using six different formulas with directly measured LDL-C across different triglyceride concentration in Nepalese population. Overall, the correlation between estimated LDL-C and measured LDL-C was good. Overall, the Friedewald formula showed the best performance for estimating LDL-C (ICC=0.917; 95% CI: 0.904-0.927) with the mean difference of -2.44 mg/dl compared to the directly-measured LDL-C. Similar to our study, previous studies

Table 3: Comparison of estimated formulas to directly-measured LDL-C according to serum TG concentration

TG level	LDL-C measurement	Mean ± SD (mg/dL)	Mean difference	ICC (confidence interval)
Overall	**Directly-measured**	**95.9±32**	NA	NA
	Friedewald	98.6±34.5	-2.66	0.917(0.904-0.927)
	Anandaraja	98.4±33.4	-2.44	0.852 (0.836-0.867)
	Chen	102±32.5	-6.04	0.910 (0.844-0.943)
	Hattori	92.4±32.5	3.58	0.916 (0.895-0.931)
	Vujovic	106.6±35.1	-10.61	0.883 (0.597-0.947)
	Modified Friedewald	104.1±34.9	-8.18	0.899 (0.763-0.946)
Normal TG	**Directly-measured**	**89±29**	NA	NA
	Friedewald	94.1±31.6	-4.66	0.914 (0.872-0.939)
	Anandaraja	97.4±30.6	-8.03	0.845 (0.715-0.905)
	Chen	88.2±29.7	-3.21	0.925 (0.903-0.941)
	Hattori	88.2±29.7	1.17	0.927 (0.917-0.937)
	Vujovic	98.9±31.9	-9.44	0.883 (0.613-0.947)
	Modified Friedewald	97.4±31.8	-7.98	0.895 (0.727-0.946)
Borderline High TG	**Directly-measured**	**104±33**	NA	NA
	Friedewald	107.6±35.8	-3.21	0.932 (0.910-0.949)
	Anandaraja	105.1±35.5	-0.71	0.885 (0.855-0.909)
	Chen	110.6±32.3	-6.58	0.921 (0.841-0.954)
	Hattori	100.8±33.6	3.58	0.933 (0.908-0.951)
	Vujovic	115.8±35.8	-11.48	0.887 (0.514-0.955)
	Modified Friedewald	113.3±35.8	-8.95	0.906 (0.722-0.955)
High TG	**Directly-measured**	**103±34**	NA	NA
	Friedewald	101.8±37.8	1.50	0.903 (0.883-0.920)
	Anandaraja	96.1±36.6	7.16	0.839 (0.771-0.883)
	Chen	114.6±33.4	-11.32	0.866 (0.563-0.940)
	Hattori	95.1±35.5	8.18	0.883 (0.772-0.930)
	Vujovic	115.6±37.3	-12.30	0.861 (0.545-0.937)
	Modified Friedewald	111.4±37.4	-8.08	0.888 (0.786-0.933)

The mean difference (directly-measured LDL-C - estimated LDL-C) represents the estimation of bias between the two observations. LDL-C: low-density lipoprotein cholesterol, TG: triglycerides, SD: standard deviation, ICC: intraclass correlation coefficient, NA: not applicable

have also reported that Friedewald calculation demonstrates better agreement with directly measured LDL-C.[12, 20] The performance of Friedewald equation was fairly constant in all the TG groups. Hattori equation was better in estimating LDL-C at normal TG group (ICC=0.927; 95% CI: 0.917-0.937) and borderline high TG group (ICC= 0.933; 95% CI: 0.908-0.951). Our finding was similar to the study done to compare the accuracy between four formulae in calculating LDL-C, which reported that the Hattori formula performed best across a range of lipid values in a large database of hospitalized patients.[14] The correlation of our study was similar to the other studies where the correlation between calculated and direct LDL-C ranged from 0.78 to 0.93.[10, 21, 22]

To find the actual relation between these methods Bland–Altman plot was used, which showed clear relationship between both the directly measured LDL-C and the calculated LDL-C. There was a minimum negative bias

between the direct measurement and measurement using most of the equations. The calculated LDL-C values using most of the equations were higher than direct measurement. This was evident in normal and borderline high TG group with the exception of Hattori equation. Similar trends of higher results with calculated LDL-C as compared to directly measured LDL-C was seen in previous studies.[13, 23-26] Most of these studies compared calculated LDL-C using Friedewald equation with direct LDL-C. In contrast to our finding, Vujovic et al. found significantly lower calculated LDL compared to direct LDL-C in Serbian population.[9] Similar finding of underestimation of calculated LDL-C using Friedewald equation was found in a study done in Pakistan.[27] This underestimation by Friedewald equation was also reported by Kamal et al. and Chen et al.[6, 22]

Differences in the results of different studies may be attributed to diversity in population, pathologies and kits used. Measurement uncertainty that arises from three
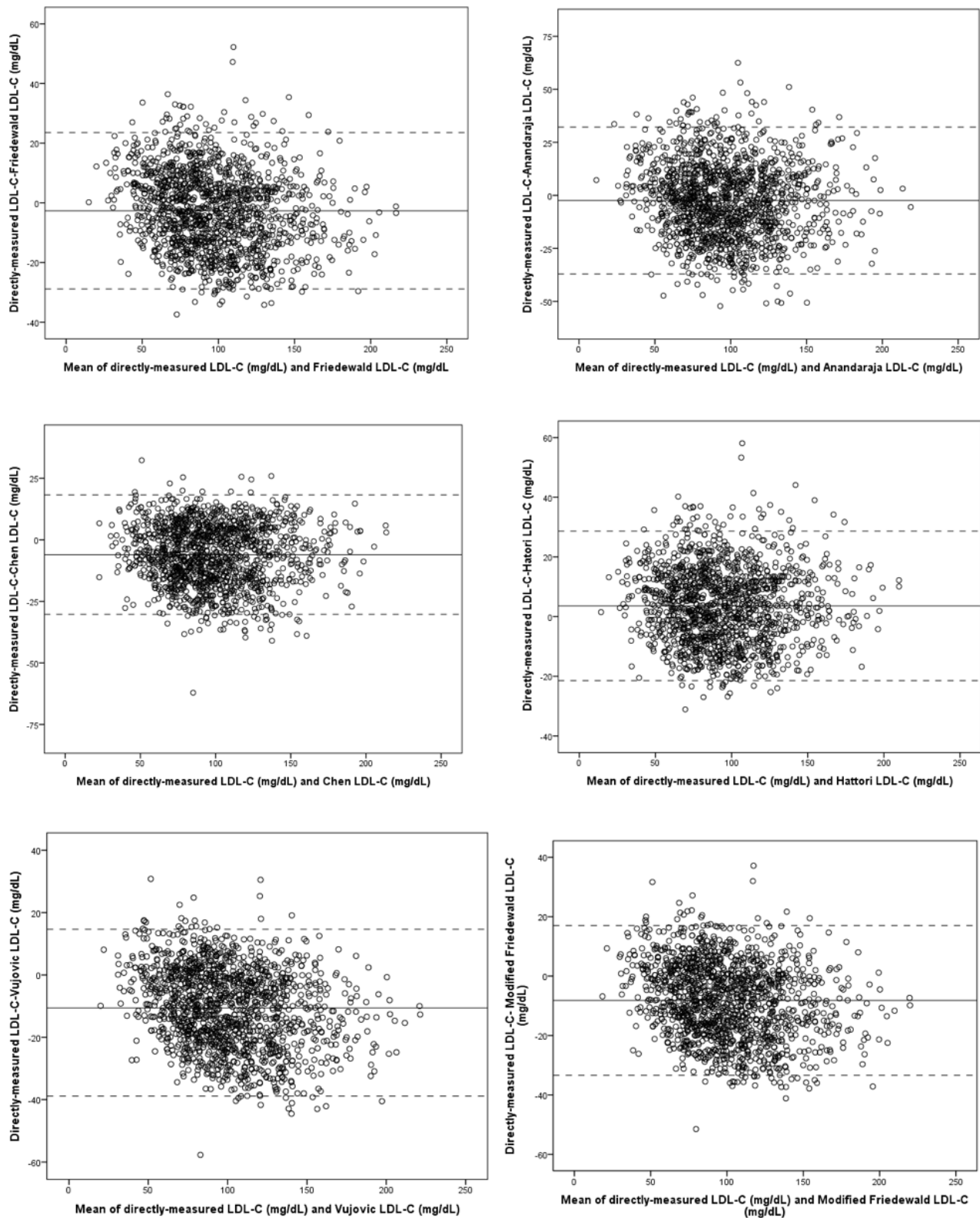
Figure 1: Bland-Altman plots of estimated formulas against directly-measured LDL-C.

independent parameters used to calculate LDL-C may have a major contribution to these differences. Arderiu and colleagues in a multicenter study reported that measurement uncertainty of direct assay was 6.9% as compared to 19.4% of calculated method and total error of calculated method was greater than the total allowable error (≤ 12) for LDL-C estimation.[28]

Friedewald equation has been shown to be relatively reliable and recommended by the NCEP as a routine

method for estimation of LDL-C despite it having several well-established constraints. It cannot be applied to samples containing TG levels > 400 mg/dL, to non-fasting samples and to samples of patients with dysbetalipoproteinemia (Fredrickson Type III).[4,29] Some authors have demonstrated that the formula should not be used in certain groups of patients with diabetes, liver and renal dysfunction even with TG levels < 400 mg/dL.[15,30,31] The results of our study showed that apart from the most commonly used Friedewald equation, Hattori equation can be used to calculate LDL-C when TG is < 200mg/dl. Unlike the Friedewald formula, the Hattori formula excludes IDL to provide a more accurate estimate of LDL-C.

The present study also had several limitations that need to be addressed. First, the beta quantification method was not used, which is considered the gold standard method for measuring LDL-C. Instead, LDL-C was measured using the enzymatic method. Second, we did not exclude participants who were taking statins or other lipid-modifying agents, which could have affected results. Other limitations of our study include the fact that racial origins were not specified and could not be considered in the analysis. However, the database is from a large hospital based population representative of the various ethnic origins of Nepal. Although patient-specific data about the disease, treatments and ethnicity was not available, our database of hospitalized patients is representative of those with diabetes, dyslipidemia and other metabolic conditions and co-morbidities.

CONCLUSIONS

Most of the LDL-C formulas correlated well with directly-measured LDL-C. Among the six LDL-C formulas, the Friedewald equation showed the best performance for estimating LDL-C, while the Hattori equation showed a higher accuracy in people with normal and borderline high TG compared with other formulas. Since the performance of calculated methods was not uniform at different TG levels, for correct cardiac risk classification, direct homogeneous assay should be the method of choice to estimate LDL-C in routine clinical laboratories. Calculation of LDL-C based on Friedewald and Hattori equation can be a good alternative for direct measurement especially in regions with limited resources.

Conflict of interest: None

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