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Comparison of calculated LDL-cholesterol using the Friedewald formula and de Cordova formula with a directly measured LDL-cholesterol in Nepalese population

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1. Introduction

Low-density lipoprotein (LDL) is one of the five major groups of lipoprotein. LDL carries cholesterol from the liver, through the blood, to the tissues of the body where it is stored [1]. High serum LDL cholesterol (LDL-C) level is associated with incidence of coronary heart disease. LDL-C lowering therapy has been shown to reduce this risk [2]. Therefore the accurate measurement of LDL-C is crucial for prevention and management of cardiovascular diseases (CVD). These reasons lead the expert panel for the detection, assessment and management of elevated blood cholesterol in adults – the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III to focus on LDL-C [3].

The gold standard for measurement of LDL-C is ultracentrifugation and beta quantification method [4]. This method is expensive and inconvenient for the routine laboratory, so it is limited to specialized lipid laboratories. Direct measurement of LDL-C using a homogeneous assay is still expensive for the labs in developing countries like ours. In addition to this, direct methods show poor performance with high triglyceride (TG) levels [5,6].

To address these problems, various formulae to calculate LDL-C have been developed [7–11]. Among these Friedewald's formula showed better performance, so it has been used widely by laboratories worldwide [12]. This formula requires fasting plasma high density lipoprotein cholesterol (HDL-C), total cholesterol (TC), and TG, and is calculated as LDL-C = TC– HDL – (TG/5) for mg/dl (TG/2.2 in mmol/l) [7]. However, this formula seems to have low accuracy in extremes of TG, presence of chylomicrons, presence of dysbetalipoproteinemia (type III hyperlipidemia) or in patients with co-morbidities including renal failure, diabetes and hepatic disease [13–15]. de Cordova et al. has developed the equation LDL-C = 0.7516 (TC - HDL-C) using10,664 Brazilian patients, including those with co-morbidities and reported to outperform several of the earlier LDL-C formulae, including Friedewald's formula [16].

This study aims to validate the application of these two formulae, Friedewald and de Cordova to calculate LDL-C in Nepalese population.

2. Materials and methods

The cross sectional study was conducted in 538 participants visiting Samyak Diagnostic Pvt. Ltd.

The samples were taken after 8 h of fasting. All samples were processed within 2 h of collection. After clotting at room temperature, serum is separated by centrifugation at 3500 rpm for 10 min. Gross hemolytic or icteric serum was rejected.

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Measurements of LDL-C, HDL-C, TC and TG were performed using reagents by Randox, according to the specification of the manufacturers using the Randox imola analyzer. The direct LDL-C was analyzed by two phase method. In first phase, chylomicron, very low density lipoprotein cholesterol (VLDL-C) and HDL-C were eliminated by esterase, oxidase and catalase then in second phase LDL-C was measured after its release by detergents. The HDL-C measurement was performed using enzymatic rate method. Total cholesterol was measured by enzymatic endpoint method. Triglyceride measurement used a glycerol phosphate oxidase – peroxidase method.

All values are expressed as mean ± standard deviation or median (range) for normally and skewed distributed variables respectively. The IBM®SPSS 22.0 statistics software was used for data analysis. The Pearson correlation test is performed to examine the various correlations. The Bland-Altman plot was employed to assess the agreement between the de Cordova formula and the direct LDL-C, and the agreement between the Friedewald formula and the direct LDL-C.

P values ≤ 0.05 were considered statistically significant.

3. Result

A total of 538 participants were included in the study. Mean age of study population being 51.45 ± 13.85 years. Among them 35.69% were male and 64.31% were female. The demographic characteristics of study population were shown in Table 1.

There was strong correlation between Friedewald LDL-C and direct LDL-C, where as slightly lower correlation was observed between de Cordova LDL-C and direct LDL-C. Pearson correlation coefficient for Friedewald and de Cordova formula was 0.93 ($p < 0.001$) and 0.88 ($p < 0.001$) respectively. The regression equation for Friedewald LDL-C is $y = 13.81 + 0.99$ x ($r = 0.85$) and that for de Cordova LDL-C is $y = 6.39 + 1.1$ x ($r = 0.77$) when calculated LDL-C is plotted in X axis and direct LDL-C is plotted in Y axis (Figs. 1 and 2). Strong correlation was noticed ($r = 0.97$) between TC and non HDL-C (TC-HDL-C).

The Bland-Altman plot of Friedewald and de Cordova LDL-C against Direct LDL-C showed mean bias of 13.08 ± 28.85 mg/dl for Friedewald LDL-C and 16.84 ± 37.16 mg/dl for de Cordova LDL-C (Figs. 3 and 4).

Friedewald LDL-C showed better correlation with direct LDL-C than de Cordova LDL-C at high and high normal TG level where as both had similar correlation with direct LDL-C at low normal and low TG level. The mean difference (measured LDL-C –calculated LDL-C)for the Friedewald formula is lower than the de Cordova formula at all levels of TG except at the TG level 200–400 mg/dl (Table 2).

4. Discussion

Measurement of LDL-C carries high importance in the management of CVD [6,17]. However accurate estimation of LDL-C is still a challenge. Direct LDL-C measurement is preferred method but this is expensive and inconvenient for the routine laboratories [18]. Friedewald formula has been used extensively for estimation of LDL-C as it performed better than several other formulae, as cost-effective and convenient method.

To estimate LDL-C from Friedewald formula fasting samples are required as it does not consider the cholesterol formed post-prandially in chylomicrons or in the intermediate-density lipoproteins or in lipoprotein (a) [19]. This formula does not distinguish between cholesterol derived from LDL-C and lipoprotein (a) leading to overestimation of the LDL-C fraction when lipoprotein (a) levels are raised [20]. Moreover, the NCEP Working Group has recommended that the LDL-C concentration should be determined with a total analytical error not exceeding ±12% (≤4% imprecision and ≤4% inaccuracy). As Friedewald formula use three parameters for LDL-C calculation, it is difficult to meet the NCEP criteria for total error and imprecision [21]. Besides, Friedewald formula considered a fixed factor of 5 for the ratio of triglycerides to very low-density lipoprotein cholesterol (TG: VLDL-C); but, the actual TG:VLDL-C ratio seems to vary significantly across the range of triglyceride and cholesterol levels [22]. These limitations of Friedewald formula lead to search for numbers of other formula but they failed to outperform Friedewald formula. However, newer formulas, Martin's formula which considered adjustable factor for TG:VLDL-C ratio and de cordova formula claim the advantage over these drawbacks of Friedewald formula.

It has been recommended that the verification of the formulae for LDL-C calculation should be done in populations different from those of the study [12]. Some part of the world has already started replacing Friedewald formula with Martin's and de cordova due to the better performance. As this study is focused at comparing formulae by de Cordova and the Friedewald against LDL-C determined by a direct method in a Nepalese population, other formulae have not been considered.

In our study both Friedewald and de Cordova formulae were highly correlated with direct LDL-C but Friedewald formula seems to

Table 1
Demographic and biochemical characteristics of study population expressed as mean ± SD or Median (Range) as possible.

Variables	Mean ± SD/Median(Range)
Age (years)	51.45 ± 13.85
Total Cholesterol (mg/dL)	181.93 ± 42.28
Triglyceride (mg/dL)	122(26–1125)
HDL- Cholesterol (mg/dL)	43(18–87)
Non HDL-Cholesterol (mg/dL)	136.69 ± 41.53
LDL- Cholesterol (Direct) (mg/dL)	118.99 ± 38.85
LDL-Cholesterol (Friedewald Formula) (mg/dL)	106.28 ± 36.46
LDL- Cholesterol (de Cordova Formula) (mg/dL)	101.25(30.8–230.3)

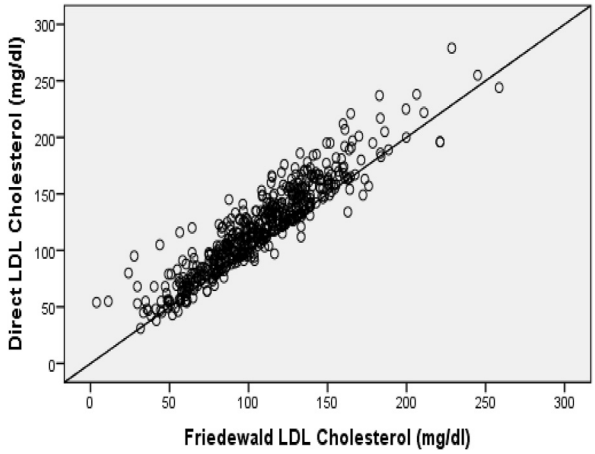

Fig. 1. Correlation between calculated LDL-C by Friedewald formula and direct LDL-C.

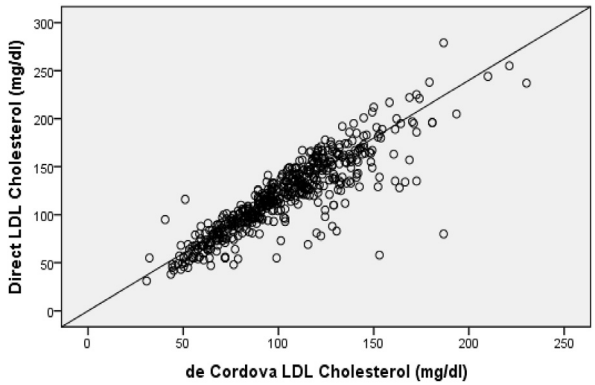

Fig. 2. Correlation between calculated LDL-C by de cordova formula and direct LDL-C.

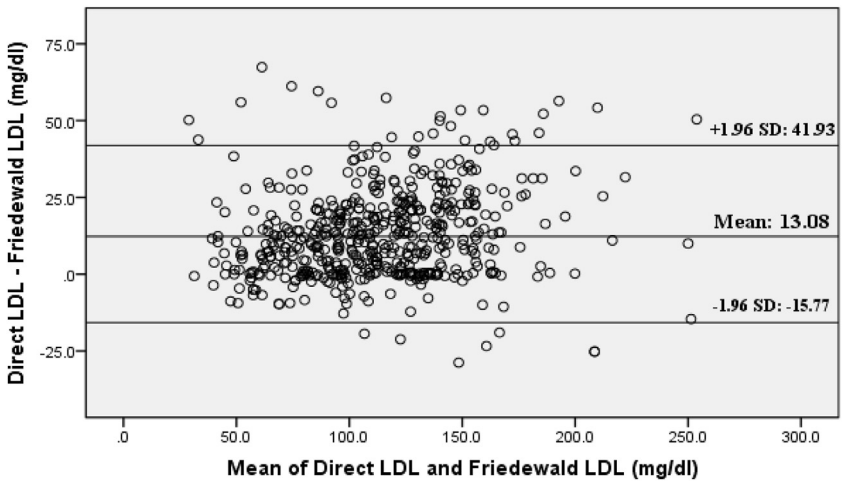

Fig. 3. Bland-Altman plots of direct LDL-C and Friedewald calculated LDL-C.

have better correlation with direct LDL-C than de Cordova formula. As high correlation coefficients do not necessarily reflect the agreement of methods, we use the Bland-Altman difference plot [23]. The Bland-Altman plot suggests a better agreement between the Friedewald formula and the directly measured LDL-C. With the de Cordova formula, LDL-C seems to be underestimated by 16.84 mg/dL and can vary from -20.32 to 54.0 mg/dL, whereas with the Friedewald formula LDL-C seems to be underestimated by 13.08 mg/dL and

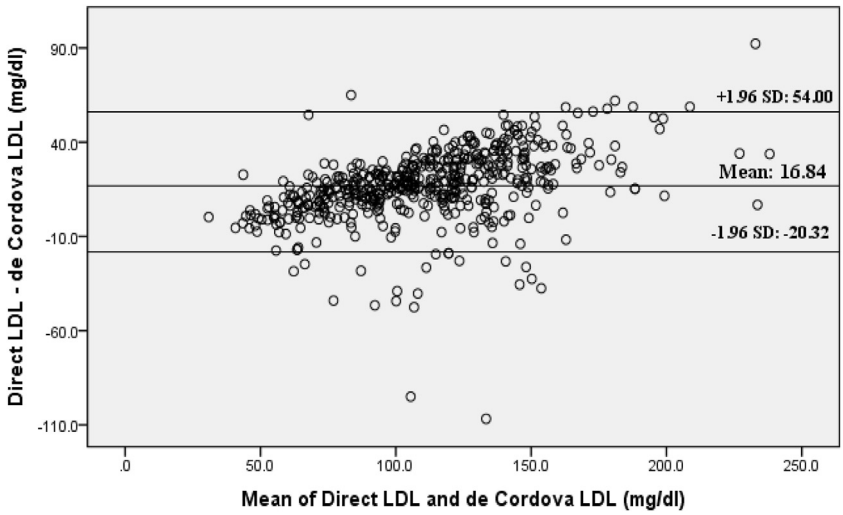

Fig. 4. Bland-Altman plots of direct LDL-C and de Cordova calculated LDL-C.

Table 2
Comparison of Friedewald LDL-C and de Cordova LDL-C with direct LDL-C at different level of TG.

LDL-C measurement	Mean ± SD (mg/dL)	Mean Difference	Correlation Coefficient (P-Value)
TG <100 (n=187)			
Direct LDL-C	107.43 ± 35.1	NA	NA
Friedewald LDL-C	101.57 ± 34.4	5.86	r = 0.95 (<0.01)
de Cordova LDL-C	87.58 ± 26.3	19.85	r = 0.95 (<0.01)
TG 100–200 (n=240)			
Direct LDL-C	123.36 ± 37.8	NA	NA
Friedewald LDL-C	109.9 ± 35.8	13.46	r = 0.94 (<0.01)
de Cordova LDL-C	103.26 ± 27.6	20.10	r = 0.94 (<0.01)
TG 200–400 (n=91)			
Direct LDL-C	132.6 ± 41.7	NA	NA
Friedewald LDL-C	111.83 ± 37.5	20.77	r = 0.90 (<0.01)
de Cordova LDL-C	122.76 ± 29.5	9.84	r = 0.87 (<0.01)
TG >400 (n=20)			
Direct LDL-C	112.6 ± 43.3	NA	NA
Friedewald LDL-C	81.71 ± 44.5	30.89	r = 0.89 (<0.01)
de Cordova LDL-C	141.18 ± 34.3	28.58	r = 0.71 (<0.01)

can vary from -15.77 to 41.93 mg/dL. The variation of 28.85 mg/dL observed with Friedewald formula is narrower than the variation of 37.16 mg/dL observed with the de Cordova formula.

These two formulae were highly correlated with the direct LDL-C at different level of TG. Similar correlation was observed between Friedewald and de Cordova formula for Triglyceride level <100 mg/dL and even up to 200 mg/dL. But Friedewald formula showed better correlation with for TG level >200 than de Cordova formula. In this study Friedewald formula performed better even at TG level greater than 400 mg/dL in contrast to the well known fact that this formula shows poor correlation at these TG level. The reason behind this might be the small sample size (n = 20) in this group compared to others. In addition to this most of the readings in this group were close to 400 mg/dl, ranging from 400 mg/dL to 450 mg/dL. Furthermore, this study showed good correlation between TC and non-HDL-C as in previous studies [6,16], which supported the evidence that non HDL-C may provide additive value to CVD risk assessment [24].

This study had several limitations. Beta quantification method, which is considered the gold standard method for measuring LDL-C, was not used to compare the calculated LDL-C. Another limitation is that this study did not include other available formulae to calculate LDL-C which might prove better for the selected population.

5. Conclusion

In a Nepalese population, there is better agreement of the Friedewald formula with a directly measured LDL-C compared to the de Cordova formula. However, this has to be verified in a larger population, including different conditions in which the Friedewald formula was found to be inaccurate.

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Declaration of competing interest

The authors declare that they have no conflict of interests.

CRediT authorship contribution statement

Santosh Pradhan: Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Keyoor Gautam:** Conceptualization, Resources, Project administration, Supervision. **Devish Pyakurel:** Conceptualization, Resources, Supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2020.e00165.

References

[1] K.R. Feingold, C. Grunfeld, "Introduction to lipids and lipoproteins", endotext [internet]. https://www.ncbi.nlm.nih.gov/books/NBK305896/, 2000.
[2] R.K. Wadhera, D.L. Steen, I. Khan, R.P. Giugliano, J. M Foody, A review of low density lipoprotein cholesterol, treatment strategies, and its impact on cardiovascular disease morbidity and mortality, J. Clin. Lipidol. 10 (3) (2016) 472–489.
[3] National Cholesterol Education Program (NCEP), Executive summary of the third report, expert panel on "Detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III)'', JAMA 285 (19) (2001) 2486–2497.
[4] M. Nakamura, Y. Kayamori, H. Iso, et al., LDL cholesterol performance of beta quantification reference measurement procedure, Clin. Chim. Acta 431 (2014) 288–293.
[5] M. Nauck, R. Warnick, N. Rifai, Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation, Clin. Chem. 48 (2002) 236–254.
[6] J. Martins, S.A.S. Olorunju, L.M. Murray, T.S. Pillay, Comparison of equations for the calculation of LDL-cholesterol in hospitalized patients, Clin. Chim. Acta 444 (2015) 137–142.
[7] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (1972) 499–502.
[8] S. Anandaraja, R. Narang, R. Godeswar, R. Laksmy, K.K. Talwar, Lowdensity lipoprotein cholesterol estimation by a new formula in Indian population, Int. J. Cardiol. 102 (2005) 117–120.
[9] Y. Chen, X. Zhang, B. Pan, et al., A modified formula for calculating low-density lipoprotein cholesterol values, Lipids Health 9 (2010) 52.
[10] Y. Hattori, M. Suzuki, M. Tsushima, et al., Development of approximate formula for LDL-chol, LDL-apo B and LDL-chol/LDL-apo B as indices of hyperapobetalipoproteinemia and small dense LDL, Atherosclerosis 138 (1998) 289–299.
[11] H. Choi, J.S. Shim, M.H. Lee, Y.M. Yoon, D.P. Choi, H.C. Kim, Comparison of formulas for calculating low-density lipoprotein cholesterol in general population and high-risk patients with cardiovascular disease, Kor. Circ. J. 46 (5) (2016) 688–698.
[12] C.P. Onyenekwu, M. Hoffmann, F. Smit, T.E. Matsha, R.T. Erasmus, Comparison of LDL-cholesterol estimate using the Friedewald formula and the newly proposed de Cordova formula with a directly measured LDL-cholesterol in a healthy South African population, Ann. Clin. Biochem. 51 (6) (2014) 672–679.
[13] S.A. Ahmadi, M.A. Boroumand, The impact of low serum triglyceride on LDL-cholesterol estimation, Arch. Iran. Med. 11 (2014) 318–321.
[14] J.P. Rubies, J.L. Reverter, M. Senti, J.B. Pedro, I. Salinas, A. Lucas, et al., Calculated low-density lipoprotein cholesterol should not be used for management of lipoprotein abnormalities in patients with diabetes mellitus, Diabetes Care 16 (1993) 1081–1086.
[15] C. Matas, M. Cabre, A. La Ville, et al., Limitations of the Friedewald formula for estimating low-density lipoprotein cholesterol in alcoholics with liver disease, Clin. Chem. 40 (1994) 404–406.
[16] C.M.M. de Cordova, M.M. de Cordova, A new accurate, simple formula for LDL-cholesterol estimation based on directly measured blood lipids from a large cohort, Ann. Clin. Biochem. 50 (1) (2013) 13–19.
[17] N.J. Stone, J.G. Robinson, A.H. Lichtenstein, et al., Guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American college of cardiology/American heart association task force on practice guidelines, Circulation 129 (2014) S1–S45.
[18] National Cholesterol Education Program, Recommendations on Lipoprotein Measurement: from the Working Group on Lipoprotein Measurement, NIH, National Heart, Lung, and Blood Institute, Bethesda (MD), 1995, p. 186.
[19] J.H. Contois, G.R. Warnick, A.D. Sniderman, Reliability of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B measurement, J. Clin. Lipidol. 5 (2011) 264–272.
[20] F. Kronenberg, A. Lingenhel, K. Lhotta, B. Rantner, M.F. Kronenberg, Konig et.al "Lipoprotein(a)- and low-density lipoprotein-derived cholesterol in nephrotic syndrome: impact on lipid-lowering therapy?", Kidney Int. 66 (1) (2004) 348–354.
[21] P.S. Bachorik, J.W. Ross, National education Program recommendations for measurements of low-density lipoprotein cholesterol: executive summary. National cholesterol education Program working group on lipoprotein measurements, Clin. Chem. 41 (1995) 1414–1420.
[22] S.S. Martin, M.J. Blaha, M.B. Elshazly, P.P. Toth, et al., Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile, JAMA 310 (19) (2013) 2061–2068.
[23] D. Giavarina, Understanding Bland altman analysis, Biochem. Med. 25 (2) (2015) 141–151, 2015.
[24] A.D. Sniderman, K. Williams, J.H. Contois, H.M. Monroe, M.J. McQueen, J. de Graaf, et al., A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk, Circ. Cardiovasc. Qual. Outcomes 4 (3) (2011) 337–345.