Introduction

Cardiovascular diseases (CVD) have emerged as the leading cause of mortality worldwide causing death of more than 9 million people in 2019.[1] Increased levels of low-density lipoproteins cholesterol (LDL-C) in serum have been identified to be associated with higher risk of CVDs. National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) recommends clinical guidelines targeting LDL-C as a marker for diagnosis and treatment of CVD.[2] For appropriate management, the accuracy of LDL-C estimation is essential. Recent guidelines by The American College of Cardiology/American Heart Association (ACC/AHA) and European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) have recommended the use of alternative homogenous assays for LDL-C measurement, most of the laboratories still use Friedewald Equation (FE). However, various novel equations have shown better performance than FE specific to a particular population. Besides, no equation has been devised for use in Sub-Himalayan population.

Methods: A cross-sectional laboratory data-based study was conducted by recruiting lipid profiles of 1851 samples to validate 10 different equations for calculating LDL and to devise a novel Modified Friedewald Equation (MFE) specific for Sub-Himalayan population. Results: The novel MFE is presented as: LDL-C = -2.421 + (0.752 × TC) - (0.047 × TG) - (0.350 × HDL). A significant difference was observed between direct LDL-C (118.84 ± 40.39 mg/dL) and all other equations except MFE (118.84 ± 37.96 mg/dl, P > 0.999) and Puavilai Equation (117.99 ± 49.05 mg/dL, P = 0.138). Additionally, MFE showed lowest mean percentage bias of 0.14% with 95% limits of agreement within ± 2SD on Bland-Altman analysis. On ROC analysis at cut-offs of clinical decision limits of 100 mg/dl, 130 mg/dl, 160 mg/dl, and 190 mg/dl, MFE outperformed all other equations with highest AUC (0.974, 0.978, 0.982, and 0.995) respectively with specificity >95% at higher levels. MFE also showed highest correlation (r = 0.954, P < 0.001) and least rMSE (13.8) with direct LDL although all the equations showed significant positive correlation with direct LDL (p < 0.001). Conclusion: MFE derived in this study showed a better diagnostic performance as compared to other 10 equations taking Direct LDL-C as gold standard for Sub-Himalayan Population and may be used as a substitute for FE in the study population.

Keywords: Equation derivation, low density lipoprotein (LDL-C), regression analysis, sub-Himalayan population
have emphasised on aggressive lowering of LDL Cholesterol as a primary goal of management to reduce cardiovascular risk.[13] Another recent study has shown LDL-Cholesterol to be an independent risk factor for all CVD-related events like Coronary artery disease, stroke, hospitalization, and so on.[14] Gold standard method for LDL-C analysis is beta-quantification technique and ultracentrifugation.[15] These methods are tedious, time-consuming, expensive, impractical, and are not available in routine laboratories. Currently, alternative homogenous assays for measuring LDL-C directly have also been developed. However, these are expensive and are also not available everywhere especially in poor resource settings. Moreover, these methods are unreliable at high triglyceride levels, conditions with heterogeneous triglyceride, and lipid disorders.[16] Due to its impracticality of gold standard and Hattori Puavilai[17] In practice, LDL-C is most commonly reported as a primary goal of management to reduce cardiovascular risk. These methods are tedious, time-consuming, expensive, impractical, and are not available in routine laboratories. Currently, alternative homogenous assays for measuring LDL-C directly have also been developed. However, these are expensive and are also not available everywhere especially in poor resource settings. Moreover, these methods are unreliable at high triglyceride levels, conditions with heterogeneous triglyceride, and lipid disorders.[16] Due to its impracticality of gold standard methods, Friedewald Equation (FE) was developed for LDL-C calculation.[17] In practice, LDL-C is most commonly reported using the FE (LDL-C = (Total cholesterol) − (High density lipoproteins) - (Triglyceride)/5). However, there are significant shortcomings and limitations to the FE. It is not valid for Triglyceride levels more than 500 mg/dl (12.93 mmol/L). Previous studies have shown inconsistent performance of FE among different populations where FE has been shown to underestimate LDL-C even for TG levels <400 mg/dl (10.344 mmol/L) in Japanese, North African Populations.[8,19] Thus, certain other equations have been developed by researchers with regard to their populations. Puavilai Equation[20] was found to be better among the available equations, as reported by a study conducted on North African population.[20] Vujovic et al.[21] developed an equation for Serbian population. Nevertheless, it was not assessed in Indian setting. In India, Anandaraja et al.[22] and Huchegowda et al.[23] have developed equation for LDL-C estimation. However, Indian subcontinent demonstrates heterogeneity in terms of climate, cultural, and dietary habits. Previous studies in India have included population from plain regions and not from hilly and sub-Himalayan areas. Sub-Himalayan region has variation in terms of climatic changes and its population has cultural differences relative to plains. In hilly areas, higher altitude may provide different environment to induce adaptive changes at the level of metabolism of nutrients as a result of chronic hypobaric hypoxia.[19] These adaptations may have bearing upon factors determining LDL-C, implying need of derivation of new equation for LDL-C calculation for Sub-Himalayan population. Additionally, recent studies have demonstrated an increasing trend of obesity, diabetes mellitus, metabolic syndrome in Sub-Himalayan region due to urbanisation and changed life style in urban as well as semi-urban regions. Another study has pointed similar changes in BMI, abdominal obesity even in tribal population of sub-Himalayan region. Therefore, it is important to study dyslipidaemia in this relatively under-studied population so as to help in making policy decisions for control of CVD. [6-18]

Accordingly, aim of this research was to validate existing equations to determine LDL-C and devise a new equation with special consideration of Sub-Himalayan population. This study may provide guidelines to clinicians for better management of CVDs by utilising fasting lipid profile in sub-Himalayan population.

Materials and Methods

Study design
A cross-sectional comparative laboratory data based study was conducted at a tertiary care referral centre in Sub-Himalyan region after obtaining ethical clearance from Institutional Ethical Committee (AIIMS/IEC/19/1242).

Sample size
Data record of 1851 fasting blood samples submitted to Clinical Biochemistry laboratory, AIIMS Rishikesh from January 2019 to December 2019, was accessed and evaluated in the study.

Biochemical analysis
Serum total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), and direct LDL-C as measured on AU5800 Clinical Chemistry Automated System (Beckman Coulter, Inc., USA), based on cholesterol esterase-cholesterol oxidase-peroxidase (CHO-POD), glycerol phosphate oxidase-peroxidase (GPO-POD), enzymatic immunoinhibition and enzymatic selective protection respectively, as per manufacturer’s instructions (Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA). Samples were run only after two levels of internal quality controls were found satisfactory. The laboratory is registered for external quality assurance scheme (EQAS) with Christian Medical College, Vellore. EQAS results were satisfactory during that period.

Calculation of LDL-cholesterol based on previous equations
Further, LDL-C was calculated using equations proposed by Friedewald et al.,[7] Puavilai et al,[11] Vujovic et al,[21] Anandaraja et al,[3] Gowda et al,[14] Teerakancharna et al,[3] Almadi et al,[24] Cordova et al,[25] Chen et al,[22] and Hattori et al[23] and as mentioned in [Table 1].

Derivation of equation for LDL-C
A mathematical formula for LDL-C based on TC, TG, and HDL was developed through linear regression analysis. The best-fitting line was found using the method of least squares by minimising the sum of the squares of the errors in the approximations of the LDL-C. The correlation coefficient (r) of TC with LDL-C was very high (r = 0.91); however, correlation coefficient of TG as well as of HDL with LDL-C was less than 0.3. Accordingly, TC had more contribution in the prediction of LDL-C. The derived equation (Modified Friedewald’s equation) to predict LDL-C through regression is presented

\[ \text{LDL-C} = -2.421 + (0.752 \times \text{TC}) - (0.047 \times \text{TG}) - (0.350 \times \text{HDL}). \]

Statistical analysis
Direct LDL-C by homogenous assay was compared with the LDL-C obtained by aforementioned equation as well as newly devised formula using paired t-test. Percentage bias was calculated for all stated equation taking Direct LDL-C as reference using
Further, Bland-Altman plots were plotted for each formula taking direct LDL as reference method. Percentage Bias, Bias, and limits of agreement (LOA) are depicted in [Table 2]. Bland-Altman plots of differences between direct LDL-C and MFE LDL-C, expressed as bias [(direct LDL-C) – (calculated LDL-C)] versus mean of direct LDL-C and calculated LDL-C for Chen equation, Teerakanchana equation, Puavilai Equation and MFE are shown in [Figure 1]. MFE showed lowest mean percentage bias of 0.14% with 95% limits of agreement within ± 2 SD on Bland-Altman analysis. On correlation analysis, calculated LDL-C by all the equations showed significantly high correlation with direct LDL-C (p < 0.001). Again, correlation coefficient, rMSE was also calculated for all stated equations and is shown in [Table 3]. Highest correlation with direct LDL-C was observed by MFE and Chen equation (r = 0.954). Again, correlation coefficient, rMSE was also calculated for all stating equations and is shown in [Table 3]. Highest correlation with direct LDL-C was observed by MFE and Chen equation (r = 0.954). Again, correlation coefficient, rMSE was also calculated for all stating equations and is shown in [Table 3]. Highest correlation with direct LDL-C was observed by MFE and Chen equation (r = 0.954). Again, correlation coefficient, rMSE was also calculated for all stating equations and is shown in [Table 3]. Highest correlation with direct LDL-C was observed by MFE and Chen equation (r = 0.954). Again, correlation coefficient, rMSE was also calculated for all stating equations and is shown in [Table 3]. Highest correlation with direct LDL-C was observed by MFE and Chen equation (r = 0.954). Again, correlation coefficient, rMSE was also calculated for all stating equations and is shown in [Table 3]. Highest correlation with direct LDL-C was observed by MFE and Chen equation (r = 0.954). Again, correlation coefficient, rMSE was also calculated for all stating equations and is shown in [Table 3]. Highest correlation with direct LDL-C was observed by MFE and Chen equation (r = 0.954). Again, correlation coefficient, rMSE was also calculated for all stating equations and is shown in [Table 3]. Highest correlation with direct LDL-C was observed by MFE and Chen equation (r = 0.954). Again, correlation coefficient, rMSE was also calculated for all stating equations and is shown in [Table 3]. Highest correlation with direct LDL-C was observed by MFE and Chen equation (r = 0.954). Again, correlation coefficient, rMSE was also calculated for all stating equations and is shown in [Table 3]. Highest correlation with direct LDL-C was observed by MFE and Chen equation (r = 0.954). Again, correlation coefficient, rMSE was also calculated for all stating equations and is shown in [Table 3].

Correlation coefficient was determined between all the calculated LDL-C and direct LDL-C. Again, root mean square error (rMSE) was calculated to assess error in the derived LDL-C by various equations. The P value < 0.05 was considered as statistically significant. Statistical software, STATA/SE version 14.2 (Stata Corp LP, College Station, TX, USA), SPSS v. 23, and Graphpad prism v 5 was used for the analysis.

Results

Population consisted of 891 females and 960 males, with mean age of 47.60 (±14.37) years. Mean (±SD) of Serum TC, TG, HDL, and direct LDL-C were obtained as 194.01 (±55.60) mg/dl, 170.57 (±15.24) mg/dl, 47.59 (±20.80) mg/dl, and 118.84 (±40.39) mg/dl respectively. The predicted values of LDL-C through our equation (referred to as “Modified-Friedwald Equation” or “MFE” henceforth) and by various other equations were compared with direct LDL-C value and presented in [Table 2]. In case of FE, 1779 samples were used for analysis because 72 samples had TG >400 mg/dl and hence were excluded from analysis.

It was observed that all equations showed significantly different LDL-C values from direct LDL-C value (118.84 ± 40.39) including FE (113.57 ± 41.05, P < 0.001) except MFE with mean LDL-C (118.84 ± 37.96, P > 0.999) and Puavilai Equation (117.99 ± 49.05, P = 0.138) as depicted in [Table 2].

Percentage bias was calculated as depicted in [Table 3]. MFE showed lowest mean percentage bias of 0.14%, whereas LDL-C calculated by equation of Teerakanchana, Puavilai, and Vujovic also showed acceptable bias of less than 3% as per NCEP recommendations. Further, Bland-Altman plots were plotted for each formula taking direct LDL as reference method. Percentage Bias, Bias, and limits of agreement (LOA) are depicted in [Table 2]. Bland-Altman plots of differences between direct LDL-C and MFE LDL-C, expressed as bias [(direct LDL-C) – (calculated LDL-C)] versus mean of direct LDL-C and calculated LDL-C for Chen equation, Teerakanchana equation, Puavilai Equation and MFE are shown in [Figure 1]. MFE showed lowest mean percentage bias of 0.14% with 95% limits of agreement within ± 2 SD on Bland-Altman analysis. On correlation analysis, calculated LDL-C by all the equations showed significantly high correlation with direct LDL-C (p < 0.001). However, highest correlation coefficient was observed with MFE and Chen equation (r = 0.954, P < 0.001). Again, correlation coefficient, rMSE was also calculated for all stated equations and is shown in [Table 3]. Highest correlation with direct LDL-C was observed by MFE and Chen equation (r = 0.954). Again, MFE was also showed best in terms of least rMSE among all the other equations.

As per NCEP treatment guidelines, clinical decision levels for LDL Cholesterol at 100 mg/dl (2.58 mmol/L), 130 mg/dl (3.36 mmol/L), 160 mg/dl (4.13 mmol/L), and 190 mg/dl (4.91 mmol/L) were used as cut-off to determine AUC, sensitivity and specificity. Clinical decision levels for LDL-C through our equation (referred to as “Modified-Friedwald Equation” or “MFE” henceforth) and by various other equations.

### Table 1: Mathematical Equations for calculated LDL-C by different formulae

| Formula for calculated LDL-C | Mathematical Equation | Reference No. |
|-----------------------------|----------------------|---------------|
| Ahmadi’s equation           | LDL-C=TC/1.19 + TG/1.9 - HDL/1.1 - 38 | [20] |
| Anandaraja’s equation       | LDL-C=(0.9TC) - (0.9XTG)/5 - 28 | [13] |
| Chen’s equation             | LDL-C=Non-HDL-C × 90% - TG × 10% | [22] |
| Cordova’s equation          | LDL-C=3/4 (TC - HDL) or 0.7516 × TC - HDL | [21] |
| Friedewald’s equation       | LDL-C=TC - HDL - (TG/5) | [7] |
| Gowda’s equation            | LDLcal= (0.88 × TC) - (0.02 × TG) - (0.03 × HDL) - 37.48 | [14] |
| Hattori’s equation          | LDLc=Chol-HDLc - VLDLc - IDLc=0.94 Chol - 0.94 HDLc - 0.19 TG | [23] |
| Puavilai’s equation         | LDL=Total cholesterol-HDL-1/6 triglyceride | [11] |
| Teerakanchana’s equation    | LDL-C=0.910 TC-0.111 TG - 0.634 HDL-C - 6.755 | [19] |
| Vujovic’s equation          | S-LDL-C (mmol/L) = TC - TG/3 - HDL-C | [12] |

### Table 2: Comparison of mean direct LDL and mean of LDL calculated using various equations

| Study Formulae                  | LDL (mg/dl) (Mean±SD) | P     |
|--------------------------------|-----------------------|-------|
| Friedewald’s equation          | 113.57±41.05          | <0.001|
| Chen’s equation                | 114.72±44.24          | <0.001|
| Teerakanchana’s equation       | 120.69±44.30          | <0.001|
| Hattori’s equation             | 105.23±46.79          | <0.001|
| Anandaraja’s equation          | 115.91±46.39          | <0.001|
| Chen’s equation                | 171.55±108.45         | <0.001|
| Gowda’s equation               | 128.41±47.58          | <0.001|
| Puavilai’s equation            | 117.99±49.05          | 0.138 |
| Cordova’s equation             | 110.05±40.35          | <0.001|
| Vujovic’s equation             | 121.52±48.91          | <0.001|
| Modified Friedewald’s equation | 118.84±37.96          | >0.999|
which was the highest just after Chen equation with AUC of 0.975. However, at cut-off 130 mg/dl (3.36 mmol/L), 160 mg/dl (4.13 mmol/L), and 190 mg/dl (4.91 mmol/L) MFE outperformed all other equations with AUC of 0.978, 0.982, and 0.995 respectively as shown in Table 4. MFE showed >95% sensitivity at 100 mg/dl (2.58 mmol/L). It showed highest specificity of >95% at 160 mg/dl (4.13 mmol/L) and >99% specificity at 190 mg/dl (4.91 mmol/L) among all calculated equations as depicted in Table 4 and AUC at various clinical decision limits is shown in Figure 2.

**Discussion**

Cholesterol plays essential role in maintaining good health. It is indispensable component of cell membranes and precursor to many steroid hormones and bile acids required for lipid digestion. Nevertheless, it has earned bad reputation for its morbid role in CVDs at higher levels, as evidenced from epidemiological studies like Framingham and MRFIT. Cholesterol being nonpolar is transported along with proteins in blood circulation, together forming lipoproteins. Though there are different classes of lipoproteins, LDL has been identified as a risk factor for CVD.

Most of the treatment guidelines including NCEP recommends LDL levels for therapeutic decisions. Determination of LDL-C by its reference method is not possible in routine clinical laboratory as it involves preparative ultracentrifugation. Though alternative methods have been developed, many peripheral laboratories still rely on FE for LDL-C determination. Reliability of FE has been reviewed by many previous studies and has shown contradictory results. Consequently, researchers have developed their own equation for their population-specific use.

In India also, two studies have derived equation for LDL-C calculation. However, India has a heterogeneous population due to wide variation in geographical area and ethnicity. Previous equations are based on population from the plains.
Uttarakhand is a state with distinct geographic and cultural habits that may have bearing on body’s metabolism. A recent upward trend has been observed with respect to central obesity, hypertension, Diabetes Mellitus, metabolic syndrome in Uttarakhand, and adjoining sub-Himalayan population due to increasing urbanisation and consequent life-style modification.[16] An early assessment of the risk factors has a key role in prevention of CVD. Therefore, a population-specific equation for LDL estimation may help a primary care physician to make an appropriate management decision despite lack of sophisticated and expensive instrumentation in far flanged areas.[16,18]

As no such study was conducted in study population, current study was planned to compare diagnostic performance of 10 different available equations with direct LDL and to devise a novel equation in this particular population and compare its performance with 10 other equations.
A significant difference was observed between direct LDL and most of the equations except MFE and Puavilai equation. This corroborates with a study in North African Population where they have observed Puavilai equation to be comparable to direct LDL among other equations. The plausible explanation given is lower contribution of TG in Puavilai equation. In MFE derived in current study, the contribution of TG is even lesser than Puavilai equation. Similarly, equation by Gowda also has very little contribution with respect to TG. However, it failed to show comparability with direct LDL in current population.

As per NCEP recommendations, for method validation of LDL, bias of >3% is not acceptable. In the current study, acceptable bias was observed with Puavilai, Teerakanchana, Vujovic, and MFE. Among them also, MFE showed least %bias of 0.14% with respect to direct LDL Cholesterol. On Bland-Altman analysis, only MFE and Puavilai showed definitive agreement with 95% limits of agreement within ± 2SD.

As per NCEP-ATP III guidelines, clinical decision limits of 100 mg/dl, 130 mg/dl, 160 mg/dl, and 190 mg/dl have been considered for classifying LDL Cholesterol into optimal or high/very high and to decide whether lifestyle modification or drug therapy is required for such patients.

Considering these levels, ROC analysis was performed to evaluate diagnostic performance in terms of sensitivity and specificity for each equation with respect to direct LDL. It was observed that MFE showed highest AUC with sensitivity >95% at cut-off of 100 mg/dl (2.58 mmol/L) and 130 mg/dl (3.36 mmol/L). Specificity improved with increasing cut-off levels with >95% specificity at 160 mg/dl (4.13 mmol/L), and >99% at 190 mg/dl (4.91 mmol/L). Although Chen equation was primarily devised for TG >400 mg/dl, it failed to show that kind of accuracy and specificity in the study population. Equation by Friedewald, Ahmadi, and Anandaraja however showed the least AUC in study population. The possible reason for this observation may be the higher contribution of TG in all these equations, which become inconsistent and heterogeneous as TG concentrations increases.

On correlation analysis and on calculation of rMSE, MFE showed highest correlation with direct LDL although all the equations showed significant positive correlation with direct LDL.

Taking all this together, MFE gave most accurate and reliable results. Puavilai equation also showed good results in terms of less bias; however, it had a poor diagnostic sensitivity, specificity, larger rMSE, and weaker correlation than MFE. FE was also not found appropriate and gave significantly different values of LDL-C from direct values. When we checked for correlation between the calculated LDL-C and direct LDL-C for various equations, MFE and Chen equation showed best correlation. Huchegowda et al. support our result and found Chen equation
to have best correlation among previous equations in their study. However, under present consideration Chen equation gave significantly different LDL-C values as compared to direct LDL-C values. Besides, Chen equation showed a larger rMSE as well as bias by Bland-Altman analysis. Moreover, its diagnostic performance was found to be lower than MFE at higher clinical decision limits for which it was primarily devised. None of the Indian study however showed comparable results.

The strength of the present study lies in its sample size (n = 1851), being a first study in sub-Himalayan population and it is also the first study where diagnostic performance at clinical decision levels of LDL Cholesterol has been considered. Moreover, 10 different previously available equations have been compared to validate the study equation. The derived equation also has very less contribution of TG in the equation, which may be helpful in dyslipidaemias and higher TG values. However, further study needs to be done to evaluate validity of this equation in dyslipidemias.

Similar to any study, this study also has certain limitations. Our data did not represent the general population as it was taken from the patients who attended hospital and it is imperative to validate this equation in general population of this region in community-based study and also in other similar group of populations as well as dyslipidemia patients, in particular.

Conclusions

Modified Friedewald Equation derived in this study showed a better diagnostic performance as compared to other 10 equations taking Direct LDL-C as gold standard for Sub-Himalayan population and may be used as a substitute for Friedewald Equation in the study population. Considering the effect of climatic condition, racial and ethnic background, this study emphasises the need to validate any equation for LDL calculation before being put into clinical use.

List of abbreviations

CVD - cardiovascular diseases
LDL-C - low-density lipoproteins cholesterol
NCEP ATP - National Cholesterol Education Program Adult Treatment Panel
FE - Friedewald Equation
TG - Triglyceride
TC - total cholesterol
HDL - high density lipoproteins
CHO-POD - cholesterol esterase-cholesterol oxidase-peroxidase
GPO-POD - glycerol phosphate oxidase-peroxidase
EQAS - external quality assurance scheme
ROC - Receiver operating characteristics
AUC - area under curve
rMSE - root mean square error
MFE - Modified-Friedewald Equation
LOA - limits of agreement.

Impact statement

The strength of the present study lies in its sample size (n = 1851), being the first study in sub-Himalayan population and it is also the first study where diagnostic performance at clinical decision levels of LDL Cholesterol has been considered. Moreover, 10 different previously available equations have been compared to validate the study equation. Considering the effect of climatic condition, racial and ethnic background, this study emphasises the need to validate any equation for LDL calculation before being put into clinical use.

Highlights

- First study to derive equation for LDL calculation in sub-Himalayan region
- Validation of the equation in large cohort at different clinical decision limits
- Accurate population specific LDL estimation may help primary care physician as well as policy makers in making decisions in management of NCDs
- Increasing urbanisation and life style modification in Sub-Himalayan region necessitates population-based studies.

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Conflicts of interest

There are no conflicts of interest.

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