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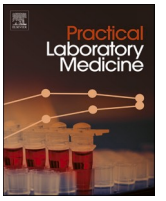

Registered Report Stage II

Choosing the right equation for calculating indirect LDL-Cholesterol (LDL-C) in adult Pakistani population: Evaluation of seven equations using big data analytics

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ABSTRACT

Objective: Cardiovascular diseases (CVDs) are a leading cause of mortality worldwide. Low density lipoprotein cholesterol (LDL-C) contributes to the atherogenic process. However, direct LDL-C (d-LDL) has rarely been estimated by the gold standard method because it is cumbersome and expensive. We aim to evaluate calculated low density lipoprotein (LDL-c) by various equations with reference to directly measured LDL-C in the Pakistani adult population as a cost-effective alternative.

Methods: We retrospectively evaluated the validity of seven equations for estimating calculated LDL-C by computing correlation coefficients (r) and Bland Altman plots to assess agreement (mean %) for (d-LDL) and calculated (LDL-c) on all seven equations. Statistical analysis was performed in Stata Statistical Software: Release 17, College Station, TX: StataCorp LLC.

Results: We analyzed 247082 direct assays of lipid profiles of adults aged ≥18 years. The mean LDL-C levels computed on Friedewald, de Cordova, Chen, Hattori, Vujovic, Teerakanchana, Sampson equations were 106.8 ± 31.4, 103.7 ± 25.0, 108.6 ± 28.2, 100.1 ± 29.5, 115.2 ± 31.2, 113.1 ± 28.3 and 110.3 ± 30.6 respectively. Friedewald and Hattori equations correlated strongly with direct LDL-C (r = 0.937) for each followed by Sampson (r = 0.935) and Vujovic (r = 0.931). However, the median bias was least for the Friedwald equation (−1.6) compared to the other equations.

Conclusion: In contrast to the global literature advocating for the use of newer equations, although the conventional and widely utilized Friedewald equation remains the best alternative for calculated LDL-C estimation in adult Pakistani population.

Abbreviation:

LDL-C	Low density lipoprotein cholesterol
CVDs	Cardiovascular diseases
d-LDL	Direct low density lipoprotein

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(*continued*)

LDL-c	Calculated low density lipoprotein
CAP	College of American Pathologists
HDL-C	High density lipoprotein cholesterol
TG	Triglycerides
TC	Total cholesterol
NCEP	National Cholesterol Education Program
AKUH	Aga Khan University Hospital
ASCVD	Atherosclerotic Cardiovascular Disease
ILMS	Integrated Laboratory Management System
CLIA	Clinical Laboratory Improvement Amendments

1. Introduction

Globally, the most common cause of morbidity and mortality is cardiovascular disease (CVD) [1]. Various epidemiological and clinical studies have shown that raised level of low-density lipoprotein cholesterol (LDL-C) is a major risk factor for plaque formation and development of atherosclerotic cardiovascular disease (ASCVD) [2]. A few clinical trials have demonstrated that LDL-C lowering therapy can reduce the risk cardiovascular disease [3]. There is a positive correlation between LDL-C and CVDs, and many guidelines have focused on LDL-C as a major clinical parameter for categorization and treatment of dyslipidemia [4,5]. Accurate LDL-C estimation is of paramount importance and the more LDL-C is reduced on statin therapy, the greater will be subsequent risk reduction in patients with clinical ASCVD. For individuals with diabetes mellitus who are at higher risk, particularly those with multiple risk factors or those between 50 and 75 years old, it is advisable to use a high-intensity statin to achieve a reduction in LDL-C levels of at least 50 % [6]. South Asians also tend to develop more severe coronary artery disease at a younger age and may also suffer from earlier myocardial infarction and heart failure. Low-density lipoprotein (LDL) is a particle that consists of outer phospholipids, apolipoproteins, free cholesterol, inner triglycerides, and cholesterol ester, which carries cholesterol from the liver to the peripheral tissues [7] (see Figs. 1 and 2).

Accurate and precise measurement of LDL-C is an essential step in clinical practices for evaluation of CVD risk stratification, prevention, treatment and assessing effect intervention such as statins. For estimation of LDL-C levels in blood, various direct automated measurement methods are available. Beta quantification, which involves ultracentrifugation and polyanion precipitation, is the gold standard method for accurately measuring LDL-C by separating lipoproteins [8]. LDL-C is not affected by chylomicrons or other lipoproteins in this method. This method is not suitable for routine laboratory analysis because it is labor-intensive, costly, cumbersome, time consuming and requires large specimen volumes [9]. Over the period, many direct homogenous assays have been developed for the determination of LDL-C in serum with satisfactory degree of accuracy but still they are expensive for routine use [10].

However, mainly due to economic reasons, instead of the direct measurement of LDL-C, the calculation methods are extensively used in clinical laboratories usually in developing countries. More than 40 years ago, Friedewald equation was developed for the indirect estimation of LDL-C [11]. Friedewald equation is simple and cost effective, but it cannot be utilized in non-fasting samples as it

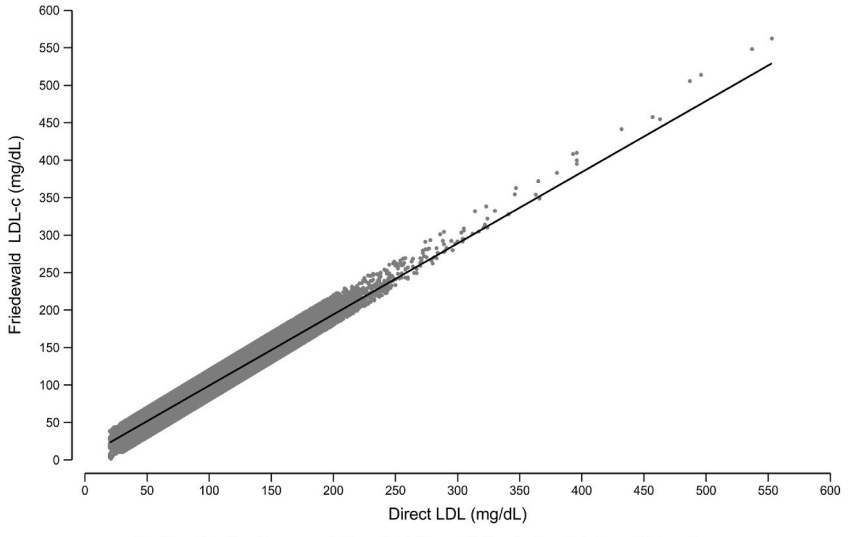
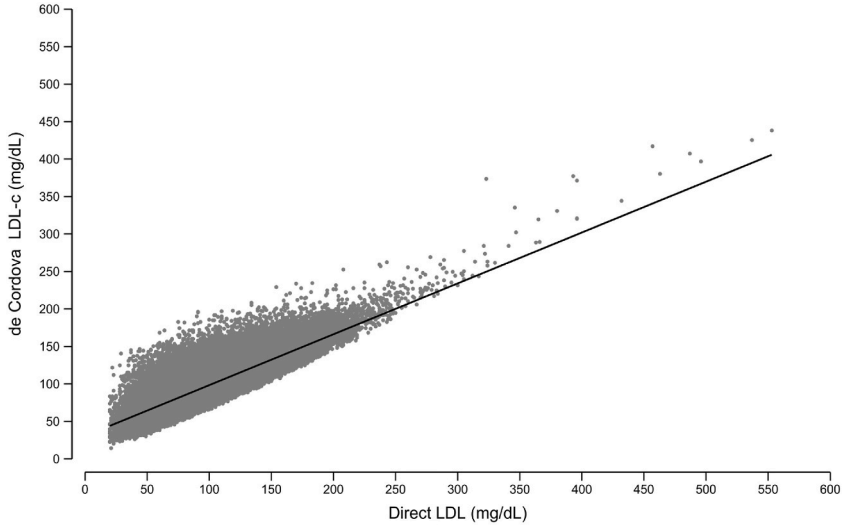
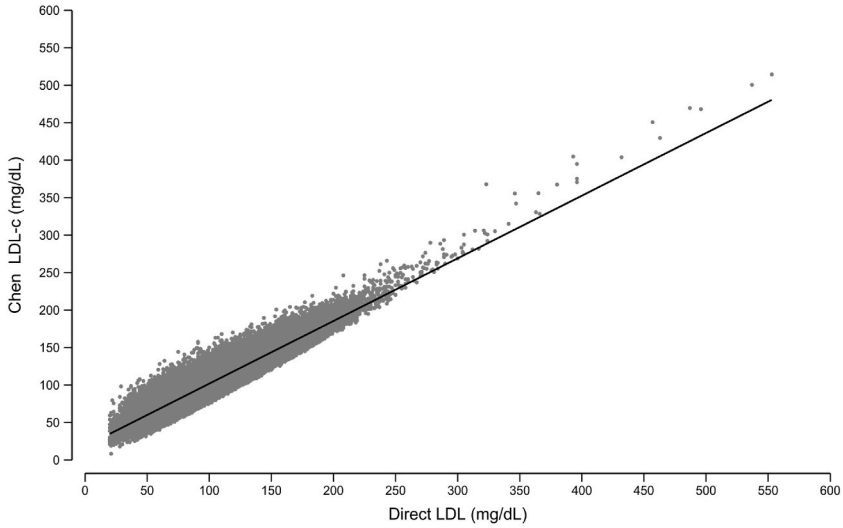


Fig. 1. Scatter plots showing association of d-LDL with LDL-c by different equations.



b. Scatter plot showing association of d-LDL vs. LDL-c by the de Cordova Equation



c. Scatter plot showing association of d-LDL vs. LDL-c by the Chen Equation

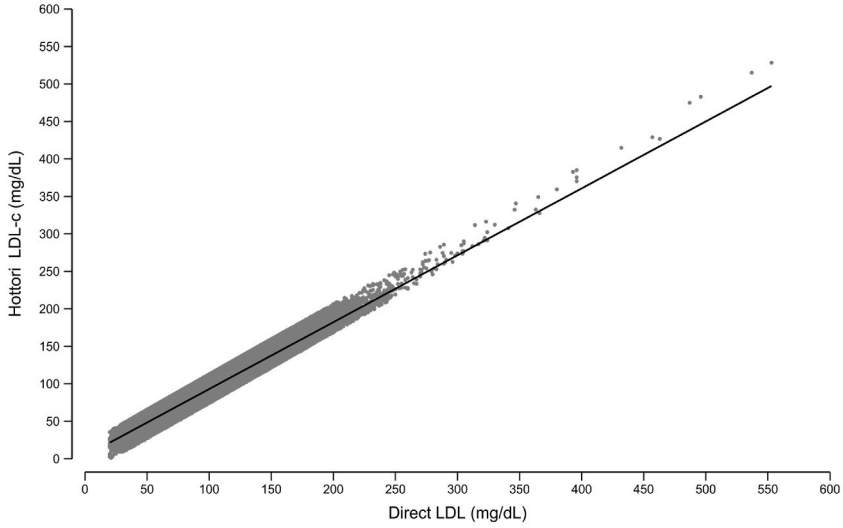
Fig. 1. (*continued*).

does not consider the cholesterol formed post-prandially in chylomicrons, intermediate density lipoproteins or in lipoprotein (a). Some conditions such as very high or low triglyceride levels, diabetes, renal disease, metabolic syndrome, and disorders of lipoproteins diminish the accuracy of this formula [11,12]. There are many other formulas for the calculation of LDL-C such as Chen, de Cordova and Hattori which have not been validated in diverse populations [13–15]. There is an ongoing search for new equations and most equations have been developed to calculate population specific LDL-C. We aim to evaluate various calculated LDL-C equations with reference to directly measured LDL-C in the Pakistani adult population as a cost-effective alternative.

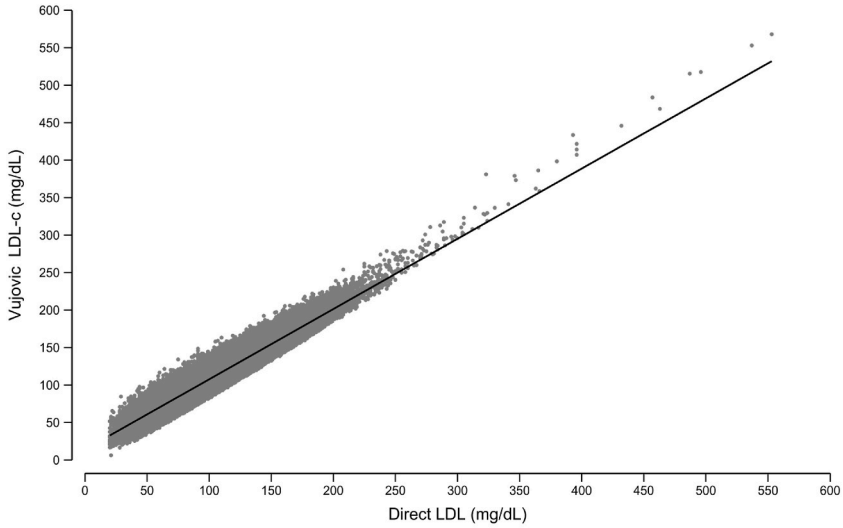
2. Materials and methods

2.1. Study design

A cross sectional analysis was conducted at the Section of Chemical Pathology, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi from 2021 to 2023 after approval from the ethical review committee of Aga Khan University Hospital (AKUH). The laboratory is accredited by College of American Pathologists (CAP) and serves a large tertiary care academic hospital. The study was reviewed and approved as an exemption from the ethical review committee of Aga Khan University Hospital (2022-8284-



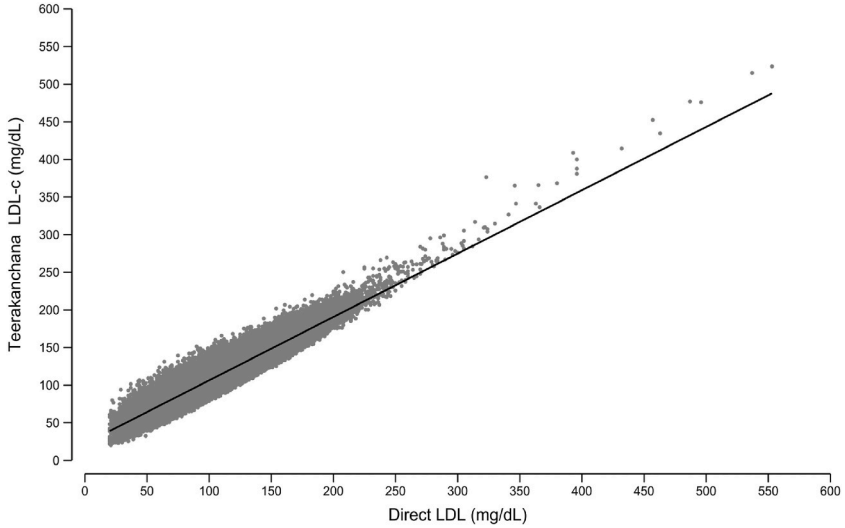
d. Scatter plot showing association of d-LDL vs. LDL-c by the Hottori Equation



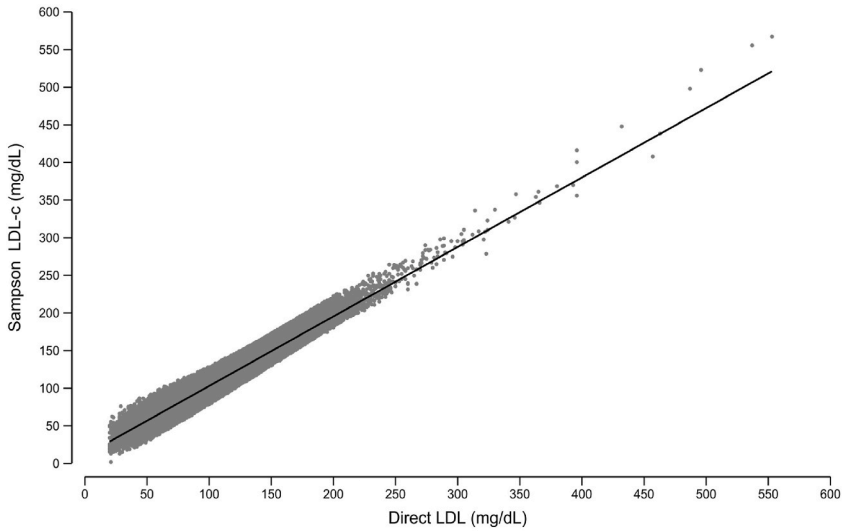
e. Scatter plot showing association of d-LDL vs. LDL-c by the Vujovic Equation

Fig. 1. (*continued*).

23577). Lipid profile (LDL-C, HDL-C, TG and TC) of 247082 individuals was extracted from the integrated laboratory management system (ILMS) of Aga Khan University Hospital by a team of investigators. Duplicate results were not analyzed. For the lipid profiles at our center, blood samples are obtained in the morning after an overnight fast in serum separator tubes to determine low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), triglycerides (TG) and total cholesterol (TC). Samples were centrifuged after collection and analyzed immediately. Measurements of LDL-C, HDL-C, TG and TC are done by ADVIA 1800 chemistry analyzer. The ADVIA 1800 Chemistry System is a high-throughput clinical chemistry analyzer that performs photometric analysis of blood samples. The first step of the reaction eliminates cholesterol associated with lipoproteins other than low-density lipoprotein. A selective surfactant releases cholesterol preferentially from non-LDL particles. Hydrogen peroxide produced by cholesterol esterase and cholesterol oxidase in the first step is eliminated by catalase. Another surfactant in Reagent 2 releases cholesterol from the low-density lipoprotein. Azide in ADVIA Chemistry Reagent 2 inhibits the catalase. Hydrogen peroxide generated by cholesterol esterase and cholesterol oxidase is quantified using a Trinder endpoint (596 nm). The measurement of uncertainty of the four analytes was within the allowable limits as given by CLIA (10 % for total cholesterol, 25 % for triglycerides, 30 % for high density lipoprotein, and 12 % for low density lipoprotein). For targeted comparison of calculated LDL (LDL-c) estimation, a total of seven equations were used in this study. These equations are summarized in Table 1.



f. Scatter plot showing association of d-LDL vs. LDL-c by the Teerakanchana Equation



g. Scatter plot showing association of d-LDL vs. LDL-c by the Sampson Equation

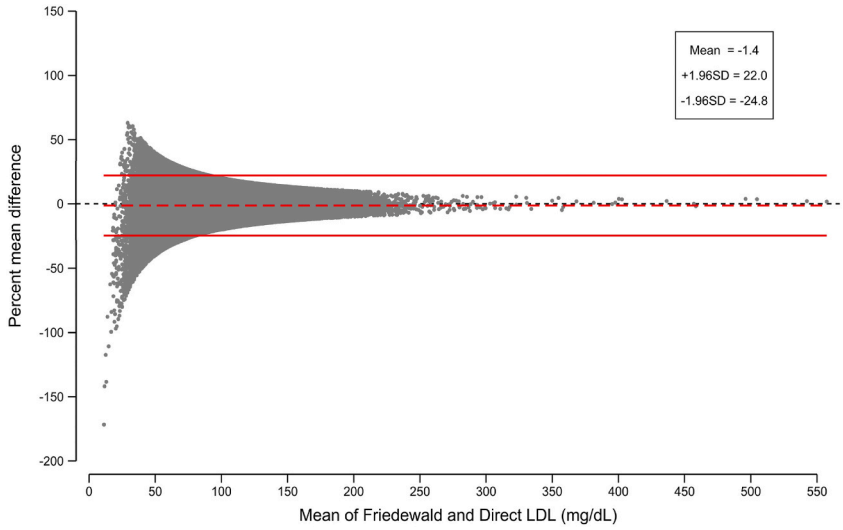
Fig. 1. (*continued*).

2.2. Statistical analysis

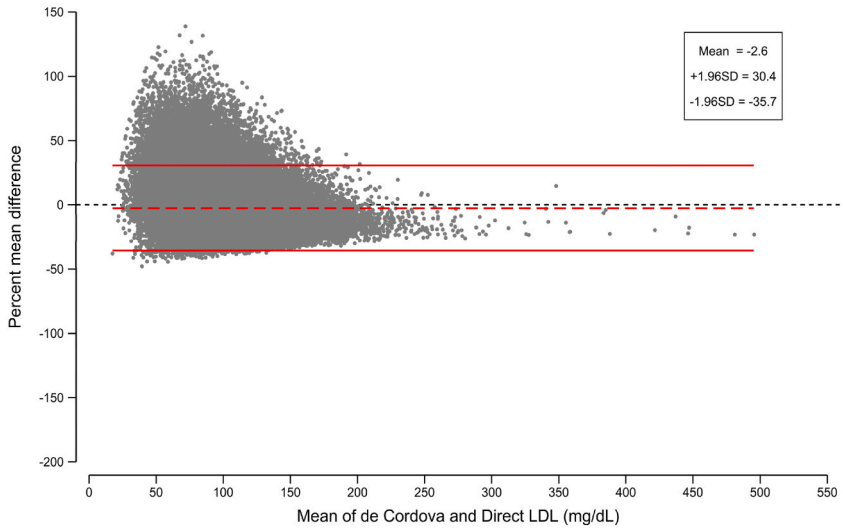
All statistical analysis was performed in Stata Statistical Software: Release 17, College Station, TX: StataCorp LLC. LDL-C was calculated for all seven equations if data on total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) was available. Any missing value required for the equation or repeated measurements of same individuals were excluded. Non-HDL was calculated by subtracting HDL from TC. Mean and SD or median and interquartile ranges were computed for direct LDL (d-LDL) obtained from the laboratory measurements and for all the seven equations (LDL-c). Median difference was computed for d-LDL and all LDL-c using clinical cut points of TC, TG and HDL. Scatter plots were built, and correlation coefficients (r) computed. Bland Altman plots were built to assess agreement (mean %) for d-LDL and LDL-c of all equations. A p value of <0.05 was considered as significant.

3. Results

We analyzed 247082 direct assays of lipid profiles, of which 42.8 % were male and 57.2 % were female. The mean age of participants was 46.7 $\pm$ 19 years. The mean level of direct LDL-C was 108.0 $\pm$ 31.0. The mean LDL-C levels computed on Friedewald, de Cordova, Chen, Hattori, Vujovic, Teerakanchana, Sampson equations were 106.8 $\pm$ 31.4, 103.7 $\pm$ 25.0, 108.6 $\pm$ 28.2, 100.1 $\pm$ 29.5,



a. Bland-Altman plot of Direct LDL and Friedewald Equation



b. Bland-Altman plot of Direct LDL and de Cordova Equation

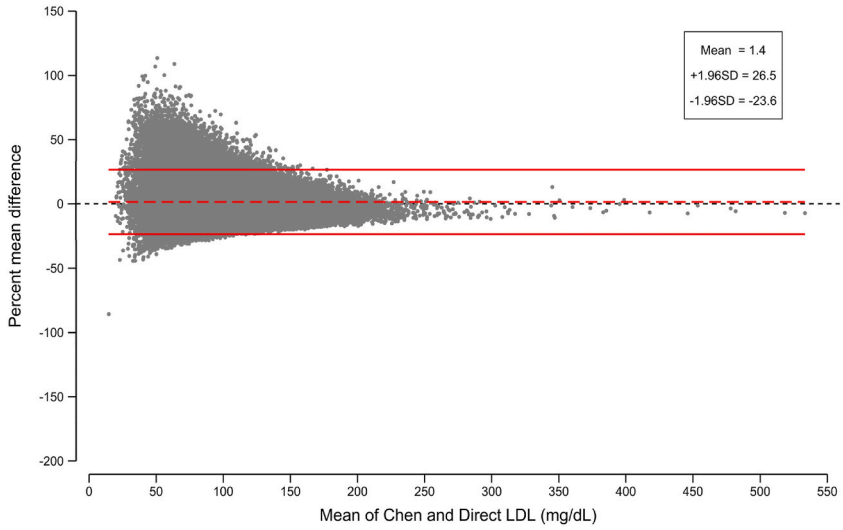
Fig. 2. Bland-Altman plots showing agreement (mean percent) of d-LDL and the LDL-c derived from seven equations.

115.2 ± 31.2, 113.1 ± 28.3 and 110.3 ± 30.6 respectively. Friedwald and Hattori equations correlated strongly with direct LDL-C (r = 0.937) followed by Sampson (r = 0.935) and Vujovic (r = 0.931). However, the median bias was least for the Friedwald equation (−1.6). The median bias was highest for Vujovic equation (15.1) as shown in Table-2.

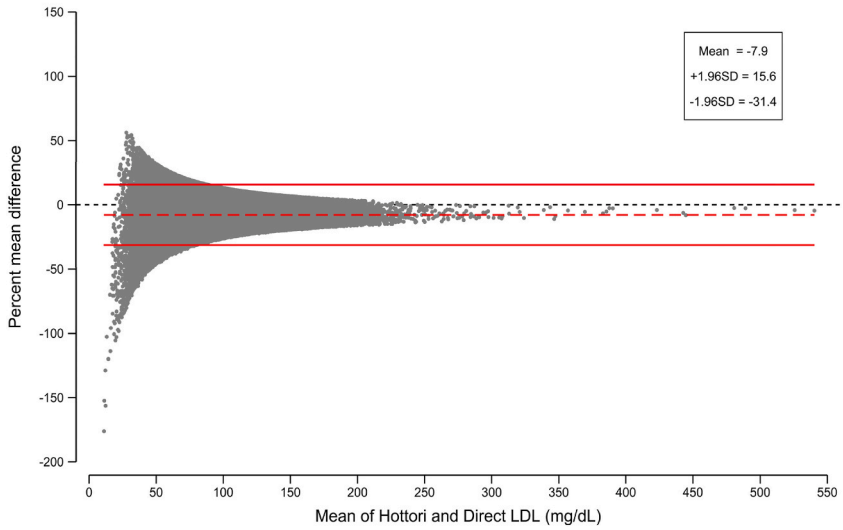
The Friedewald and Hattori equation correlated strongly with direct LDL-C levels across various ranges of total cholesterol (TC) followed by Sampson and Vujovic equation whereas de Cordova equation showed least correlation with direct LDL-C levels. All the equations showed strong correlation across various triglyceride (TG) levels. Friedewald equation showed the best correlation (r = 0.938) when triglycerides were less than 150 mg/dl and (r = 0.943) when triglycerides were greater than 500 mg/dl. Friedwald followed by Hattori and Sampson equation showed the strong correlation across various HDL levels.

4. Discussion

Our study is one of its first attempt to evaluate the accuracy and reliability of various equations including Friedewald, de Cordova, Chen, Hattori, Vujovic, Teerakanchana, Sampson equations in Pakistani population. The present study aims to answer the question of which of these formulae that are used to estimate best LDL-C levels that are more accurate compared to the direct measurement of LDL-



c. Bland-Altman plot of Direct LDL and Chen Equation



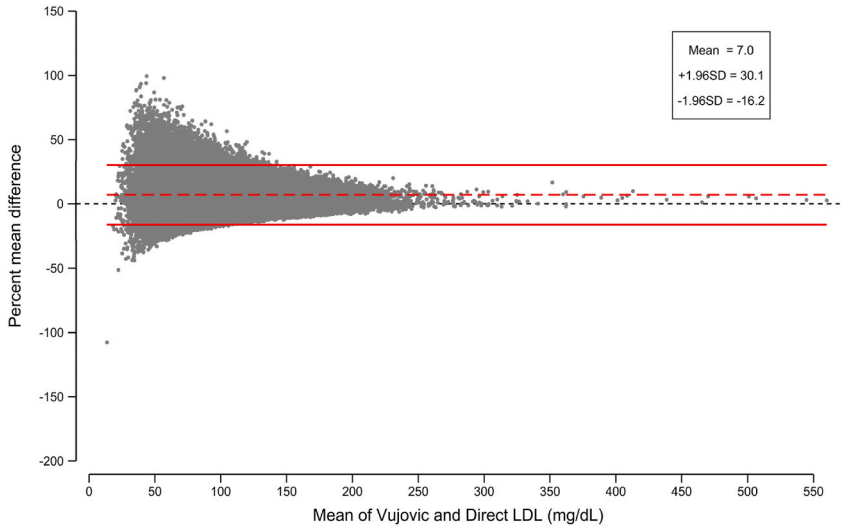
d. Bland-Altman plot of Direct LDL and Hottori Equation

Fig. 2. (*continued*).

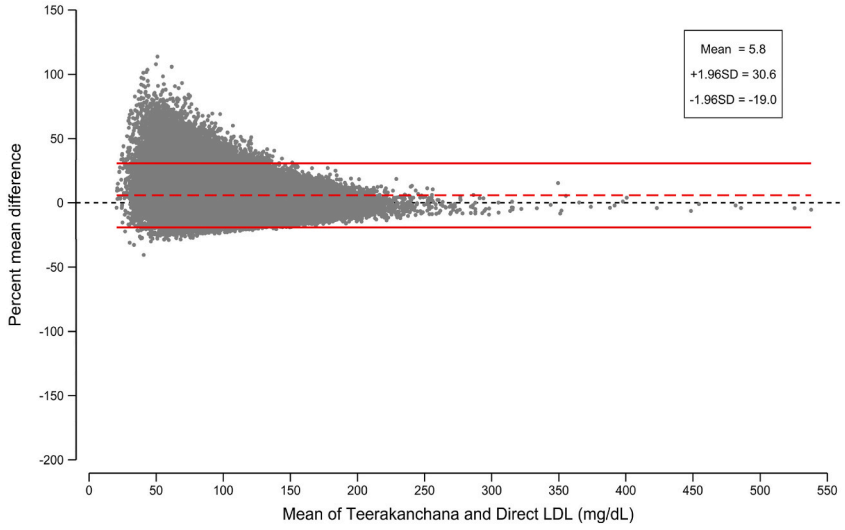
C using a homogeneous assay. Evaluation of LDL-C is fundamental in cardiovascular risk assessment for initiating dietary modifications, drug interference and supervision. Inaccurately determined LDL-C has adverse effect on CVD classification, therapy, and consequences in patients [19]. The reference method for determining LDL-C is cumbersome and not appropriate for clinical laboratory [9]. Therefore, precise LDL-C assessment is one of the most common encounters in the medical laboratory. The current NCEP Adult Treatment Panel suggestions for cardiovascular risk assessment are mostly grounded on early epidemiologic studies that used the Friedewald equation to estimate LDL-C [19].

The Friedewald equation is the most applied to estimate LDL-C, but this equation has integral limitations, such as inaccurate LDL-C calculations in patients with hypertriglyceridemia, in those with very low levels of LDL-C (<2.4 mmol/L), in patient with disorders related to lipoproteins (type III hyperlipoproteinemia), in patients with renal and liver failure, and in those with diabetes and other metabolic abnormalities [12–14,20,21]. Additionally, several other equations have been presented to address the downsides of the Friedewald equations [22–24]. The underestimated LDL-C can lead to delay in initiation of suitable lipid-lowering therapy in high-risk patients, whereas the overestimation of LDL-C will spark excessive pharmacological therapy by placing the patient in higher risks strata. For this reason, finding an equation for the estimation of LDL-C in numerous populations with the best performance comparable to the direct measurement is of paramount importance. In a research study conducted in a clinical laboratory database of 5051467 patients, all the equations that estimated LDL-C directly, except Sampson, performed poorly compared to Friedewald [25].

Our results showed the strong correlation between direct LDL-C and calculated LDL-C by Friedewald equation as depicted in



e. Bland-Altman plot of Direct LDL and Vujovic Equation



f. Bland-Altman plot of Direct LDL and Teerakanchana Equation

Fig. 2. (*continued*).

Table 2. This finding does not concur with other studies, which report a good correlation of calculated LDL-C by modified Friedwald's with measured LDL, which assumes that VLDL constitutes one-sixth of total triglycerides, and it is costly for serum LDL test from direct measurement, especially if it must be tested several times in a year [17,26].

The result from the present study shows that overall, Friedewald and Hattori equations correlated strongly and provided higher correlation with d-LDL followed closely by Sampson and Vujovic equation. This is contrary to the study conducted on North Indian population, which shows that the Teerakanchana equation provided stronger correlation with the measured LDL-C, followed by Vujovic equation [27]. In another study conducted in Indian population Vujovic equation was preferred over others for calculating LDL-C [28]. Martin et al., analyzed four equations including Friedewald, Chen, de Cordova, and Hattori to direct measurement in hospitalized patients in South Africa [21]. They found a good correlation between the de Cordova formula and Friedewald at low TG concentrations. However, the Hattori formula was the best equation to estimate LDL-C in hospitalized patients, even at extreme lipid values which is also present in our study [21].

According to Krishnaveni et al., Friedwald equation correlated maximally with direct measurement of LDL-C at all levels of TG except at TG $<$ 100 mg/dL in Indian adult population. They found that for subjects with serum levels of TG $<$ 100 mg/dl, Anandaraja's Formula was the most accurate equation [29]. In a study done in Iran, Chen and Vujovic equations overestimated LDL-C at all TG levels and de Cordova equation showed underestimation for TG $<$ 150 mmol/L strata and overestimation for TG $>$ 1.69 mmol/L levels. The Hattori formula overestimated LDL-C at TG $<$ 1.69 mmol/L levels and underestimated LDL-C at TG $>$ 1.69 mmol/L levels [30].

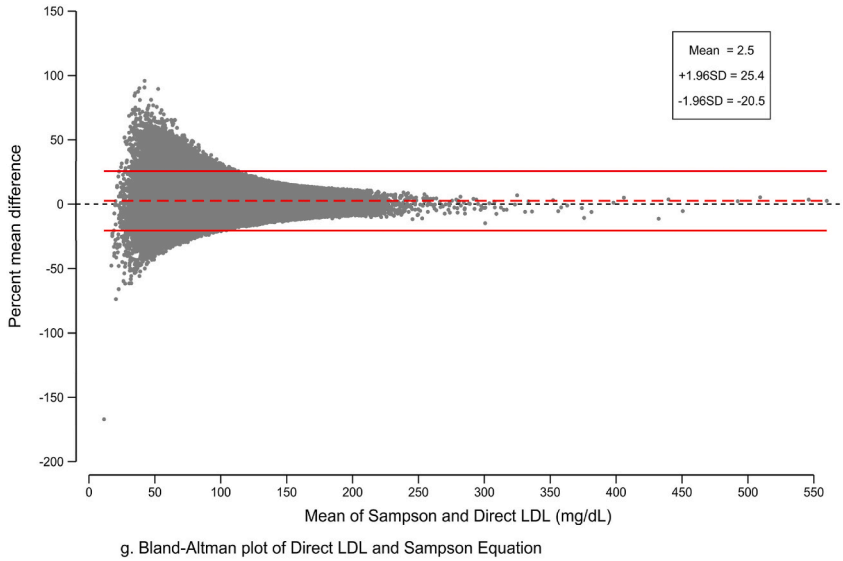


Fig. 2. (*continued*).

Table 1
Seven equations used to calculate Low-density lipoprotein cholesterol in this study.

Name	Equation
Friedwald (1972) [11]	LDL-C = TC-HDL-C-TG/5
Hattori (1998) [13]	LDL-C = 0.94*TC-0.94*HDL-C-0.19*TG
Chen (2010) [14]	LDL-C= (TC-HDL)*0.9-(TG*0.1)
de Cordova (2013) [15]	LDL-C = 0.7516(TC-HDL-C)
Teerakanchana (2007) [16]	LDL-C = 0.910*TC-0.634*HDL-C-0.111*TG-6.75
Vujovic (2010) [17]	LDL-C = TC-HDL-C-TG/6.85
Sampson (2020) [18]	LDL-C = TC/0.948−HDL-C/0.971−(TG/8.56+TG × non-HDL-C/2140 −TG^2/16100)−9.44

Table 2
Median difference, correlation coefficient of d-LDL and LDL-c of all seven equations, n = 247082.

Equation	Mean ± SD	Median (IQR)	Median difference	PW Corr (rho)	PW Corr (Sig)
d-LDL	108.0 ± 31.047	108.0 (86.0, 128.0)		1.000	
Friedewald (1972)	106.8 ± 31.438	106.4 (84.8, 127.6)	−1.6	0.937	<0.001
de Cordova (2013)	103.7 ± 25.036	103.7 (86.4, 120.3)	−2.7	0.842	<0.001
Chen (2010)	108.6 ± 28.239	108.3 (89.1, 127.1)	4.6	0.919	<0.001
Hattori (1998)	100.1 ± 29.570	99.8 (79.5, 119.7)	−8.5	0.937	<0.001
Vujovic (2010)	115.2 ± 31.225	114.9 (93.6, 135.8)	15.1	0.931	<0.001
Teerakanchana (2007)	113.1 ± 28.356	112.8 (93.7, 131.7)	−2.1	0.922	<0.001
Sampson (2020)	110.3 ± 30.662	110.0 (89.1, 130.6)	−2.8	0.935	<0.001

*Pairwise correlation coefficient.

Ahmadi et al., reported that in Iranian adult subjects with low TG concentrations and undesirably high TC, Friedewald equation may overestimate LDL-C. Therefore, they suggested a new equation for such subjects and named it as Ahmadi equation [31].

5. Limitations

Our cross sectional, single centre and retrospective study has several inherent limitations. We had only access to the lipid profile data of the subjects, and clinical characteristics or outcomes of patients in our sample were unknown. In addition, patients who received statin therapy and other cholesterol lowering drugs were not excluded and there was missing information about renal, hepatic, or other comorbidities of subjects. In this study, calculated LDL-C by various formulas were not compared with the reference method i.e., ultracentrifuge and precipitation. Lipoprotein (a) level was not measured in blood samples, so the effect of lipoprotein (a) has not been studied. However, prospective, observational, and multicenter research studies which capture all clinical, pharmacological details and patient outcomes will verify and validate our results.

6. Conclusion

In contrast to the global literature advocating for the use of newer equations for calculation of indirect LDL-C in adult population, the conventional and widely utilized Friedewald equation remains the best alternative and cost-effective way for indirect LDL-C estimation in Pakistani adult population. Multi-center studies are warned to verify our studies results.

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CRediT authorship contribution statement

Syed Bilal Hashmi: Writing – review & editing, Writing – original draft. **Sibtain Ahmed:** Visualization, Supervision, Data curation, Conceptualization. **Shiraz Hashmi:** Writing – review & editing, Formal analysis. **Rasool Bux:** Formal analysis. **Imran Siddiqui:** Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2024.e00418.

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