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Biochemistry Section

Assessing the Validity of Nine Different Formulae for LDL-C Estimation in a Tertiary Care Centre, Hyderabad, India

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ABSTRACT

Introduction: Conventionally, Friedewald's formula has been used to calculate Low Density Lipoprotein- Cholesterol (LDL-C) due to its simplicity and convenience although it has limitations. Many researchers have proposed different formulae to increase the accuracy of calculated LDL-C, but none of those have concluded about a single best formula owing to differences in selected study populations. As LDL-C measurement is of utmost importance for assessing the cardiovascular risk according to National Cholesterol Education Programme's (NCEP) Adult Treatment Panel III (ATP III), a search for a better formula to improve accuracy of cardiovascular disease (CVD) risk prediction is essential.

Aim: To assess the validity of calculated LDL-C by nine formulae and compare them to values obtained by the direct method.

Materials and Methods: A total of 324 participants were assessed retrospectively for serum lipid profile by standard methods from December 2020 to February 2021 at Employee State Insurance Corporation Medical College and Hospital, Sanathnagar, Hyderabad, Telangana, India. LDL-C was calculated using nine different formulae (Ahmadi, Anand, Chen, de Cordova, Friedewald, Hattori, Martin-Hopkins, Puavillai and Vujovic) and correlated with direct LDL-C. For further analysis, subjects were divided into five groups based on the Triglyceride levels (TG) viz; group 1 (TG <100 mg/dL), group 2 (TG: 100-150 mg/dL), group 3 (TG: 151-200 mg/dL), group 4 (TG: 201-400 mg/dL), group 5 (TG >400 mg/dL). Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 23.0.

Results: Total of 324 lipid profile reports were analysed and calculated LDL-C by nine formulas were compared. At TG levels <100 mg/dL, Puavillai was the most accurate. Between TG levels 100-200 mg/dL, Martin-Hopkins showed better accuracy and correlation with direct LDL-C. At TG levels 201-400 and >400 mg/dL, Puavillai had better accuracy. But, none of the formulae showed strong correlation with Direct LDL-C at TG >400 mg/dL. ROC curves also showed that Puavillai performed better among all formulae, at all TG levels.

Conclusion: Among the nine equations, Puavillai and Martin-Hopkins showed highest accuracy and better performance than others in the present study population. Martin-Hopkins can be used at TG levels of 100-200 mg/dL while Puavillai can be used at lower and higher TG levels in this demographic population for estimating LDL-C.

Keywords: Cardiovascular, Calculated low, Cardiovascular disease, Low density lipoprotein cholesterol, Triglyceride

INTRODUCTION

Cardiovascular diseases are the leading cause of global mortality [1,2]. Lipoprotein levels have been touted to be markers of cardiovascular risk assessment from a long time [3,4]. Among the lipoprotein subtypes, Low Density Lipoproteins- Cholesterol (LDL-C) carries cholesterol from liver to peripheral tissues and hence has pro-atherogenic properties. So, LDL-C is one of the crucial biochemical parameters which has been used to assess the cardiovascular risk according to the National Cholesterol Education Programme's (NCEP) Adult Treatment Panel III (ATP III) [5]. Therefore, the accuracy of LDL-C measurement plays an important role.

The gold standard for measurement of LDL-C is by ultracentrifugation and beta-quantification which are laborious, time taking and expensive to be used in routine laboratory practice [6]. Other methods used are direct estimation by homogenous assays or indirectly by calculating with use of various formulae that incorporate different lipoprotein levels like Triglycerides (TG) and non High Density Lipoprotein (NHDL) cholesterol which are measured by standard methods. In developing countries, where resources are limited, many laboratories cannot afford to perform direct assay of LDL-C as it is expensive. Hence, various clinical laboratories use a less expensive and easy method where LDL-C is calculated by using different formulae. There are several published equations for calculating LDL-C like Ahmadi, Anandraja, Chen, de Cardovo, Friedwald's, Hattori, Martin-Hopkins, Puavillai and Vujovik equations [7-15].

The most commonly used formula is Friedewald's equation which incorporates Total Cholesterol (TC), Triglyceride (TG) and High-Density Lipoprotein (HDL) cholesterol. It assumes that Very Low-Density Lipoprotein Cholesterol (VLDL-C) greatly influences TG levels and that the ratio between TG and VLDL-C is fixed as 5, but the actual ratios may vary. Different studies have shown that Friedwald's method yields better results in patients with serum TG concentrations less than 400 mg/dL [16,17]. Many researchers have stated that Friedewald's equation tends to either overestimate or underestimate LDL-C in individuals with conditions such as diabetes mellitus, alcoholic liver disease, and chronic renal failure who are on dialysis [18-21]. Both overestimation and underestimation of LDL-C can pose problems to patients. While overestimation leads to prescription of unnecessary medication; underestimation can delay proper treatment, increasing cardiac risk in them. For this reason, many researchers have attempted to modify the equation by changing the TG: VLDL-C ratio.

But, each equation provides a different result. This variation might be probably due to limitations of the selected study population that was used to derive the equation as they differ in demography, ethnicity and environmental influences. This indicates the need to develop a more local approach to the formula which can be used to calculate LDL-C. Therefore, the present study attempted to compare nine different formulae i.e., Ahmadi, Anand, Chen, de Cardovo, Friedwald, Hattori, Martin-Hopkins, Puavillai and Vujovic equations in order to analyse which formula best suits Indian population.

MATERIALS AND METHODS

This study was a retrospective analytical study comprising of 324 lipid profile reports. The data was collected from the laboratory database from the subjects attending Outpatient Department of Employee State Insurance Corporation (ESIC) Medical College and Hospital, Sanathnagar, Hyderabad, Telangana, India and analysed for a period of three months from December 2020 to February 2021, after obtaining Institutional Ethical Committee clearance (ESICMC/SNR/IEC-F0238/12-2020). The laboratory serves a large tertiary care academic hospital. Patient details were anonymised except for age and gender. All subjects aged above 18 years who came to the biochemistry laboratory for a complete lipid profile investigation were included in the study.

All the 324 laboratory reports of the participants included, were divided into five groups based on their TG levels (Group 1: TG <100 mg/dL, Group 2: TG=100-150 mg/dL, Group 3: TG=151-200 mg/dL, Group 4: TG=201-400 mg/dL, Group 5: TG >400 mg/dL).

Blood samples were collected as per the standard protocol i.e., after an overnight fast of 10-12 hours, 3 mL of venous blood in a plain tube, serum separated and analysed immediately to determine direct LDL-C (Homogeneous Enzymatic Colorimetric Assay), HDL-C (Homogeneous Enzymatic Colorimetric Assay), TG {Glycerine Phosphate Oxidase Peroxidase (GPO-PAP)} and TC (Cholesterol Oxidase Peroxidase (CHOD-POD) Method), on Roche Cobas C311 Chemistry Analysers (Roche Diagnostics GmbH, Mannheim, Germany). Apart from direct homogenous assay, calculated LDL-C was also determined using the following nine formulae:

1. Ahmadi: LDL-C=TC/1.19+TG/1.9–HDL-C/1.1 [7]
2. Anandaraja: LDL-C=(0.9×TC)–(0.9×TG/5)–28 [8]
3. Chen: LDL-C=(TC–HDL-C)×0.9–(TG×0.1) [9]
4. de Cordova: LDL-C=0.7516×(TC–HDL-C) [10]
5. Friedewald: LDL-C=TC–HDL-C–TG/5 [11]
6. Hattori: LDL-C=(0.94×TC)–(0.94×HDL-C)–(0.19×TG) [12]
7. Martin-Hopkins: LDL=TC–HDL–TG/novel factor derived using an LDL-C calculator [13]
8. Puavillai: LDL-C=TC–HDL-C–TG/6 [14]
9. Vujovic: LDL-C=TC–HDL-C–(TG/6.85) [15]

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistical Package for the Social Science (SPSS) version 23.0. Data was expressed as mean and standard deviation. Associations were analysed using Pearson's correlation test. Paired t-test was also performed to compare the means. Diagnostic Performance of the nine formulae was analysed using Area Under Curve (AUC) obtained by constructing Receiver Operating Characteristic (ROC) Curves. Two-tailed p-value <0.05 was taken as significant.

Conversion factors to SI units: To convert TG from mg/dL to mmol/L multiply by 0.01129. To convert TC, LDL-C and HDL-C from mg/dL to mmol/L multiply by 0.02586 [22].

RESULTS

A total of 324 participants of which 120 (37.04%) were females and 204 (62.96%) were males with a mean age of 45±15 years were included. The demographic distribution and lipid data of the participants are shown [Table/Fig-1]. LDL-C estimated by direct homogeneous assay and calculated using nine different formulae were compared and correlated.

Lipoprotein Concentrations

The study population was divided into 5 groups based on their TG levels (Group 1: TG <100 mg/dL, Group 2: TG=100-150 mg/dL, Group 3: TG=151-200 mg/dL, Group 4: TG=201-400 mg/dL, Group 5: TG >400 mg/dL) in order to evaluate the performance of these nine formulae at different levels of TG, especially at higher and lower levels of TG where the commonly used Friedwald formula has limitations of usage.

Variable	Mean±SD	Mean difference	t-test (vs Direct LDL-C)	Pearson correlation	
			p-value	r	p-value
Age (years)	45±15	-	-	-	-
Total Cholesterol (mg/dL)	172±44	-	-	-	-
HDL-C (mg/dL)	36±9.3	-	-	-	-
Triglycerides (mg/dL)	148±87	-	-	-	-
Direct LDL-C (mg/dL)	114±34	-	-	-	-
Comparative analysis of LDL-C by nine formulae					
Ahmadi LDL-C (mg/dL)	186±125	-72.7	**<0.001**	0.2	**<0.001**
Anadaraja LDL-C (mg/dL)	99±42	14	**<0.001**	0.744	**<0.001**
Chen LDL-C (mg/dL)	110±41	3	0.072	0.696	**<0.001**
de Cordovo LDL-C (mg/dL)	109±41	4.4	**0.025**	0.578	**<0.001**
Friedewald LDL-C (mg/dL)	104±43	8.3	**<0.001**	0.739	**<0.001**
Hattori LDL-C (mg/dL)	98±41	15	**<0.001**	0.739	**<0.001**
Martin-Hopkins LDL-C (mg/dL)	114±41	-0.86	0.573	0.751	**<0.001**
Pauvillai LDL-C (mg/dL)	118±37	1.6	0.320	0.734	**<0.001**
Vujovic LDL-C (mg/dL)	132±49	-19	**<0.001**	0.651	**<0.001**

[Table/Fig-1]: Demographic distribution and lipid data of the study subjects.
SD: Standard deviation; r=Correlation Coefficient; p<0.05 considered statistically significant

LDL-C concentrations, their distributions and correlations with direct LDL-C in the 5 groups are shown in [Table/Fig-2-13]. The

Variable	Mean±SD	Mean difference	t-test (vs Direct LDL-C)	Pearson correlation	
			p-value	r	p-value
	Group 1: TG <100 (mg/dL) (n=104)				
Direct LDL-C (mg/dL)	102±33				
Ahmadi LDL-C (mg/dL)	93±37	8.7	**<0.001**	0.784	**<0.001**
Anadaraja LDL-C (mg/dL)	98±38	3.9	0.073	0.823	**<0.001**
Chen LDL-C (mg/dL)	96±35	5.9	**0.004**	0.822	**<0.001**
de Cordovo LDL-C (mg/dL)	86 ± 29	15.7	**<0.001**	0.823	**<0.001**
Friedewald LDL-C (mg/dL)	100±38	1.5	0.475	0.820	**<0.001**
Hattori LDL-C (mg/dL)	94±36	7.7	**<0.001**	0.819	**<0.001**
Martin-Hopkins LDL-C (mg/dL)	100±36	1.2	0.102	0.981	**<0.001**
Puavillai LDL-C (mg/dL)	103±38	-0.84	0.597	0.821	**<0.001**
Vujovic LDL-C (mg/dL)	110±39	-8.2	**<0.001**	0.823	**<0.001**
	Group 2: TG=100-150 (mg/dL) (n=70)				
Direct LDL-C (mg/dL)	120±35				
Ahmadi LDL-C (mg/dL)	144±33	-23.6	**<0.001**	0.886	**p<0.001**
Anadaraja LDL-C (mg/dL)	111±34	9	**<0.001**	0.902	**p<0.001**
Chen LDL-C (mg/dL)	116±32	4	**0.007**	0.928	**p<0.001**
de Cordova LDL-C (mg/dL)	107±27	13	**<0.001**	0.927	**p<0.001**
Friedewald LDL-C (mg/dL)	117±36	2.4	0.138	0.928	**p<0.001**
Hattori LDL-C (mg/dL)	110±34	9.7	**<0.001**	0.928	**p<0.001**
Martin-Hopkins LDL-C (mg/dL)	119±35	1	0.513	0.928	**p<0.001**
Puavillai LDL-C (mg/dL)	122±36	1.7	0.298	0.928	**p<0.001**
Vujovic LDL-C (mg/dL)	134±36	14.3	**<0.001**	0.928	**p<0.001**

[Table/Fig-2]: Distribution of calculated LDL-C in TG groups <100, 100-150 mg/dL.
TG: Triglycerides; SD: Standard deviation; r=Correlation Coefficient; p<0.05 considered statistically significant

Variable	Mean±SD	Mean difference	t-test (vs Direct LDL-C) p-value	Pearson correlation r	Pearson correlation p-value
	Group 3: TG=151-200 (mg/dL) (n=44)				
Direct LDL-C (mg/dL)	120±33				
Ahmadi LDL-C (mg/dL)	175±31	-54.6	**<0.001**	0.918	**<0.001**
Anadaraja LDL-C (mg/dL)	104±37	16	**<0.001**	0.945	**<0.001**
Chen LDL-C (mg/dL)	114±34	6	**<0.001**	0.962	**<0.001**
de Cordova LDL-C (mg/dL)	110±28	10.2	**<0.001**	0.961	**<0.001**
Friedewald LDL-C (mg/dL)	111±38	8.7	**<0.001**	0.960	**<0.001**
Hattori LDL-C (mg/dL)	104±35	16	**<0.001**	0.960	**<0.001**
Martin-Hopkins LDL-C (mg/dL)	117±35	2.9	0.055	0.962	**<0.001**
Puavillai LDL-C (mg/dL)	117±38	-3.02	0.75	0.961	**<0.001**
Vujovic LDL-C (mg/dL)	135±7	-15	**<0.001**	0.962	**<0.001**
	Group 4: TG=201-400 (mg/dL) (N=63)				
Direct LDL-C (mg/dL)	124±31				
Ahmadi LDL-C (mg/dL)	228±47	-103.7	**<0.001**	0.796	**<0.001**
Anadaraja LDL-C (mg/dL)	96±37	28.3	**<0.001**	0.896	**<0.001**
Chen LDL-C (mg/dL)	115±33	9	**<0.001**	0.936	**<0.001**
de Cordova LDL-C (mg/dL)	118±29	6.3	**<0.001**	0.941	**<0.001**
Friedewald LDL-C (mg/dL)	105±36	19.1	**<0.001**	0.918	**<0.001**
Hattori LDL-C (mg/dL)	98±34	26	**<0.001**	0.917	**<0.001**
Martin-Hopkins LDL-C (mg/dL)	118±33	6.1	**<0.001**	0.932	**<0.001**
Puavillai LDL-C (mg/dL)	121±36	3.5	**<0.001**	0.946	**<0.001**
Vujovic LDL-C (mg/dL)	140±37	16.1	**<0.001**	0.940	**<0.001**

[Table/Fig-3]: Distribution of calculated LDL-C in TG groups 151-200 and 201-400 mg/dL.
TG: Triglycerides; SD: Standard deviation; r: Correlation Coefficient; p<0.05 considered statistically significant

Variable	Mean±SD	Mean difference	t-test (vs Direct LDL-C) p-value	Pearson correlation r	Pearson correlation p-value
	Group 5: TG >400 (mg/dL) (N=43)				
Direct LDL-C (mg/dL)	106±36				
Ahmadi LDL-C (mg/dL)	428±152	-322.5	**<0.001**	0.174	0.447
Anadaraja LDL-C (mg/dL)	106±36	23.5	**0.011**	0.447	**0.003**
Chen LDL-C (mg/dL)	125±68	-19	0.077	0.253	0.102
de Cordova LDL-C (mg/dL)	152±66	-46	**<0.001**	0.133	0.395
Friedewald LDL-C (mg/dL)	88±69	18	0.085	0.365	**0.016**
Hattori LDL-C (mg/dL)	82±65	24	**0.014**	0.367	**0.015**
Martin-Hopkins LDL-C (mg/dL)	129±66	-23	**0.029**	0.256	0.098
Puavillai LDL-C (mg/dL)	107±71	-1.3	0.899	0.322	**0.035**
Vujovic LDL-C (mg/dL)	166±81	-60	**<0.001**	0.199	0.202

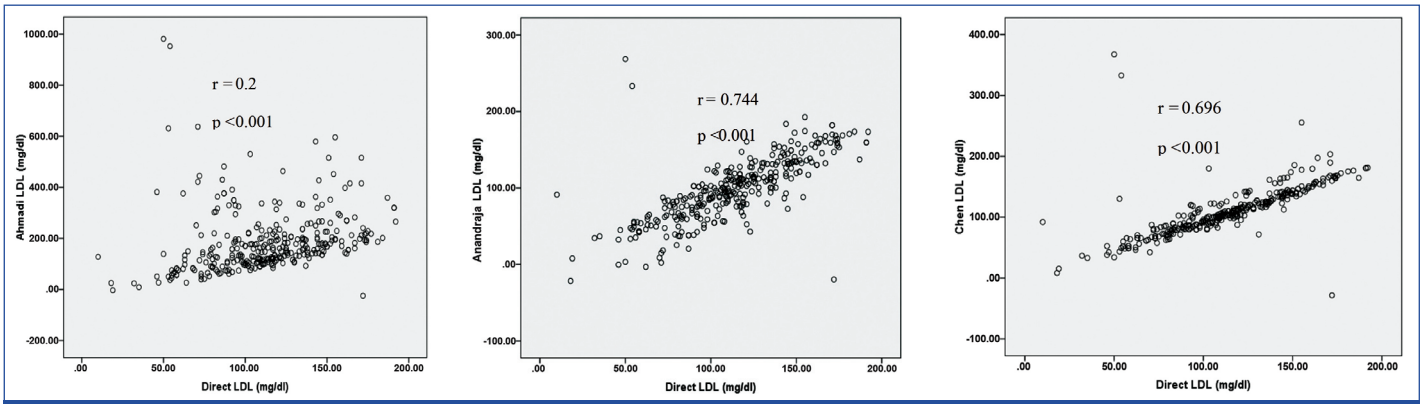
[Table/Fig-4]: Distribution of calculated LDL-C in TG groups >400 mg/dL.
TG: Triglycerides; SD: Standard deviation; r: Correlation Coefficient; p<0.05 considered statistically significant

present study showed that at TG levels <100 mg/dL, Puavillai showed the least mean difference and best correlation with direct LDL-C (r=0.821).

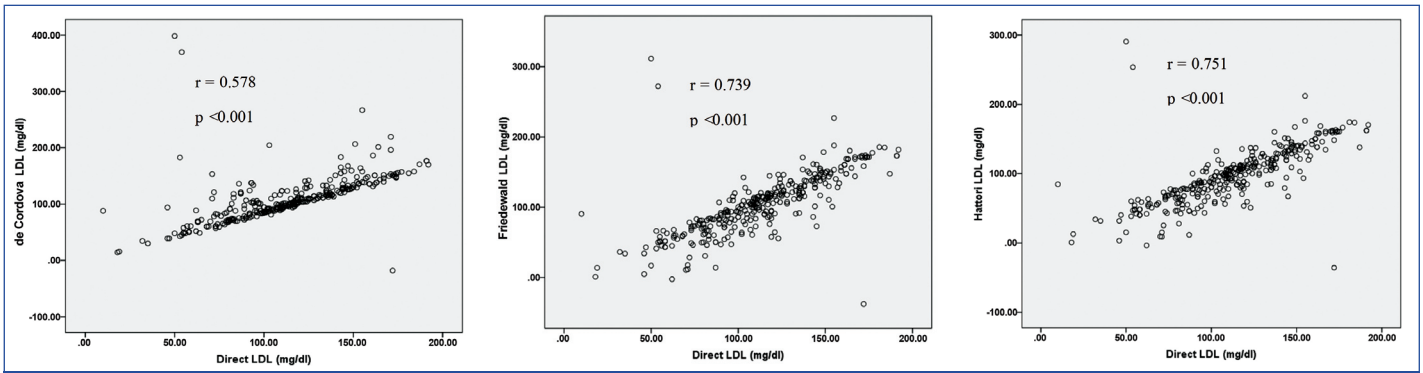
At TG levels 100-150 mg/dL and 151-200 mg/dL, Martin-Hopkins LDL-C showed the least mean difference and best correlation with direct LDL-C (r=0.928,0.962). Puavillai LDL-C showed the least mean difference and best correlation (r=0.946) with direct LDL-C followed by Martin-Hopkins (r=0.932) at TG levels 201-400 mg/dL. Also, at higher TG levels i.e., >400 mg/dL, Puavillai LDL-C had the least mean difference of -1.3, all other formulae including Martin-Hopkins were highly inaccurate.

Diagnostic Performance

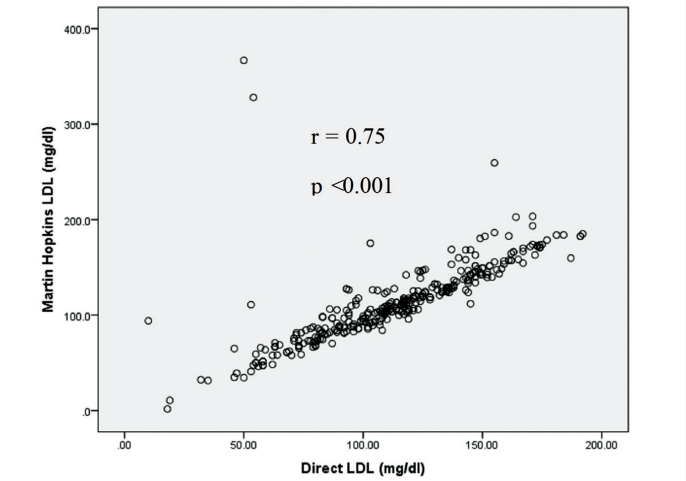
Receiver Operating Characteristic (ROC) curves were constructed to analyse the performance of calculated LDL-C using the nine



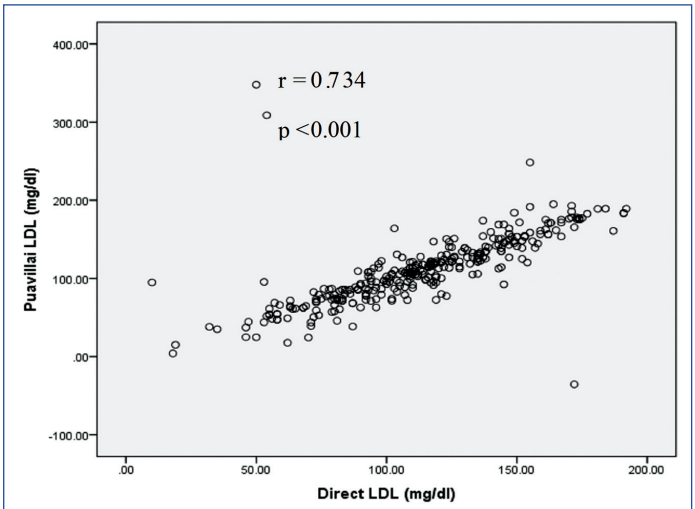
[Table/Fig-5]: Correlation of Ahmadi LDL-C with Direct LDL-C; **[Table/Fig-6]:** Correlation of Anandraja LDL-C with Direct LDL-C; **[Table/Fig-7]:** Correlation of Chen LDL-C with Direct LDL-C. (Images from left to right)



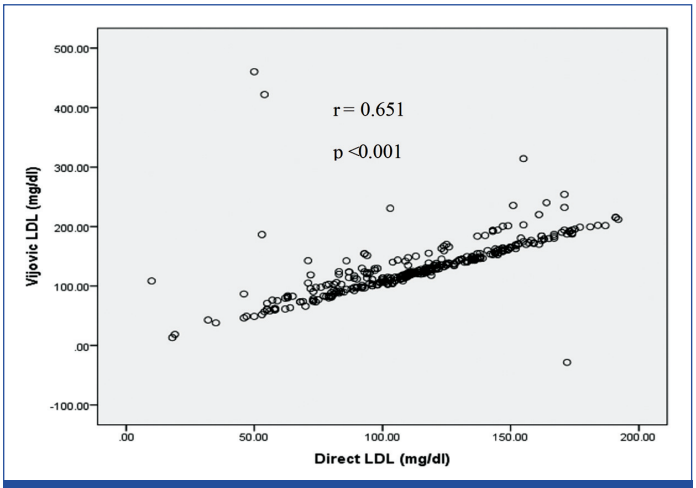
[Table/Fig-8]: Correlation of de Cordova LDL-C with Direct LDL-C; **[Table/Fig-9]:** Correlation of Friedewald LDL-C with Direct LDL-C; **[Table/Fig-10]:** Correlation of Hattori LDL-C with Direct LDL-C. (Images from left to right)



[Table/Fig-11]: Correlation of Martin-Hopkins LDL-C with Direct LDL-C.



[Table/Fig-12]: Correlation of Puavillai LDL-C with Direct LDL-C.



[Table/Fig-13]: Correlation of Vujovic LDL-C with Direct LDL-C.

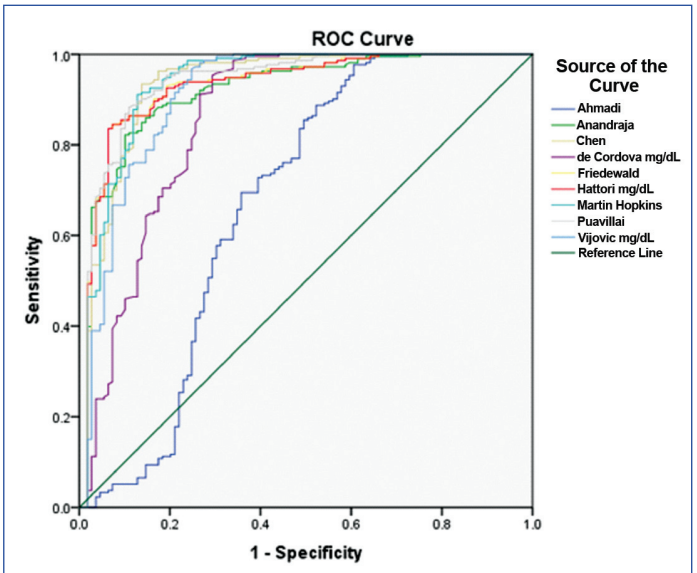
different formulae [Table/Fig-14,15]. Out of all, Puavillai showed the best performance followed by Martin-Hopkins and then Friedewald's LDL-C.

DISCUSSION

According to National Cholesterol Education Programme (NCEP) guidelines, LDL-C level is crucial for risk assessment, instituting treatment to prevent cardiovascular diseases and monitoring [23]. Despite of inherent limitations of Friedewald formula, it is one of the commonly used method, as the reference method (ultracentrifugation) to measure LDL is laborious, costly and not suitable for resource limited setting. Thus, accurate measurement of LDL-C is important to avoid adverse outcome to patients.

Calculated LDL-C (mg/dL)	AUC	p-value
Ahmadi	0.674	**<0.001**
Anandraja	0.918	**<0.001**
Chen	0.931	**<0.001**
de Cordova	0.861	**<0.001**
Friedewald	0.934	**<0.001**
Hattori	0.930	**<0.001**
Martin-Hopkins	0.937	**<0.001**
Puavillai	0.939	**<0.001**
Vujovic	0.913	**<0.001**

[Table/Fig-14]: Area Under Curves (AUCs) of calculated LDL-C using the nine formulae.
AUC: Area under curve; p<0.05 considered statistically significant



[Table/Fig-15]: Diagnostic performance of nine formulae.
ROC: receiver operating characteristic

However, many formulas were derived previously to quantify LDL-C precisely than the widely used Friedewald's formula. Furthermore, under estimate and over estimate of LDL-C leads to delay in treatment and unnecessary exposure to drugs respectively. Therefore, determining an equation for estimation of LDL-C in different population with good comparability to direct LDL-C measurement is essential.

In this study, there was a positive correlation between direct LDL-C and calculated LDL-C with all formulae. This is in line with other studies where the LDL-C was measured by different homogenous assays [15,24,16,25-28]. The study population was divided into five groups of different TG levels to validate the nine formulae at these TG levels. Most of these formulae showed high correlation with direct LDL-C at different levels of TG.

In the present study, when compared to nine common formulae, Puavillai was the best equation to estimate LDL-C in Indian population and the next best is Martin- Hopkins equation. At the TG level <100 mg/dL, Puavillai had the highest accuracy followed by Martin-Hopkins in contrary to a similar analysis where de Cordova had the highest accuracy [26]. Further, Puavillai equation correlated maximally with direct LDL-C at all levels of TG except at TG 100 mg/dL -200 mg/dL in Indian population. It was also inferred from this study results that Friedewald's formula overestimated LDL-C in almost all TG groups, the amount of overestimation increased with the increase in TG, this was in consistent with the results previously reported by Mora S et al., Martin SS et al., and Kannan S et al., [26,29,30]. However, it was next most accurate after Puavillai and Martin-Hopkins. Whereas,

Krishnaveni P and Gowda VM demonstrated that Friedewald equation correlated with direct LDL-C at all levels of TG except at TG less than 100 mg/dL in Indian population, however they did not include samples with TG>400 mg/dL [31]. According to Wadhwa N and Krishnaswamy R, vijovic formula was the most accurate equation for estimation of LDL-C in Indian population [32]. Different findings between the present study and other Indian studies may be due to differences in the age group and different estimation formulae. The Ahmadi, de Cordova and Anandraja formulae were the least accurate at almost all levels of TG. This finding is consistent with the study conducted by Martins J et al., [27].

ROC analysis reconfirmed these findings i.e., Puavillai showed the best performance followed by Martin-Hopkins and then Friedewald's formula {AUC=0.939 (p<0.001), 0.937 (p<0.001) and 0.934 (p<0.001), respectively}. In terms of correlation, accuracy and diagnostic performance, Ahmadi equation showed the highest misinterpretation with extreme overestimation in this study population similar to the results obtained by Karkhaneh A et al., [33]. This finding is contrary to the study performed by Ahmadi SA et al., [7]. While Chen, de Cordova, Anandraja and Hattori equations overestimated LDL-C, Vujovic equation underestimated it in most cases. These differences were particularly more at higher TG levels.

Limitation(s)

The present study includes several inherent limitations. Authors had only access to the lipid profiles of the subjects. Clinical outcomes of patients in present study sample were unknown. In addition, we did not have information about intake of cholesterol lowering drugs such as statins. Also, authors did not compare the calculated LDL-C by various formulae with the reference method i.e., ultracentrifugation.

CONCLUSION(S)

Among the various equations/formulae, Puavillai and Martin-Hopkins showed highest accuracy and better performance than other equations in this study population. Puavillai performed better at very low and very high TG levels (100 mg/dL, >400 mg/dL) when compared to Friedwald's formula which overestimated LDL-C at all TG levels and the accuracy decreased with increasing TG levels.

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