Research Article

Correlation Analysis of Direct LDL Measurement and Calculated LDL Methods in Lipid Profile Assessment: A Comprehensive Study

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Keywords

LDL-cholesterol, Freidewald equation, various modified formulae, Vujovic

Abstract

Introduction

Assessing LDL cholesterol is pivotal for cardiovascular risk evaluation. While direct LDL measurement is accurate, calculated LDL methods offer practicality and cost-effectiveness. This study aims to evaluate the correlation between direct LDL measurement and various calculated LDL methods, shedding light on their clinical utility.

Methods

A retrospective analysis of lipid profiles from 1075 patients was conducted, encompassing direct LDL measurement and calculation of LDL using nine different methods. Statistical analyses, including correlation coefficients and scatter plots, were employed to assess the agreement between direct LDL and calculated LDL methods.

Results

Surprisingly, all calculated LDL methods exhibited a robust correlation with direct LDL measurement across the study cohort. The Friedewald equation, as well as modified equations demonstrated particularly robust correlations. These findings indicate the reliability of calculated LDL methods in estimating LDL cholesterol levels.

Discussion

The significant correlation observed between direct LDL measurement and calculated LDL methods underscores the clinical utility of the latter. While direct LDL measurement remains the gold standard, calculated LDL methods offer practical advantages, particularly in resource-limited settings.

Conclusion

In conclusion, this study highlights the excellent correlation between direct LDL measurement and calculated LDL methods in lipid profile assessment. Clinicians can leverage calculated LDL methods as reliable alternatives for LDL

cholesterol estimation, facilitating efficient cardiovascular risk evaluation in routine clinical practice. Further research may explore the optimal use of calculated LDL methods in specific patient populations, enhancing their clinical applicability and utility.

Introduction

One among the leading cause of mortality worldwide are cardiovascular diseases. Low density lipoprotein (LDL) are considered bad cholesterol as it causes atherosclerosis, an utmost contributor to cardiovascular disease [1]. Low-density lipoprotein-cholesterol (LDL-C) remains of utmost clinical importance; it is positioned in clinical trials as a treatment target and is emphasized in worldwide guidelines as the primary cholesterol target [2]. It is mainly due to economic reasons, instead of the direct measurement of LDL-C, the calculation methods are widely used in clinical laboratories particularly in developing countries [3]. In addition to Friedewald Formula, there are several other formulas for calculation of LDL-C such as Chen, de Cordova, Vujovic, Anandaraja, Hattori, Ahmadi, Puavillai, Sampson's equation, Martin-Hopkins, Saiedullah; Planella and Wagner which have not been validated in varied populations [4-15].

Friedwald, the most commonly used formula has its own limitations as shown by earlier studies [16,17]. Over and under estimation of LDL-C in patients suffering from diabetes mellitus, alcoholic liver disease, and chronic liver failure have been seen by many [18-21], which may become a problem to patients. This can be overcome by establishing a formula for our population for which we conducted the following study.

9 different formulas as shown in Table 1. were used along with direct LDL measurement

Table 1: 9 different formulas as shown in this table were used along with direct LDL measurement.

Proposed by	**Formula**
Friedewald et al., [4]	LDL-C = TC – HDL-C – 0.2 × TG
Ahmadi et al., (5)	LDL-C = TC/1.19 + TG/1.9-HDL-C/1.1
Anandaraja et al., [6]	LDL-C = (0.9 × TC) – (0.9 × TG/5) – 28
Chen et al., (7)	LDL-C = (TC – HDL-C) × 0.9 – (TG × 0.1)
Cordova and Cordova [8]	LDL-C =3/4 (TC-HDLc)
Hattori et al., [9]	LDL-C = (0.94 × TC) – (0.94 × HDL-C) –(0.19 × TG)
Puavillai et al., [10]	LDL-C=TC-HDLc-TG/6
Sampson's equation (3)	LDL-C = [TC/0.948−HDL-C/0.971−(TG/8.56+TG × non-HDL-C/2140 −TG^2/16100)−9.44 [25]]
Vujovic et al., [11]	LDL-C=TC-TG/6.85-HDLc

Materials and methods

A retrospective analysis of lipid profiles from 1078 patients was conducted from clinical biochemistry lab database at SMCH, Trichy for 6 months encompassing direct LDL measurement and calculation of LDL using nine different methods. Institutional ethical committee clearance was obtained (IEC No. 18/2022). Care was taken to anonymised the patients except for age & gender.

All patients who came for complete lipid profile investigation were included

A total of 1075 patients out of 1078 were subdivided into various groups for further analyses based on **age, triglyceride (TG), total cholesterol (TC) & HDL- cholesterol (HDL-C) levels** as in Tables 2-5.

Table 2: Four groups based on age (<20, 20–39, 40–59 and ≥ 60 years).

Age	**No. (% age)**	**Mean Age ± SD**	**Mean TC (mmol/L) ± SD**	**Mean TG (mmol/L) ± SD**	**Mean HDL (mmol/L) ± SD**	**Mean D-LDL (mmol/L) ± SD**
< 20	14 (1.3)	13.79 ± 3.53	3.81 ± 1.05	1.33 ± 0.85	1.12 ± 0.22	2.41 ± 1.02
20-39	200 (18.6)	32.02 ± 5.39	4.55 ± 1.18	1.64 ± 0.82	1.15 ± 0.29	3.01 ± 1.02
40-59	541 (50.3)	49.81 ± 5.43	4.70 ± 1.12	1.74 ± 0.78	1.20 ± 0.68	3.12 ± 1.02
>=60	320 (29.8)	67.20 ± 6.38	4.52 ± 1.06	1.58 ± 0.72	1.14 ± 0.28	2.98 ± 0.95

Table 3: Five levels of TG (<0.56, 0.56–1.69, 1.70–3.38, 3.39–4.51 and > 4.51 mmol/L).

TG mmol/L	No. (% age)	Mean Age ± SD	Mean TC (mmol/L) ± SD	Mean TG (mmol/L) ± SD	Mean HDL (mmol/L) ± SD	Mean D-LDL (mmol/L) ± SD
< 0.56	15 (1.4)	42.27 ± 21.22	3.22 ± 0.86	0.48 ± 0.09	1.18 ± 0.28	2.05 ± 0.67
0.56–1.69	630(58.6)	51.41 ± 14.15	4.42 ± 1.06	1.19 ± 0.30	1.22 ± 0.57	2.95 ± 1.00
1.70–3.38	376 (35)	51.53 ± 13.50	4.91 ± 1.08	2.23 ± 0.42	1.11 ± 0.46	3.24 ± 0.96
3.39–4.51	54 (50.2)	48.78 ± 12.89	5.20 ± 1.21	3.77 ± 0.25	1.03 ± 0.27	3.08 ± 1.12
> 4.51	3	Data excluded due to insufficiency				

Table 4: Three levels of TC (<5.17, 5.17–6.18, >6.18 mmol/L).

TC mmol/L	No. (% age)	Mean Age ± SD	Mean TC (mmol/L) ± SD	Mean TG (mmol/L) ± SD	Mean HDL (mmol/L) ± SD	Mean D-LDL (mmol/L) ± SD
< 5.17	750 (69.8)	51.03 ± 14.62	4.06 ± 0.77	1.55 ± 0.72	1.13 ± 0.60	2.63 ± 0.77
5.17–6.18	244 (22.7)	52.08 ± 12.60	5.58 ± 0.30	1.89 ± 0.80	1.24 ± 0.28	3.77 ± 0.63
> 6.18	81 (7.5)	50.02 ± 12.16	6.81 ± 0.63	2.16 ± 0.87	1.34 ± 0.25	4.75 ± 0.82

Table 5: Three levels of HDLC (<1.03, 1.03–1.52, >1.52 mmol/L).

HDL mmol/L	No. (%age)	Mean Age ± SD	Mean TC (mmol/L) ± SD	Mean TG (mmol/L) ± SD	Mean HDL (mmol/L) ± SD	Mean D-LDL (mmol/L)± SD
< 1.03	340 (31.6)	51.24 ± 14.93	4.05 ± 1.06	1.86 ± 0.81	0.86 ± 0.15	2.75 ± 1.00
1.03–1.52	635 (59.1)	51.37 ± 13.74	4.82 ± 1.01	1.62 ± 0.75	1.22 ± 0.13	3.16 ± 0.95
>1.52	100 (9.3)	49.93 ± 12.52	5.21 ± 1.18	1.37 ± 0.65	1.92 ± 1.37	3.32 ± 1.12

Venous blood samples (3ml) of subjects under strict overnight fasting [8-10hrs] was collected under aseptic precautions. After serum separation immediate analysis of serum lipid profile including direct LDL was done.

Statistical analysis

Statistical analyses, including correlation coefficients and scatter plots, were employed to assess the agreement between direct LDL and calculated LDL methods using SPSS Software version 27.0 and Excel sheet

Mean and standard deviation was used to convey the data.

The data was more thoroughly analysed using Pearson's correlation, Bland-Altman plots and paired t-test was also utilise to compare means of different groups.

Pearson's correlation 'r' near to 1 and p <0.05 was taken as significant

Bland-Altman plots (Figure 1) were used to see the agreement or disagreement between two different methods

Two tailed p-value <0.05 was taken as significant

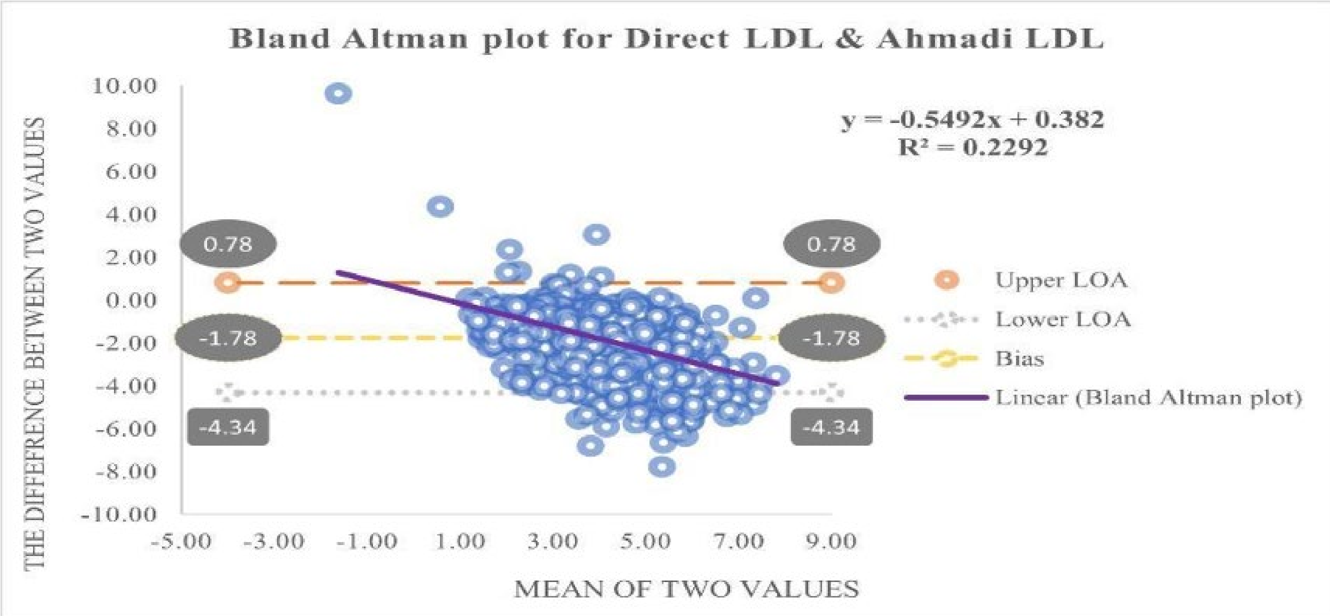
Conversion of TG in mg/dl to mmol/L was done using TG in (mg/dl) /88.57 and for TC, HDL-C and LDL-C values in mg/dl were divided by 38.67

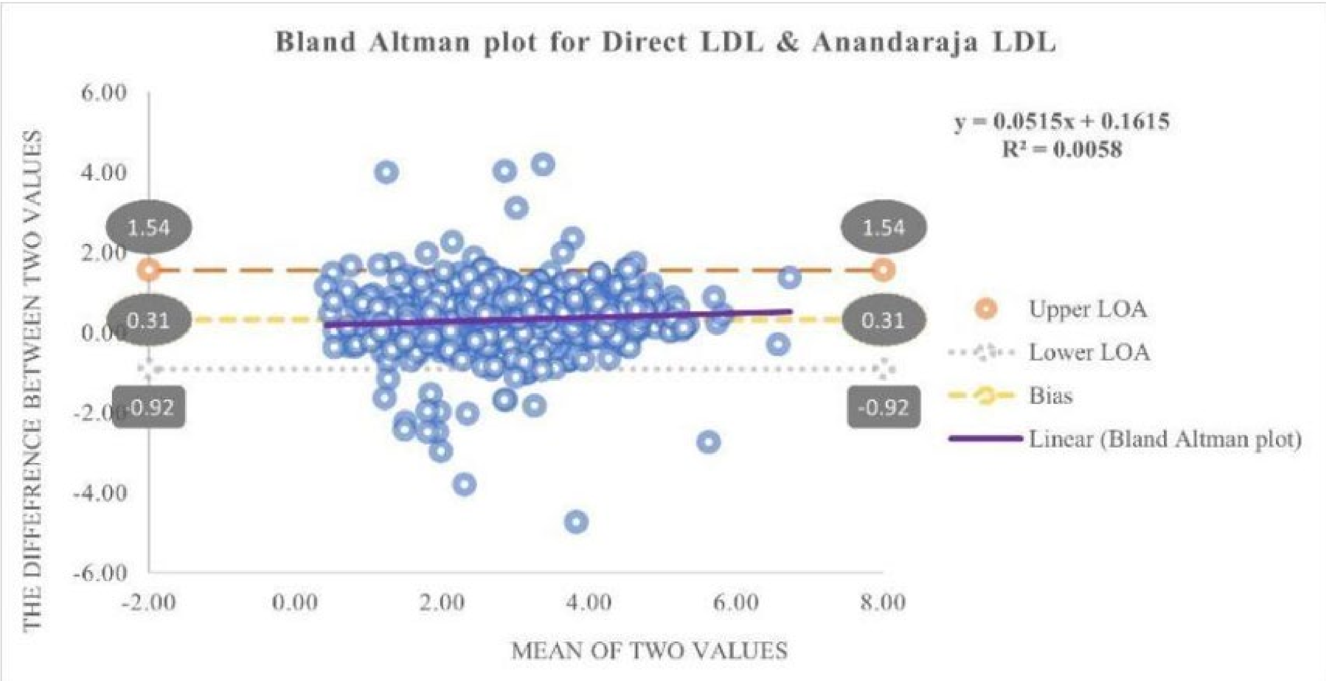
Results

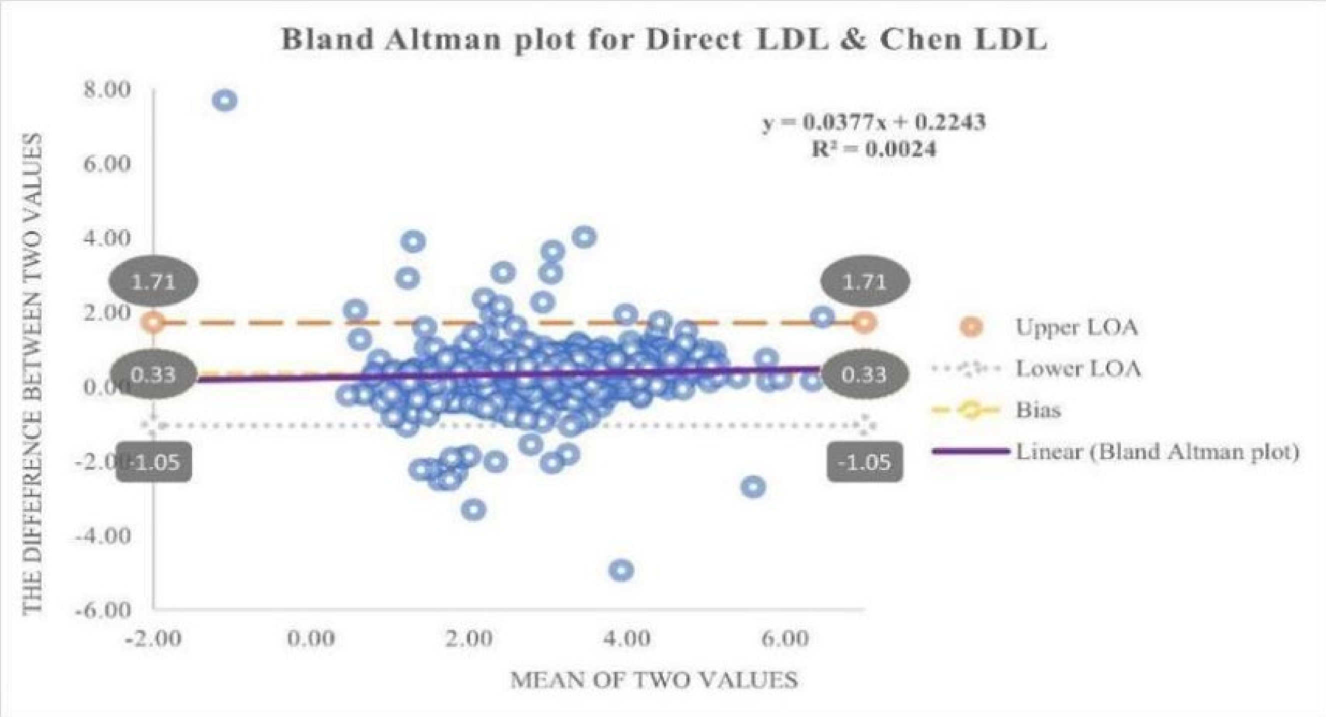
A total of 1075 patients of which 50.5% (543) were females and 49.5% (532) were males with mean age group of 51.19 ± 14.01 years were included. Table 6 shows demographic and lipid data of studied population with mean ± SD, mean difference, p value of paired t-test and r and p of Pearson correlation serving to compare and correlate different formulae

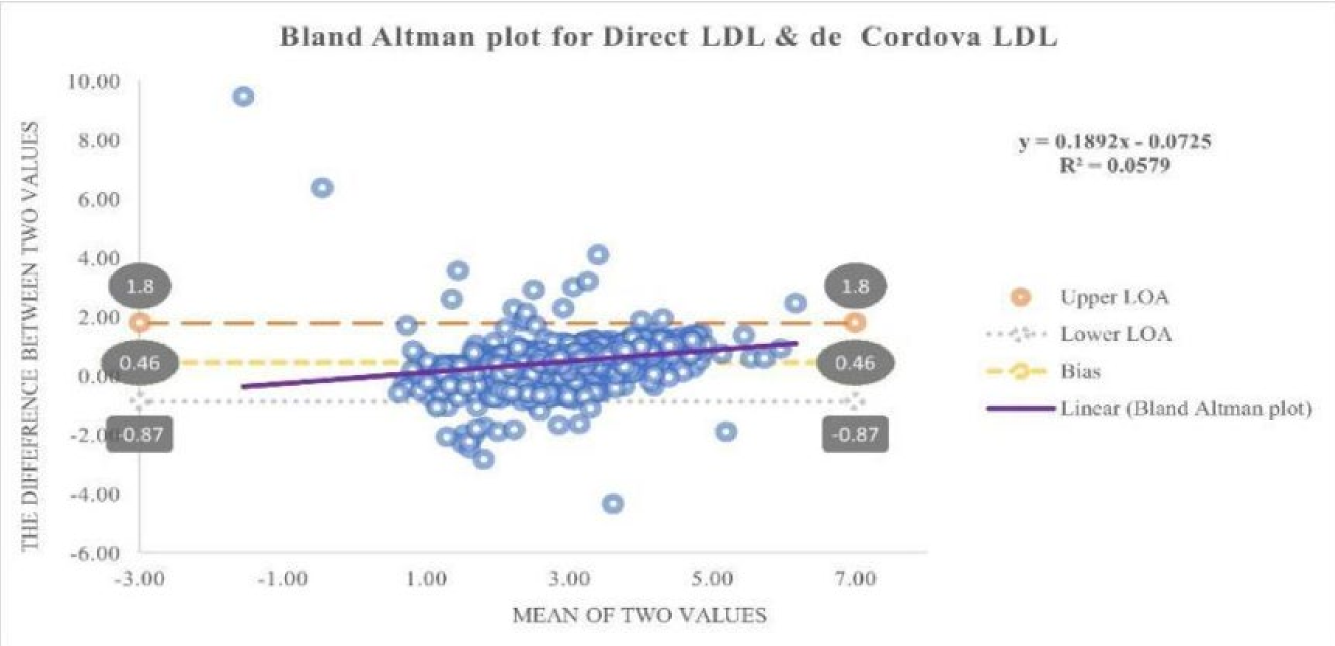
Lowest bias 0.16 is shown by Vujovic formula with lower limit being -1.3 and upper limit being 1.63. The Bland Altmann plot (Figure 1). indicates high level of agreement between Vujovic formula and Direct measurement of LDL. The small bias and narrow limits of agreement suggest that the two methods can be used interchangeably without significant concern for clinical differences. Highest mean difference is shown by Ahmadi formula which means there is small but consistent bias.

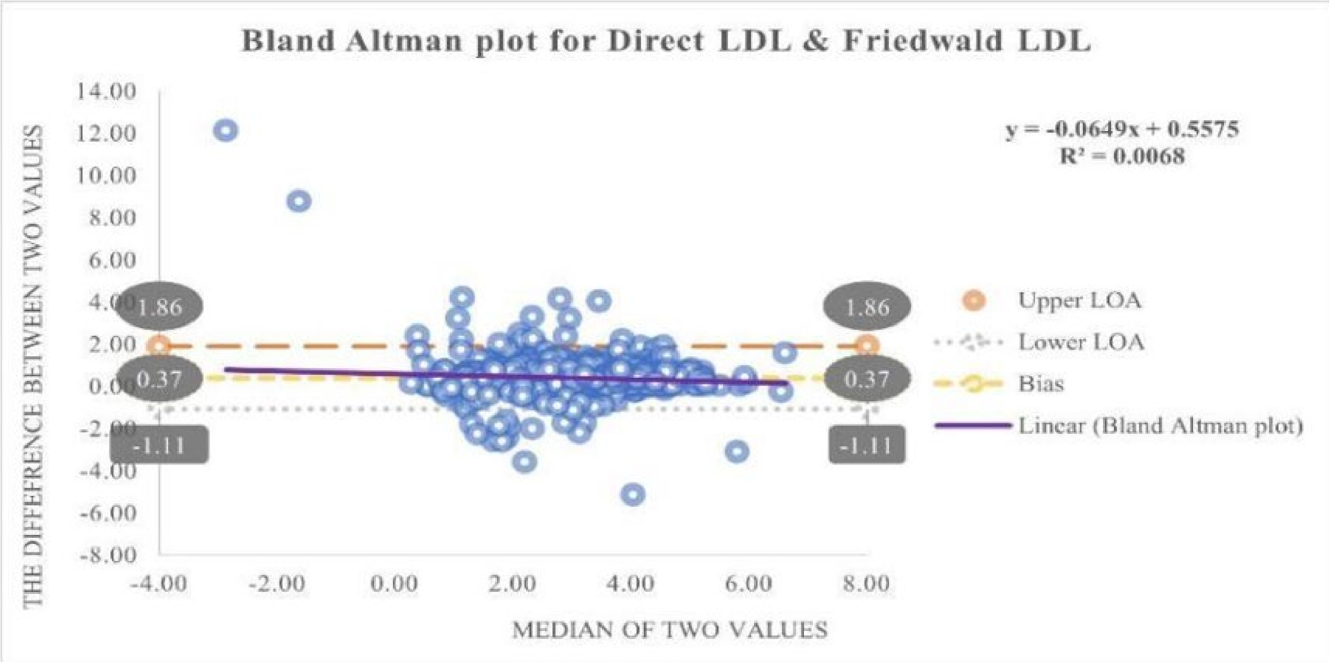
Figure 1: Bland Altman plots to look for bias between Direct-LDL and calculated-LDL's.

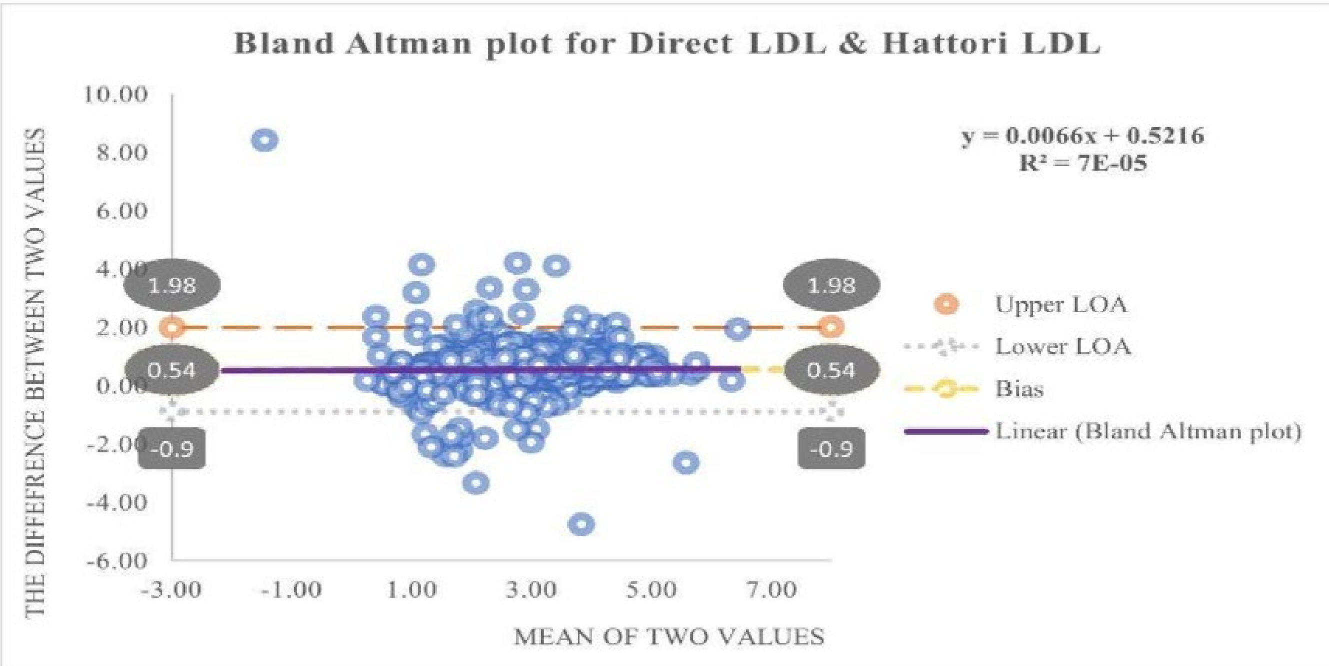


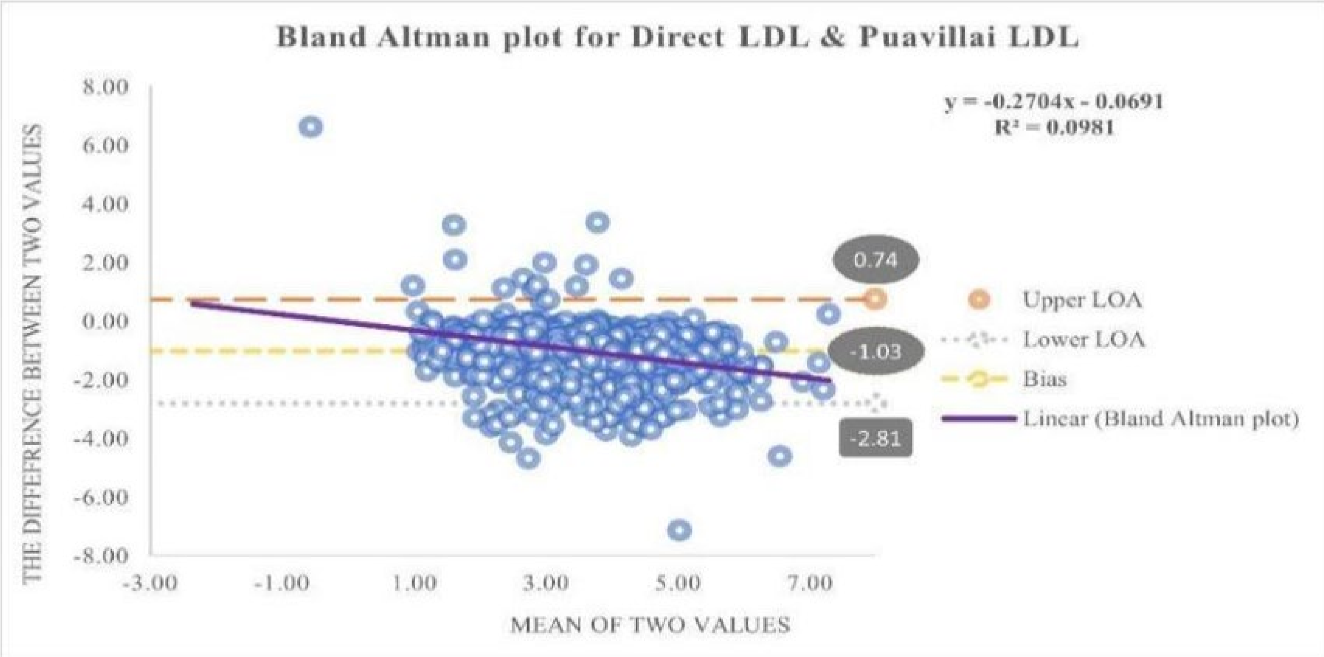


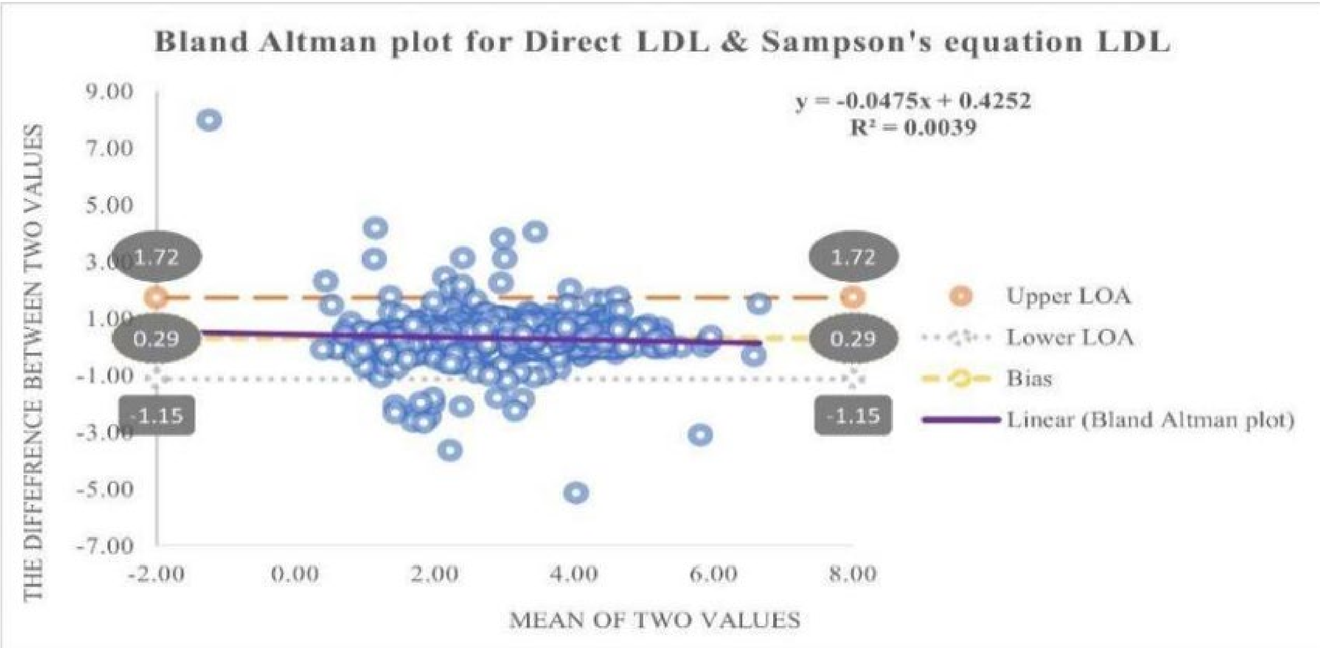

Bland Altman plot for Direct LDL & Chen LDL
y = 0.0377x + 0.2243
R² = 0.0024
1.71
0.33
-1.05
THE DIFFERENCE BETWEEN TWO VALUES
MEAN OF TWO VALUES
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)

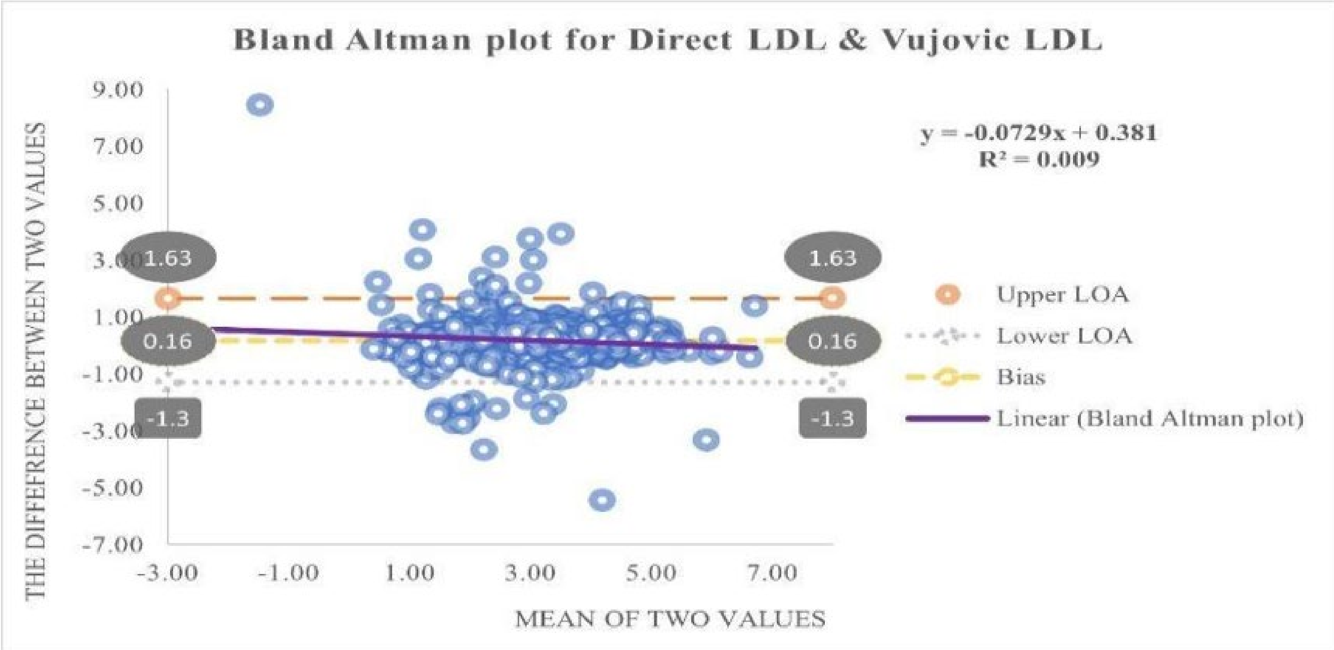

Bland Altman plot for Direct LDL & de Cordova LDL
y = 0.1892x - 0.0725
R² = 0.0579
1.8
0.46
-0.87
THE DIFFERENCE BETWEEN TWO VALUES
MEAN OF TWO VALUES
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)


Bland Altman plot for Direct LDL & Friedwald LDL
THE DIFFERENCE BETWEEN TWO VALUES
MEDIAN OF TWO VALUES
y = -0.0649x + 0.5575
R² = 0.0068
1.86
0.37
-1.11
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)


Bland Altman plot for Direct LDL & Hattori LDL
THE DIFFERENCE BETWEEN TWO VALUES
MEAN OF TWO VALUES
y = 0.0066x + 0.5216
R² = 7E-05
1.98
0.54
-0.9
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)


Bland Altman plot for Direct LDL & Puavillai LDL
y = -0.2704x - 0.0691
R² = 0.0981
THE DIFFERENCE BETWEEN TWO VALUES
MEAN OF TWO VALUES
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-3.00
-1.00
1.00
3.00
5.00
7.00
0.74
-1.03
-2.81
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)

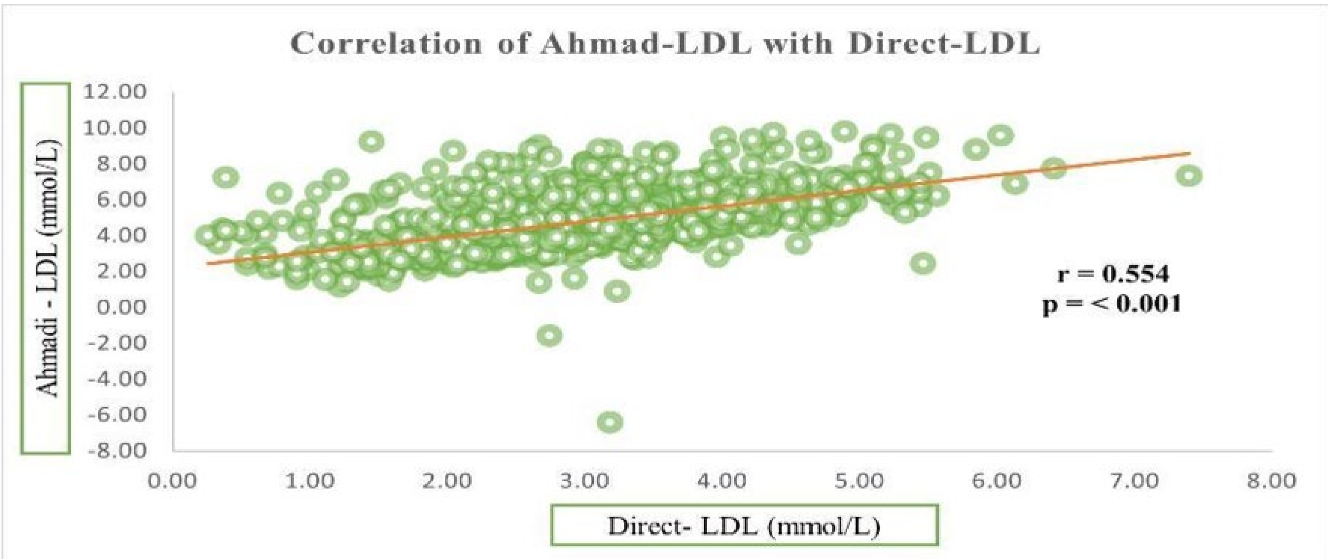

Bland Altman plot for Direct LDL & Sampson's equation LDL
y = -0.0475x + 0.4252
R² = 0.0039
THE DIFFERENCE BETWEEN TWO VALUES
MEAN OF TWO VALUES
9.00
7.00
5.00
3.00
1.00
-1.00
-3.00
-5.00
-7.00
-2.00
0.00
2.00
4.00
6.00
8.00
1.72
0.29
-1.15
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)

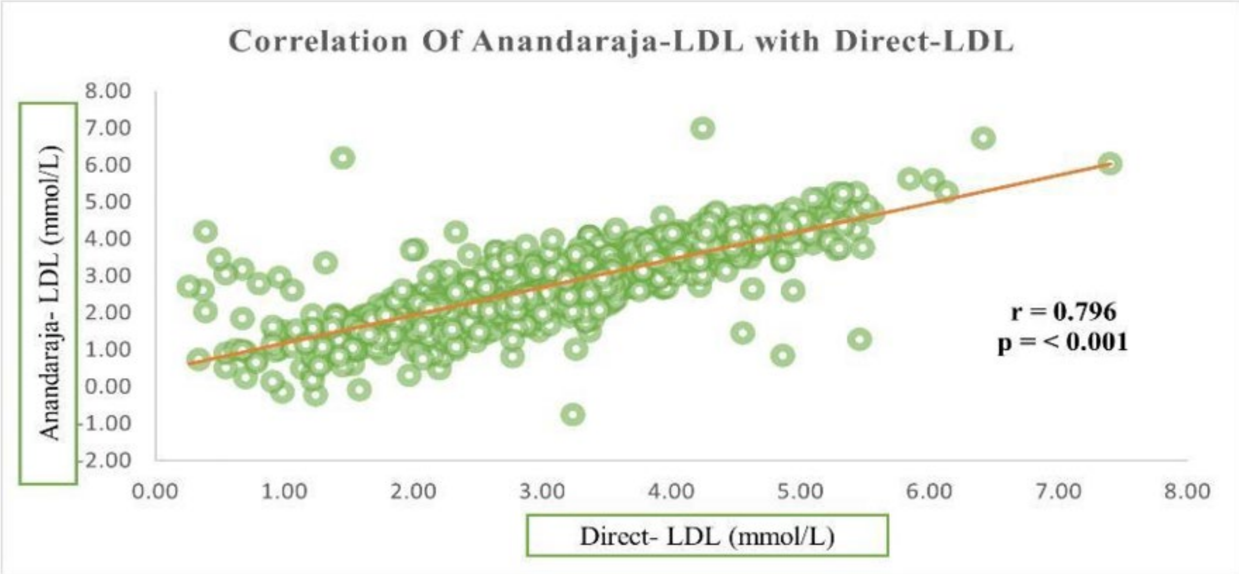


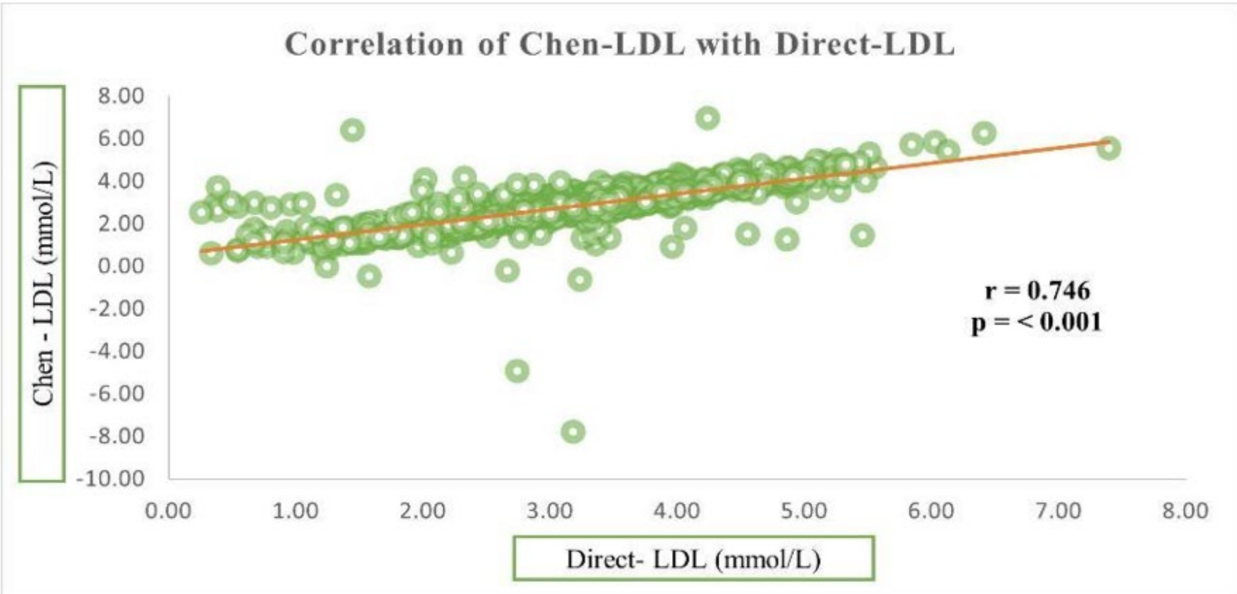
Bland Altmann plots to look for bias and agreement between Direct-LDL and calculated-LDL's

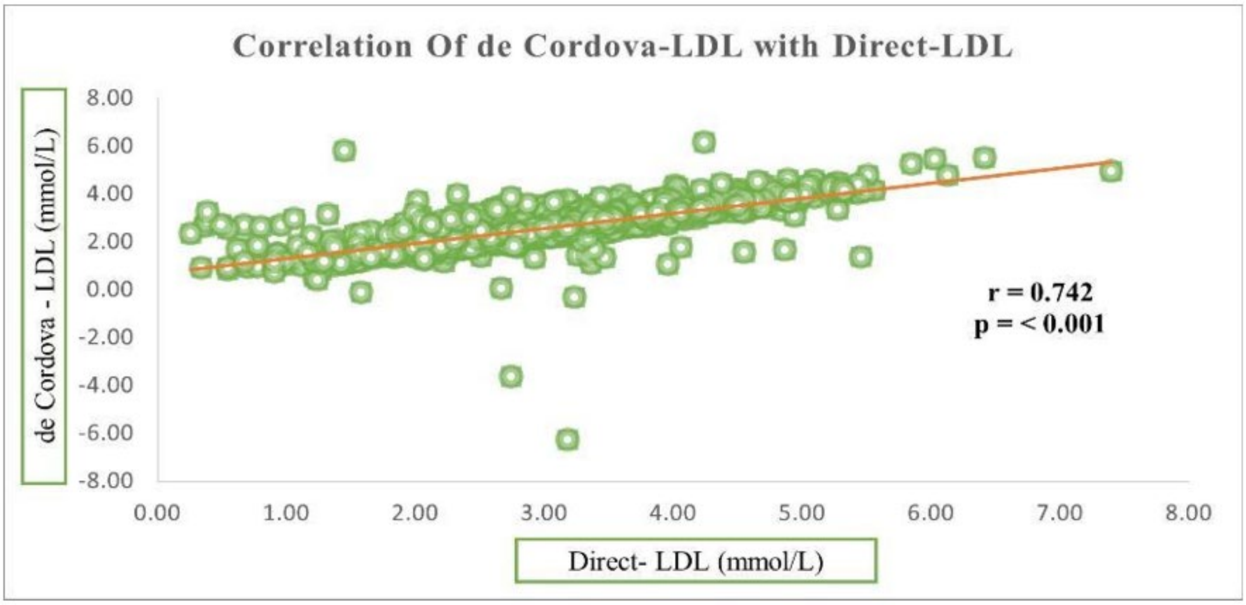
Moderate to strong relation of 0.554 – 0.796 was observed between various calculated formulae with direct LDL (Figure 2).

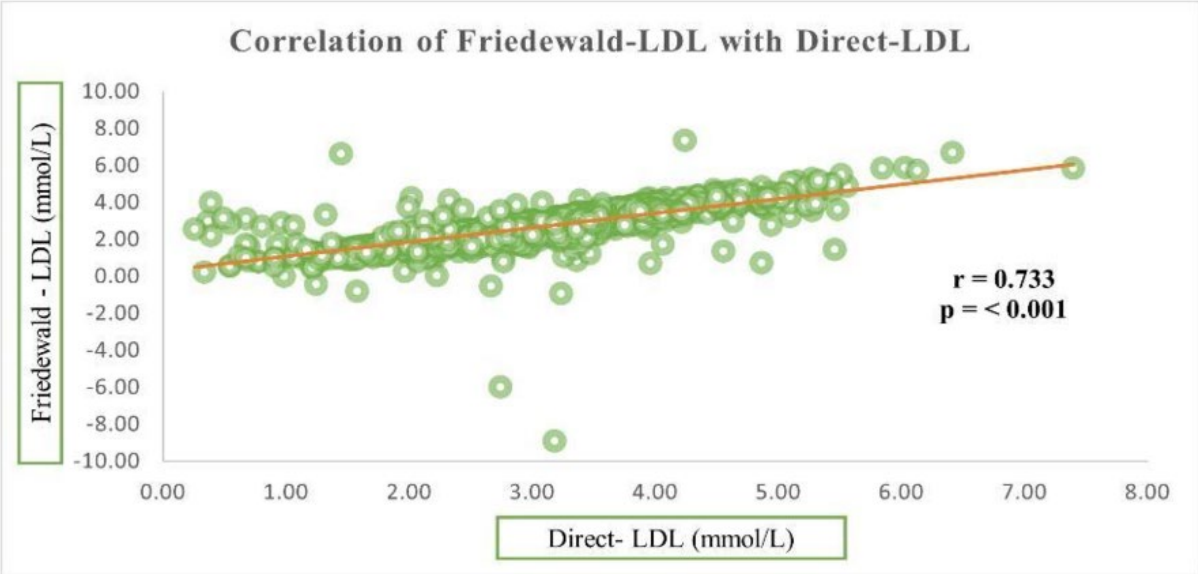
Figure 2: Correlation of various calculated formulae with direct LDL.

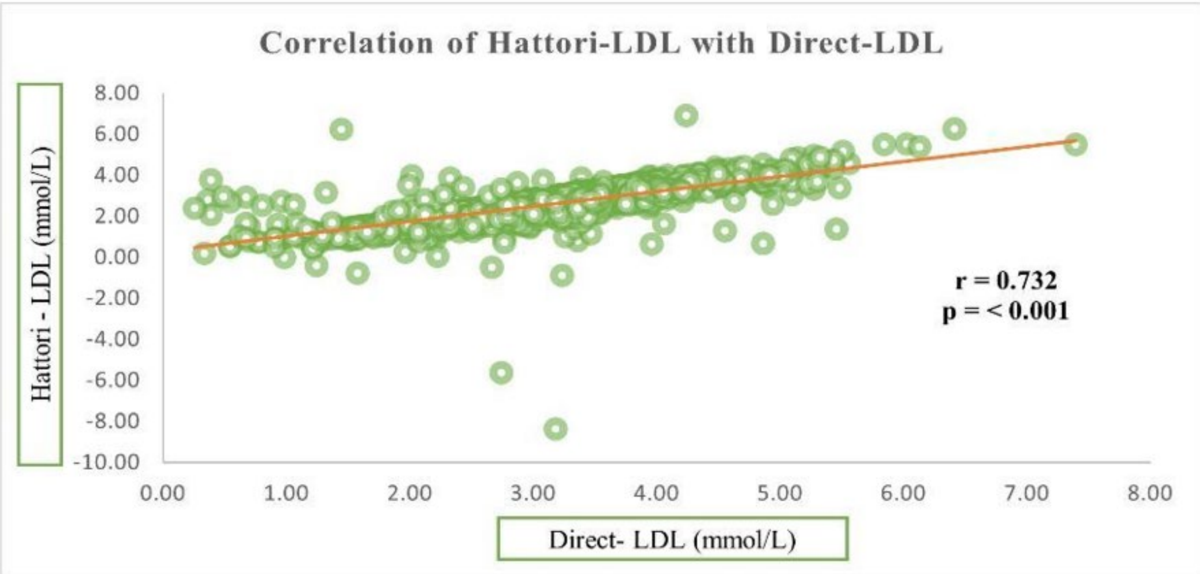


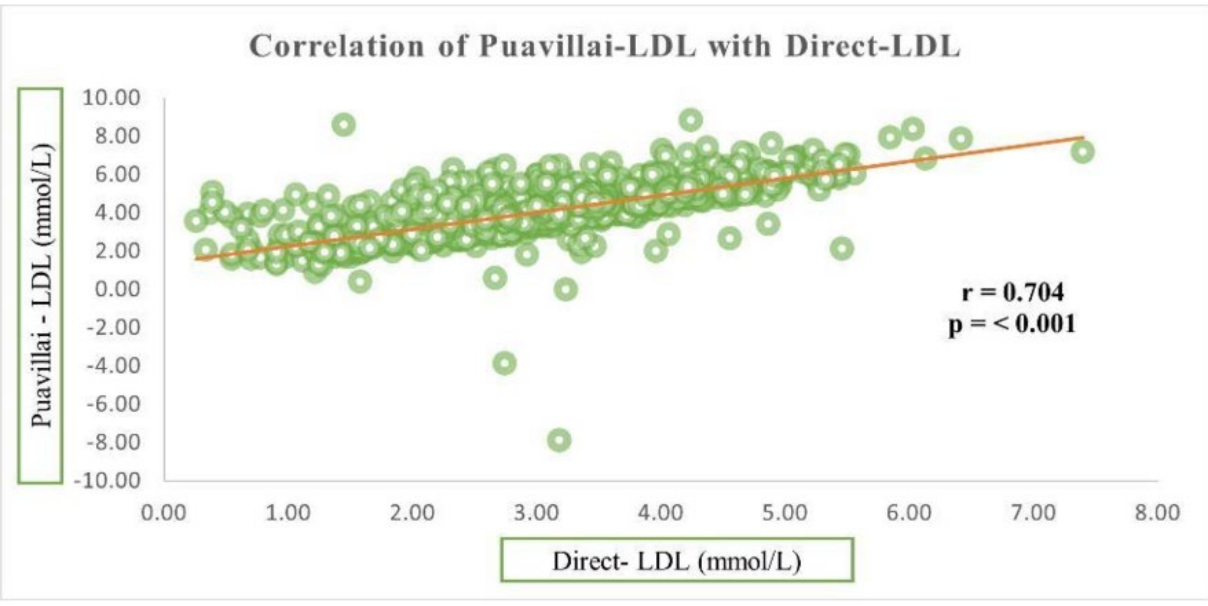

Correlation Of Anandaraja-LDL with Direct-LDL
Anandaraja- LDL (mmol/L)
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
Direct- LDL (mmol/L)
r = 0.796
p = < 0.001

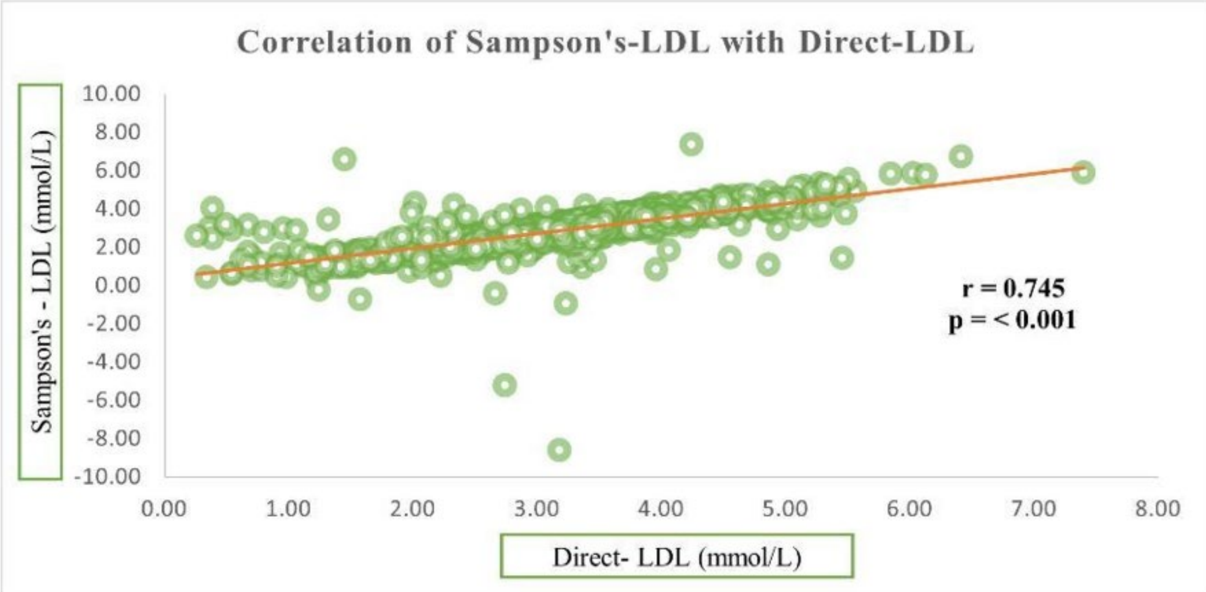

Correlation of Chen-LDL with Direct-LDL
Chen - LDL (mmol/L)
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
Direct- LDL (mmol/L)
r = 0.746
p = < 0.001

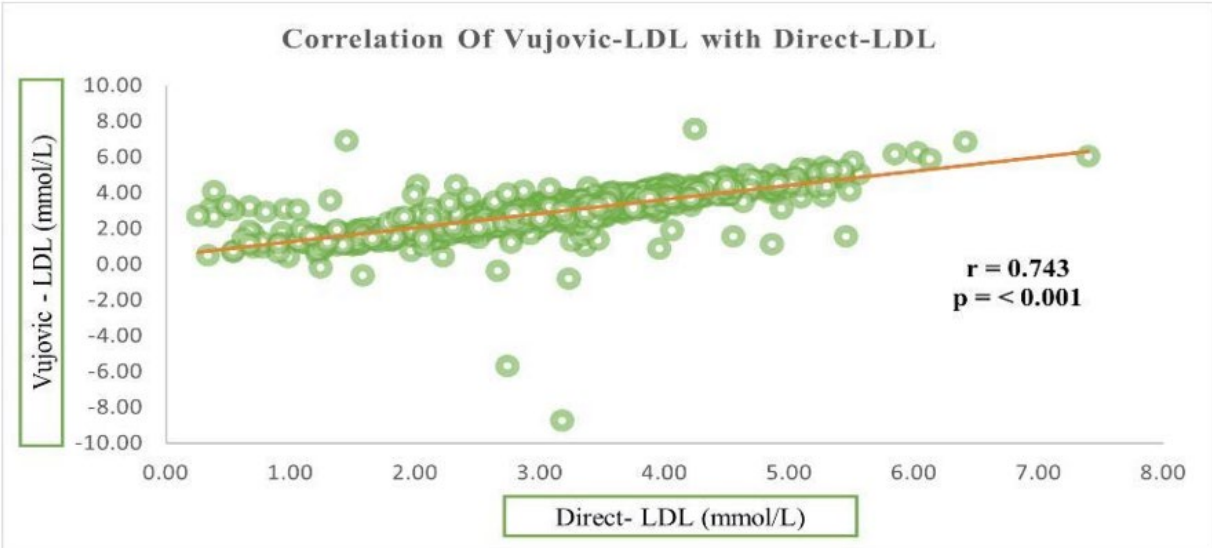

Correlation Of de Cordova-LDL with Direct-LDL
de Cordova - LDL (mmol/L)
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
Direct- LDL (mmol/L)
r = 0.742
p = < 0.001


Correlation of Friedewald-LDL with Direct-LDL
Friedewald - LDL (mmol/L)
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
r = 0.733
p = < 0.001
Direct- LDL (mmol/L)


Correlation of Hattori-LDL with Direct-LDL
Hattori - LDL (mmol/L)
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
r = 0.732
p = < 0.001
Direct- LDL (mmol/L)


Correlation of Puavillai-LDL with Direct-LDL
Puavillai - LDL (mmol/L)
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
r = 0.704
p = < 0.001
Direct- LDL (mmol/L)





Correlation of various calculated formulae with direct LDL

As shown in Table 6, Surprisingly, all calculated LDL methods exhibited a strong correlation with direct LDL measurement across the study cohort. The Friedewald equation, as well as modified equations incorporating non-HDL cholesterol or apolipoprotein B, demonstrated particularly robust correlations. These findings indicate the reliability of calculated LDL methods in estimating LDL cholesterol levels. However, the mean of calculated LDL-C by all equations showed significant mean difference with directly measured LDL-C in which least mean difference (LMD) was shown by Vujovic formula and best correlation shown by Anandaraja formula

Table 6: Demographic distribution and lipid data of the study subjects.

Variable	Mean ± SD	Mean difference	t-test (Vs Direct-LDL C)	Person correlation	
				r	P
Age	51.19 ± 14.01				
Sex	532 males 543 females				
Total cholesterol (mmol/L)	4.61 ± 1.12				
Triglycerides (mmol/L)	1.67 ± 0.78				
HDL-C (mmol/L)	1.17 ± 0.52				
Direct LDL-C (mmol/L)	3.05 ± 1.00				
Comparative analysis of LDL-C by nine formula					
Ahmadi LDL-C	4.83 ± 1.56	-1.78	**<0.001**	0.554**	**<0.001**
Anandaraja LDL-C	2.74 ± 0.96	0.31	**<0.001**	**0.796****	**<0.001**
Chen LDL-C	2.71 ± 0.97	0.33	**<0.001**	0.747**	**<0.001**
de Cordova LDL-C	2.59 ± 0.85	0.46	**<0.001**	0.742**	**<0.001**
Friedewald LDL-C	2.68 ± 1.06	0.37	**<0.001**	0.733**	**<0.001**
Hattori LDL-C	2.51 ± 1.00	0.54	**<0.001**	0.733**	**<0.001**
Puavillai LDL-C	4.08 ± 1.27	-1.03	**<0.001**	0.704**	**<0.001**
Sampson's LDL-C	2.76 ± 1.05	0.29	**<0.001**	0.745**	**<0.001**
Vujovic LDL-C	2.88 ± 1.07	**0.16**	**<0.001**	**0.743****	**<0.001**

SD: Standard deviation; r=Correlation Coefficient; p<0.05 considered statistically significant

Estimation of LDL-C in 4 subgroups based on Age (Table 7). There were four subgroups based on age (Group 1 = <20, Group 2 = 20–39, Group 3 = 40–59 and Group 4 = ≥ 60 years). in which Ahmadi and Puavillai formulae overestimated LDL values whereas all other formulae underestimated LDL values than Direct-LDL value in all age sub-groups. LMD & good correlation was shown by Vujovic formula in all subgroups

Table 7: Distribution of calculated LDL-C in age groups <20 years, 20-39 years, 40-59 years, >=60 years.

Variable	Mean ± SD	Mean difference	t-test (Vs Direct-LDL C)	Person correlation	
				r	P
Age	**Group 1: Age = <20 (years), (n= 14)**				
Direct LDL-C	2.41 ± 1.02				
Ahmadi LDL-C	3.79 ± 1.56	-1.38	**0.002**	0.547*	**0.043**
Anandaraja LDL-C	2.16 ± 0.92	0.25	**0.049**	0.904**	**<0.001**
Chen LDL-C	2.12 ± 0.81	0.29	**0.011**	0.943**	**<0.001**
de Cordova LDL-C	2.02 ± 0.73	0.39	**0.009**	0.902**	**<0.001**
Friedewald LDL-C	2.08 ± 0.88	0.33	**0.004**	0.941**	**<0.001**
Hattori LDL-C	1.95 ± 0.83	0.46	**<0.001**	0.940**	**<0.001**
Puavillai LDL-C	3.20 ± 1.14	-0.79	**0.001**	0.799**	**0.001**
Sampson's LDL-C	2.14 ± 0.89	0.27	**0.010**	0.944**	**<0.001**
Vujovic LDL-C	2.25 ± 0.89	**0.16**	**0.092**	**0.946****	**<0.001**
	Group 2: Age = 20-39 (years), (n= 200)				
Direct LDL-C	3.01 ± 1.02				
Ahmadi LDL-C	4.77 ± 1.64	-1.76	**<0.001**	0.585**	**<0.001**
Anandaraja LDL-C	2.70 ± 0.99	0.31	**<0.001**	0.734**	**<0.001**
Chen LDL-C	2.69 ± 0.94	0.32	**<0.001**	**0.770****	**<0.001**
de Cordova LDL-C	2.56 ± 0.84	0.45	**<0.001**	0.764**	**<0.001**
Friedewald LDL-C	2.65 ± 1.01	0.36	**<0.001**	0.754**	**<0.001**
Hattori LDL-C	2.49 ± 0.95	0.52	**<0.001**	0.753**	**<0.001**
Puavillai LDL-C	4.03 ± 1.29	-1.03	**<0.001**	0.724**	**<0.001**
Sampson's LDL-C	2.73 ± 1.01	0.28	**<0.001**	0.767**	**<0.001**
Vujovic LDL-C	2.86 ± 1.03	**0.15**	**<0.001**	**0.766****	**<0.001**
	Group 3: Age = 40-59 (years), (n= 541)				
Direct LDL-C	3.12 ± 1.02				
Ahmadi LDL-C	4.97 ± 1.57	-1.85	**<0.001**	0.510**	**<0.001**
Anandaraja LDL-C	2.79 ± 0.99	0.33	**<0.001**	**0.804****	**<0.001**
Chen LDL-C	2.76 ± 1.06	0.37	**<0.001**	0.699**	**<0.001**
de Cordova LDL-C	2.64 ± 0.91	0.49	**<0.001**	0.696**	**<0.001**
Friedewald LDL-C	2.71 ± 1.18	0.42	**<0.001**	0.687**	**<0.001**
Hattori LDL-C	2.54 ± 1.10	0.59	**<0.001**	0.687**	**<0.001**
Puavillai LDL-C	4.17 ± 1.32	-1.05	**<0.001**	0.663**	**<0.001**
Sampson's LDL-C	2.80 ± 1.15	0.32	**<0.001**	0.698**	**<0.001**
Vujovic LDL-C	2.92 ± 1.17	**0.20**	**<0.001**	**0.696****	**<0.001**
	Group 4: Age = >=60 (years), (n= 320)				
Direct LDL-C	**2.97 ± 0.95**				
Ahmadi LDL-C	**4.67 ± 1.45**	-1.70	**<0.001**	0.600**	**<0.001**
Anandaraja LDL-C	**2.70 ± 0.88**	0.28	**<0.001**	0.815**	**<0.001**
Chen LDL-C	**2.68 ± 0.82**	0.29	**<0.001**	**0.836****	**<0.001**
de Cordova LDL-C	**2.54 ± 0.74**	0.43	**<0.001**	0.819**	**<0.001**
Friedewald LDL-C	**2.66 ± 0.88**	0.31	**<0.001**	0.830**	**<0.001**
Hattori LDL-C	**2.49 ± 0.83**	0.48	**<0.001**	0.829**	**<0.001**

Puavillai LDL-C	**3.99 ± 1.14**	-1.02	**<0.001**	0.763**	**<0.001**
Sampson's LDL-C	**2.74 ± 0.88**	0.24	**<0.001**	**0.836****	**<0.001**
Vujovic LDL-C	**2.86 ± 0.90**	**0.11**	**<0.001**	**0.836****	**<0.001**

SD: Standard deviation; r=Correlation Coefficient; p<0.05 considered statistically significant

Estimation of LDL-C in 4 subgroups based on TG ranges (Table 8). Since we had only 3 values whose TG was > 4.51 mmol/dL, we removed these readings from database so we had only 4 sub-groups Group 1: TG <0.56 mmol/L, Group 2: TG 0.56-1.69 mmol/L, Group 3: TG = 1.70-3.38 mmol/L & Group 4: TG = 3.39-4.51 mmol/L

Table 8: Estimation of LDL-C in 4 subgroups based on TG ranges (<0.56, 0.56–1.69, 1.70–3.38, 3.39–4.51 and > 4.51 mmol/L).

Variable	**Mean ± SD**	**Mean difference**	**t-test (Vs Direct-LDL C)**	**Person correlation**	
				r	**P**
	Group 1: TG <0.56 (mmol/L), (n=15)				
Direct LDL-C	2.05 ± 0.67				
Ahmadi LDL-C	2.21 ± 0.62	-0.16	0.002	0.723**	0.002
Anandaraja LDL-C	1.97 ± 0.77	**0.08**	**<0.001**	**0.851****	**<0.001**
Chen LDL-C	1.72 ± 0.64	0.33	**<0.001**	0.800**	**<0.001**
de Cordova LDL-C	1.53 ± 0.54	0.52	**<0.001**	0.792**	**<0.001**
Friedewald LDL-C	1.81 ± 0.71	0.24	**<0.001**	0.807**	**<0.001**
Hattori LDL-C	1.70 ± 0.67	0.35	**<0.001**	0.807**	**<0.001**
Puavillai LDL-C	2.22 ± 0.72	-0.17	**0.001**	0.778**	**0.001**
Sampson's LDL-C	1.77 ± 0.74	0.28	**<0.001**	0.805**	**<0.001**
Vujovic LDL-C	1.87 ± 0.71	0.18	**<0.001**	0.803**	**<0.001**
	Group 2: TG 0.56-1.69 (mmol/L), (n=630)				
Direct LDL-C	2.95 ± 1.00				
Ahmadi LDL-C	4.04 ± 1.07	-1.09	**<0.001**	0.711**	**<0.001**
Anandaraja LDL-C	2.76 ± 0.93	0.19	**<0.001**	**0.818****	**<0.001**
Chen LDL-C	2.61 ± 0.95	0.34	**<0.001**	0.739**	**<0.001**
de Cordova LDL-C	2.40 ± 0.81	0.55	**<0.001**	0.745**	**<0.001**
Friedewald LDL-C	2.66 ± 1.04	0.29	**<0.001**	0.732**	**<0.001**
Hattori LDL-C	2.49 ± 0.98	0.46	**<0.001**	0.732**	**<0.001**
Puavillai LDL-C	3.65 ± 1.11	-0.7	**<0.001**	0.747**	**<0.001**
Sampson's LDL-C	2.70 ± 1.05	0.25	**<0.001**	0.740**	**<0.001**
Vujovic LDL-C	2.80 ± 1.05	**0.15**	**<0.001**	**0.737****	**<0.001**
	Group 3: TG = 1.70-3.38 (mmol/L), (n=376)				
Direct LDL-C	3.24 ± 0.96				
Ahmadi LDL-C	5.80 ± 1.08	-2.56	**<0.001**	0.617**	**<0.001**
Anandaraja LDL-C	2.77 ± 0.98	0.47	**<0.001**	**0.790****	**<0.001**
Chen LDL-C	2.91 ± 0.98	0.34	**<0.001**	0.735**	**<0.001**
de Cordova LDL-C	2.85 ± 0.82	0.39	**<0.001**	0.733**	**<0.001**
Friedewald LDL-C	2.77 ± 1.09	0.47	**<0.001**	0.731**	**<0.001**
Hattori LDL-C	2.60 ± 1.03	0.65	**<0.001**	0.730**	**<0.001**
Puavillai LDL-C	4.65 ± 1.11	-1.40	**<0.001**	0.719**	**<0.001**
Sampson's LDL-C	2.90 ± 1.04	0.34	**<0.001**	0.737**	**<0.001**

Vujovic LDL-C	3.05 ± 1.09	**0.19**	**<0.001**	**0.734****	**<0.001**
	Group 4: TG = 3.39-4.51 (mmol/L) , (n=54)				
Direct LDL-C	3.08 ± 1.12				
Ahmadi LDL-C	7.98 ± 1.01	-4.90	**<0.001**	0.734**	**<0.001**
Anandaraja LDL-C	2.41 ± 1.07	0.67	**<0.001**	0.757**	**<0.001**
Chen LDL-C	2.90 ± 0.93	0.18	0.07	0.758**	**<0.001**
de Cordova LDL-C	3.14 ± 0.79	**-0.06**	0.54	**0.760****	**<0.001**
Friedewald LDL-C	2.45 ± 1.02	0.63	**<0.001**	0.754**	**<0.001**
Hattori LDL-C	2.29 ± 0.96	0.79	**<0.001**	0.754**	**<0.001**
Puavillai LDL-C	5.61 ± 1.09	-2.54	**<0.001**	**0.760****	**<0.001**
Sampson's LDL-C	2.70 ± 0.93	0.37	**<0.001**	0.754**	**<0.001**
Vujovic LDL-C	2.92 1.03	0.16	0.12	0.756**	**<0.001**

TG: Triglycerides; SD: Standard deviation; r=Correlation Coefficient; p<0.05 considered statistically significant

Overestimation of LDL was shown by Ahmadi, Puavillai in all TG subgroups while reverse ie underestimation of LDL was shown by all others except de Cordova which showed underestimated LDL at TG < 3.38 mmol/L & overestimation was seen at TG >3.38 mmol/L. LMD & best correlation was shown by Anandaraja formula at TG < 0.56 mmol/L. Vujovic formula showed LMD & good correlation at TG levels in between 0.56-3.38 mmol/L and best correlation at this level was shown by Anandaraja formula which was little higher than Vujovic formula. LMD & best correlation was shown by de Cordova formula at TG > 3.38 mmol/L. Puavillai formula also showed best correlation although it had significant mean difference

Estimation of LDL-C in 3 subgroups based on TC ranges (Table 9a, 9b)

We had 3 subgroups Group 1: TC = < 5.17 mmol/L, Group 2: TC 5.17-6.18 mmol/L & Group 3: TG = > 6.18 mmol/L, in which Ahmadi and Puavillai formulae overestimated LDL values whereas all other formulae underestimated LDL values than Direct-LDL value in all TC sub-groups. LMD & good correlation was shown by Vujovic formula in all subgroups of TC except subgroup 2 where best corelation was seen while Anandaraja showed best correlation ('r' = 0.659 and 'r' = 0.338 in subgroups 1 and 3). Very poor correlation was shown by Ahmadi formula at TC >5.17 mmol/L.

Table 9a: Estimation of LDL-C in 3 subgroups based on TC ranges < 5.17 mmol/L, 5.17-6-18 mmol/L and > 6.18 mmol/L.

Variable	**Mean ± SD**	**Mean difference**	**t-test (Vs Direct-LDL C)**	**Person correlation**	
				r	**P**
	Group 1: Total cholesterol <5.17 (mmol/L), (n= 750)				
Direct LDL-C	2.63 ± 0.77				
Ahmadi LDL-C	4.25 ± 1.29	-1.63	**<0.001**	0.360**	**<0.001**
Anandaraja LDL-C	2.29 ± 0.70	0.34	**<0.001**	**0.659****	**<0.001**
Chen LDL-C	2.28 ± 0.76	0.34	**<0.001**	0.543**	**<0.001**
de Cordova LDL-C	2.20 ± 0.66	0.42	**<0.001**	0.542**	**<0.001**
Friedewald LDL-C	2.22 ± 0.86	0.41	**<0.001**	.0526**	**<0.001**
Hattori LDL-C	2.08 ± 0.80	0.55	**<0.001**	0.525**	**<0.001**
Puavillai LDL-C	3.52 ± 0.99	-0.90	**<0.001**	0.506**	**<0.001**
Sampson's LDL-C	2.30 ± 0.83	0.32	**<0.001**	0.545**	**<0.001**
Vujovic LDL-C	2.41 ± 0.85	**0.21**	**<0.001**	**0.538****	**<0.001**
Table 9b: Estimation of LDL-C in 3 subgroups based on TC ranges < 5.17 mmol/L, 5.17-6-18 mmol/L and > 6.18 mmol/L.					
	Group 2: Total cholesterol = 5.17-6.18 (mmol/L), (n= 244)				
Direct LDL-C	3.77 ± 0.63				
Ahmadi LDL-C	5.84 ± 1.12	-2.06	**<0.001**	-0.025	0.699

Anandaraja LDL-C	3.52 ± 0.40	0.26	<0.001	0.378**	<0.001
Chen LDL-C	3.47 ± 0.35	0.30	<0.001	0.504**	<0.001
de Cordova LDL-C	3.26 ± 0.30	0.51	<0.001	0.402**	<0.001
Friedewald LDL-C	3.47 ± 0.45	0.30	<0.001	0.498**	<0.001
Hattori LDL-C	3.25 ± 0.43	0.52	<0.001	0.497**	<0.001
Puavillai LDL-C	5.06 ± 0.57	-1.28	<0.001	0.189**	0.003
Sampson's LDL-C	3.57 ± 0.42	0.21	<0.001	0.501**	<0.001
Vujovic LDL-C	3.70 ± 0.41	0.07	0.051	0.509**	<0.001
	Group 3: Total cholesterol = >6.18 (mmol/L), (n= 81)				
Direct LDL-C	4.75 ± 0.82				
Ahmadi LDL-C	7.11 ± 1.29	-2.36	<0.001	-0.053	0.636
Anandaraja LDL-C	4.51 ± 0.65	0.23	0.016	0.338**	0.002
Chen LDL-C	4.43 ± 0.61	0.32	0.001	0.301**	0.006
de Cordova LDL-C	4.11 ± 0.51	0.63	<0.001	0.237*	0.033
Friedewald LDL-C	4.48 ± 0.51	0.27	0.009	0.328**	0.003
Hattori LDL-C	4.20 ± 0.68	0.54	<0.001	0.329**	0.003
Puavillai LDL-C	6.30 ± 0.81	-1.55	<0.001	0.120	0.288
Sampson's LDL-C	4.56 ± 0.70	0.19	0.055	0.333**	0.002
Vujovic LDL-C	4.75 ± 0.69	0.00	0.990	0.315**	0.004

TC: Total cholesterol; SD: Standard deviation; r=Correlation Coefficient; p<0.05 considered statistically significant

Estimation of LDL-C in 3 subgroups based on HDL ranges (Table 10).

We had 3 subgroups Group 1: HDL = < 1.03 mmol/L, Group 2: TC 1.03-1.52 mmol/L & Group 3: TG = > 1.52 mmol/L, in which Ahmadi and Puavillai formulae overestimated LDL values whereas all other formulae underestimated LDL values than Direct-LDL value in all HDL sub-groups except Anandaraja formula which showed underestimation of LDL values at HDL < 1.52 mmol/L & overestimated at HDL >1.52 mmol/L. LMD & good correlation was shown by Vujovic formula at HDL < 1.52 mmol/L. Best correlation was exhibited by Chen formula at HDL < 1.52 mmol/L. LMD & best correlation was shown by Anandaraja at HDL > 1.52 mmol/L.

Table 10: Estimation of LDL-C in 3 subgroups based on HDL ranges <1.03 mmol/L, 1.03-1.52 mmol/L and >1.53 mmol/L.

Variable	Mean ± SD	Mean difference	t-test (Vs Direct-LDL C)	Person correlation	
				r	P
	Group 1: Total cholesterol <5.17 (mmol/L), (n= 750)				
Direct LDL-C	2.75 ± 1.00				
Ahmadi LDL-C	4.87 ± 1.50	-2.11	<0.001	0.545**	<0.001
Anandaraja LDL-C	2.15 ± 0.90	0.60	<0.001	0.803**	<0.001
Chen LDL-C	2.45 ± 0.86	0.31	<0.001	0.812**	<0.001
de Cordova LDL-C	2.40 ± 0.76	0.36	<0.001	0.799**	<0.001
Friedewald LDL-C	2.34 ± 0.94	0.42	<0.001	0.795**	<0.001
Hattori LDL-C	2.19 ± 0.89	0.56	<0.001	0.795**	<0.001
Puavillai LDL-C	3.90 ± 1.15	-1.15	<0.001	0.738**	<0.001
Sampson's LDL-C	2.44 ± 0.93	0.31	<0.001	0.807**	<0.001
Vujovic LDL-C	2.57 ± 0.95	0.19	<0.001	0.808**	<0.001
	Group 1 : HDL = < 1.03 (mmol/L), (n=340)				
Direct LDL-C	3.16 ± 0.95				

Ahmadi LDL-C	4.89 ±1.51	-1.73	**<0.001**	0.595**	**<0.001**
Anandaraja LDL-C	2.94 ± 0.82	0.22	**<0.001**	0.797**	**<0.001**
Chen LDL-C	2.87 ± 0.83	0.29	**<0.001**	**0.813****	**<0.001**
de Cordova LDL-C	2.70 ± 0.75	0.46	**<0.001**	0.797**	**<0.001**
Friedewald LDL-C	2.86 ± 0.89	0.30	**<0.001**	0.804**	**<0.001**
Hattori LDL-C	2.68 ± 0.83	0.48	**<0.001**	0.804**	**<0.001**
Puavillai LDL-C	4.22 ± 1.17	-1.06	**<0.001**	0.745**	**<0.001**
Sampson's LDL-C	2.93 ± 0.89	0.23	**<0.001**	0.811**	**<0.001**
Vujovic LDL-C	3.06 ± 0.91	**0.10**	**<0.001**	**0.812****	**<0.001**
	Group 3: HDL = >1.53 (mmol/L), (n= 100)				
Direct LDL-C	3.32 ± 1.12				
Ahmadi LDL-C	4.28 ± 1.92	-0.96	**<0.001**	0.596**	**<0.001**
Anandaraja LDL-C	3.41 ±1.02	**-0.08**	**0.188**	**0.832****	**<0.001**
Chen LDL-C	2.65 ± 1.70	0.67	**<0.001**	0.541**	**<0.001**
de Cordova LDL-C	2.48 ± 1.43	0.85	**<0.001**	0.565**	**<0.001**
Friedewald LDL-C	2.67 ± 1.89	0.65	**<0.001**	0.520**	**<0.001**
Hattori LDL-C	2.50 ± 1.77	0.82	**<0.001**	0.519**	**<0.001**
Puavillai LDL-C	3.81 ± 1.94	-0.49	**0.002**	0.591**	**<0.001**
Sampson's LDL-C	2.75 ± 1.84	0.57	**<0.001**	0.538**	**<0.001**
Vujovic LDL-C	2.84 ± 1.89	0.49	**0.003**	0.533**	**<0.001**

HDL : High Density lipoprotein; SD: Standard deviation; r=Correlation Coefficient; $p<0.05$ considered statistically significant

Discussion

Serum LDL-C level not only plays a crucial role in development of atherosclerosis which is proved to be a well-known factor in development of coronary heart disease but it also plays a role assessing the treatment session of these patients [1,3]. Estimation of LDL to a very precise level is therefore necessary but a difficult task when direct LDL measurement facility is not available in the lab setup. To overcome this situation many formulae have been developed and surprisingly they show a good positive correlation with direct LDL measurement just like this and other studies [11,12,16,22-26]. In this study the study population was subdivided into various subgroups based on age, TG, TC and HDL levels to validate 9 different formulae. Most of these formulae showed good correlation with D-LDL in between the subgroups. In this study Vujovic formula came out to show least mean difference and good correlation in various subgroups based on different criterias when compared to routinely used Friedewald formula which is in line with Vujovic et al. study in Serbian population and Wadhwa N and Krishnaswamy R study in Indian population. In Wadhwa study Vujovic formula came out to the best at all levels of TG, but in our study at lower TG level ie <0.56 mmol/L Anandaraja formula showed the best correlation which might be due to lesser no of individuals in this subgroup and at higher TG level > 3.38 de Cordova along with Puavillai showed best correlation.

In this study after Vujovic formula some other formulae like Anandaraja, Chen, de Cordova, Puavillai showed best correlation in one or other subgroups. Friedewald formula which is used routinely cannot be used at higher TG, higher total cholesterol or lower HDL levels [27,28].

Different studies are conducted to evaluate effectiveness of formulae alternative to direct LDL estimation by comparing one to two formulae with direct and commonly used Friedewald formula.

Most of these studies evaluated one or two formulae with very few taking more than two formulae like our study in which we compared and correlated 9 formulae. Also the study population was subdivided into various subgroups based on age, TG, TC and HDL levels in our study but the study population was grouped based on TG levels in most of the studies.

The major findings of different studies is listed in Table 11.

Table 11: Major findings of different studies.

Name of the study using author's name	Area of studied population	Comparison of Friedewald formula with newer formula which is modified Friedewald formula
Sha MFR et al [12]	Bangladeshi	Regression equation is more accurate to D-LDL when compared with Friedewald
de Cordova [8]	Brazilian	de Cordova formula is better than Friedewald formula
Ahmadi [5]	Iranian	Ahmadi formula is better at lower TG values
Gupta et al [22]	Indian	Friedewald formula is better than Anandaraja formula
Anandaraja et al [6]	Indian	Anandaraja formula is better in Indian population
Puavillai et al [10]	Thailand	Puavillai formula is better than Friedewald formula
Vujovic et al [11]	Serbian	Vujovic formula is better than Friedewald
Wadhwa et al [29]	Indian	Vujovic Formula is better than any other formula for Indian population which is similar to our study
Hattori et al [9]	Japanese	Hattori formula is better than Friedewald
Garule et al [30]	Indian	Puavillai formula is better than any other in Indian population at most TG levels but best is different for different TG levels
Karkhaneh et al [31]	Iranian	Here groups were divided based on other biochemical parameters of lipid profile too just like our study With difference in formula that came out to be best alternative to D-LDL was Hattori and de Cordova and our study was Vujovic
Krishnaveni et al [32]	Indian	Friedewald formula correlated maximally with D-LDL at all TG levels except < 100mg/dL where Anandaraja formula is better
Teerakanchana et al [33]	Thailand	Friedewald Formula gave inconsistent results at different level of TGs when compared to D-LDL
Sahu et al [34]	Indian	Friedewald formula gave inconsistent result still remains the choice after D-LDL due to cost effectiveness in country like India
Warade et al [35] **Sudha et al [36]**	Indian	D-LDL assay should be considered as and when possible due to variability in results with commonly used Friedewald formula

Limitations

Though the sample size was good enough overall when the population was subdivided into subgroups some of them had a very low data. The study compared and correlated various formulae of LDL-C with direct assay of LDL by only one method and no ultracentrifugation or precipitation was done which is known as reference method. Individuals having age group < 20 , TG < 0.56 and > 4.51 mmol/L were very less so there are chances of bias. > 4.51 mmol/L of TG level data was very in significant and so was excluded. Total cholesterol at higher level >6.18 mmol/L was seen in only 7.5% of whole population which is again low to increase chance of bias. Also, HDL > 1.53 mmol/L was seen in 9.3% individuals again small number of samples. One possibility of not getting higher level of TG, TC or HDL is that patients were on treatment with statins. Lastly only 9 formulae were considered for the study which omitted other formulae which could have given different result.

Conclusion

We are in favour of Vujovic formula for Indian population as it looked like a better alternative when compared with most commonly used Friedewald formula and other formulae. However more studies using more sample size particularly taking lower TG and higher TG levels into consideration, and from different ethnicities and geographical areas must be done to be able to use the above method confidently in Indian population.

Abbreviations

Chol: Cholesterol; FBS: Fasting blood sugar; HDL: High-density lipoprotein; LDL-C: Low-density lipoprotein-cholesterol; TC: Total cholesterol; TG: Triglyceride

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Authors' contributions

RK– research study plan. KDS, RKPK, BP research data collection. MN, AS Data analysis, statistical work and manuscript preparation. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1975 Helsinki declaration as revised in 2008. This study was approved by the Ethics Committee of Srinivasan Medical College and Hospital (IEC No. 18/2022).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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