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Original Article

Biochemistry Section

Comparison of LDL-Cholesterol Estimate using Various Formulae with Directly Measured LDL-Cholesterol in Indian Population

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ABSTRACT

Introduction: Low-Density Lipoprotein Cholesterol (LDLc) is widely recognized as an established cardiovascular risk marker. Different formulae have been proposed for calculation of LDLc but have not been validated in Indian population and over a wide range of Triglycerides (TG). Friedewald formula is most commonly used which has various shortcomings.

Aim: To calculate LDLc using various formulae and compare it with directly measured LDLc at various ranges of TG concentration in Indian population.

Materials and Methods: One year lipid profile data of 21,503 samples was taken. Calculation of LDLc was done by the following formulae: Friedewald; Cordova and Cordova; Vujovic; Ahmadi; Anandaraja; Puavillai and Hattori. Comparison of calculated LDLc with directly measured LDLc was done at following TG ranges: <150mg/dL; 151-199mg/dL; 200-399mg/dL and >400mg/dL using Pearson's correlation coefficient and two-paired t-test.

Results: For TG range <150mg/dL, Puavillai formula correlated best with direct measurement (r = 0.978). For TG range 151-199mg/dL, Vujovic formula correlated best with direct measurement (r = 0.977and mean difference of -1.2 mg/dL). For TG range 200-399mg/dL, Vujovic formula correlated best with direct measurement (r = 0.968). For TG range >400 mg/dL, Vujovic formula correlated best with direct measurement (r = 0.791).

Conclusion: Vujovic formula appears to be more accurate than any other formula when applied to Indian population.

Keywords: Cardiovascular disease, Direct low-density lipoprotein cholesterol, Vujovic formula

INTRODUCTION

Cardiovascular diseases are the leading cause of death in the world. The concentration of circulating Low Density Lipoprotein cholesterol (LDLc) is a predictor for assessing the risk for Coronary Heart Disease (CHD) [1]. It is considered as the primary basis for accurate classification into risk categories [2]. β-quantification is the reference method for the quantitative estimation of LDLc in circulation. It requires ultracentrifugation, uses large volumes of samples and is a time consuming and expensive technique. Therefore, this method is not suitable for routine laboratory testing [3].

The other recommended methods include homogeneous direct measurement [4,5]. The direct methods require expensive automation and are not affordable by most laboratories in the developing countries. Because of these limitation many clinical laboratories throughout the world use a less expensive and easy approach for the estimation of LDLc i.e., Friedewald formula [6,7].

National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) guidelines [2] recommend the use of LDLc calculated by Friedewald formula for determination of LDLc treatment goals for prevention of cardiovascular diseases. However, there are several shortcomings of this formula, mainly the underestimation of LDL cholesterol at high Triglyceride (TG) levels and overestimation at low TG levels [7].

Several other formulae have been proposed over the years for calculation of LDLc such as Cordova and Cordova; Vujovic; Ahmadi; Anandaraja; Puavillai; Hattori; Chen; Saiedullah; Planella and Wagner [7-16] but have not been validated in varied populations.

A survey of the College of American Pathologists (CAP) reported more than 2,200 laboratories use, several different assays for direct LDLc measurements and more than 3,300 laboratories reported calculating LDLc using the Friedewald's calculation [17,18].

The present study was designed to compare the LDLc calculated by several formulae which use High Density Lipoprotein cholesterol (HDLc), Total Cholesterol (TC) and TG to calculate LDLc with directly measured LDLc over a wide range of TG levels in Indian population with the assumption that the results obtained by direct assays are the most accurate.

MATERIALS AND METHODS

This study is a retrospective analytical study. One year lipid profile data of 21,503 samples, measured by direct homogenous method on Siemens Dimensions RxL Max and EXL were taken from the laboratory database from September 2014 to August 2015. Calculation of LDLc was then done using the following seven formulae mentioned in [Table/Fig-1] [6,9-13,16].

STATISTICAL ANALYSIS

Mean and SD were calculated by various formulae. The mean values obtained from various formulae were compared with the mean value of LDLc obtained by direct measurement using Pearson's correlation coefficient. Paired t-test was also performed to compare the means. A p-value of <0.05 was considered as statistically significant. Statistical analyses were performed using SPSS version 16.0.

RESULTS

A total of 21,503 lipid profiles were grouped into four TG ranges i.e., <150mg/dL (N=13,982), 151-199mg/dL (N=3449), 200-399mg/dL (N= 3515) and >400mg/dL (N=557). For TG range <150mg/dL, presented in [Table/Fig-2], the mean value obtained from all formulae showed good correlation with value of LDLc obtained by direct method, but Puavillai formula correlated best with direct measurement with r = 0.978 and mean difference of 0.08 which was statistically insignificant (p= 0.284). For TG range 151-199mg/dL, presented in [Table/Fig-3], the mean value obtained from all formulae showed good correlation with value of LDLc obtained by

Proposed by:	Formula:
Friedewald et al., [6]	LDLc=TC-HDLc-TG/5
Cordova and Cordova [9]	LDLc =3/4 (TC-HDLc)
Vujovic et al., [10]	LDLc=TC-TG/6.85-HDLc
Ahmadi et al., [11]	LDLc=TC/1.19+TG/1.9-HDLc/1.1
Anandaraja et al., [12]	LDLc=0.9TC-0.9TG/5-28
Puavillai et al., [13]	LDLc=TC-HDLc-TG/6
Hattori et al., [16]	LDLc=0.94TC-0.94HDLc-0.19TG

[Table/Fig-1]: Different formulas for Calculation of LDLc [6,9-13,16].

Method	Mean±SD (mg/dl)	Mean Difference (mg/dl)	Correlation (r)	p-value obtained by paired t-test
Direct	104.24±39.03			
Friedewald	101.14±38.87	3.1	0.977	<0.001
Cordova	90.15±30.53	14.09	0.975	<0.001
Vujovic	**106.29±39.29**	**-2.05**	**0.978**	**<0.001**
Ahmadi	110.45±42.79	-6.21	0.883	<0.001
Anandaraja	98.77±39.08	5.47	0.937	<0.001
Puavillai	104.32±39.12	0.08	0.978	0.284
Hattori	94.88±36.53	9.36	0.977	<0.001

[Table/Fig-2]: Calculation by different formulas, At TG range <150mg/dl; N=13,982.

Method	Mean±SD (mg/dl)	Mean Difference (mg/dl)	Correlation (r)	p-value obtained by paired t-test
Direct	120.44±40.78			
Friedewald	112.37±41.82	8.07	0.977	<0.001
Cordova	110.04±31.49	10.40	0.976	<0.001
Vujovic	**121.64±41.85**	**-1.2**	**0.977**	**<0.001**
Ahmadi	173.32±36.48	-52.88	0.945	<0.001
Anandaraja	104.08±42.08	16.36	0.965	<0.001
Puavillai	118.09±41.84	23.51	0.977	<0.001
Hattori	105.28±39.31	15.16	0.977	<0.001

[Table/Fig-3]: Calculation by different formulas, At TG range 151mg/dl to 199 mg/dl; N= 3449.

Method	Mean±SD (mg/dl)	Mean Difference (mg/dl)	Correlation (r)	p-value obtained by paired t-test
Direct	120.56±45.74			
Friedewald	107.43±47.83	13.13	0.966	<0.001
Cordova	119.78±35.89	0.78	0.957	0.003
Vujovic	**121.55±47.63**	**-0.99**	**0.968**	**<0.001**
Ahmadi	231.67±50.26	-149.11	0.736	<0.001
Anandaraja	95.98±47.92	24.57	0.960	<0.001
Puavillai	116.14±47.69	4.42	0.967	<0.001
Hattori	100.46±44.97	20.10	0.966	<0.001

[Table/Fig-4]: Calculation by different formulas. At TG range 200mg/dl to 399 mg/dl; N= 3515.

direct method, but Vujovic formula correlated best with r = 0.977 and mean difference of -1.2 mg/dL (p= <0.001).

For TG range 200-399mg/dL, presented in [Table/Fig-4], the mean value obtained from Vujovic formula correlated best with the mean value of LDLc obtained by direct method (r = 0.968). For TG range >400 mg/dL, presented in [Table/Fig-5], all formulae showed high significant mean difference. Among the various formulae, the mean value obtained by Vujovic formula correlated best with direct measurement r = 0.791 and mean difference of 5.89mg/dL.

DISCUSSION

Friedewald formula is the formula of choice for LDLc calculation in most laboratories across the world. Many studies have shown its limitation and some have shown that other equations perform better for certain groups of populations.

Method	Mean±SD (mg/dl)	Mean Difference (mg/dl)	Correlation (r)	p-value obtained by paired t-test
Direct	106.99±46.95			
Friedewald	67.87±79.97	39.12	0.701	<0.001
Cordova	143.18±43.18	-36.19	0.761	<0.001
Vujovic	**101.10±66.69**	**5.89**	**0.791**	**0.001**
Ahmadi	444.46±217.37	-337.47	0.019	<0.001
Anandaraja	56.28±75.88	50.71	0.727	<0.001
Puavillai	88.38±71.38	18.61	0.756	<0.001
Hattori	62.57±75.72	44.42	0.697	<0.001

[Table/Fig-5]: Calculation by different formulas, At TG range >400mg/dl; N= 557.

This study compared several formulae including Friedewald formula with direct LDLc measurement. Our results indicate that Friedewald formula fails to provide a good result at TG> 400mg/dL. This is contradictory to the study done by Sha MFR et al., in Bangladeshi population which concluded that Friedewald formula can be used up to serum TG concentration of 700mg/dL [7].

Cordova and Cordova suggested a new formula that performed better than Friedewald formula in Brazilian population over a wide TG range [9]. Our results show that Cordova formula does not provide any significant advantage over Friedewald formula in Indian population.

Ahmadi formula has been validated in Iranian subjects at TG<300mg/dL [11]. In our study, it performed well only at TG ranges <150mg/dL. At all other TG ranges it performs poorly. Hence, it is not suitable to be used in Indian population.

This study supports the study done by Gupta S et al., which concluded that Anandaraja formula does not provide any advantage over Friedewald formula for LDLc estimation in Indian population [19]. This is contradictory to the study done by Anandaraja et al., which found their formula more accurate than Friedewald formula for TG <350mg/dL [12]. The modified Friedwald equation developed by Puavillai et al., also correlated well with direct measurement and performed better than Friedewald formula at TG range >200mg/dL in Indian population [13].

Hattori formula developed by Hattori et al., shown to perform better than Friedwald formula in Japanese population does not provide any advantage over Friedewald formula in Indian population [16].

Our study supports the study done by Vujovic et al., which validated a modified formula in Serbian population with TG<400mg/dL [10]. They concluded that there is no significant difference between LDLc calculated by Vujovic formula and directly measured LDLc. Our results show that Vujovic formula shows good correlation at TG range <150mg/dL. It performed better than any other formulae at TG ranges 151-199mg/dL, 200-399mg/dL and TG> 400mg/dL. Hence, in this group of Indian population, it performed better than any other formula.

LIMITATION

This study compares calculated LDLc with direct LDLc assay and not with the reference method i.e., ultracentrifuge and precipitation for comparison. Also, the study uses only one assay for TG, TC, LDLc and HDLc and other assay methods have not been considered. Another limitation is that the number of samples with TG>400mg/dL was small. Finally, several other equations for LDLc calculation besides the ones used here have been described which have not been taken into consideration.

CONCLUSION

We propose that Vujovic formula is most suitable for estimation of LDLc in Indian population. It can be used over a wide TG range and should be preferred over other formulae for calculation of LDLc in resource- poor settings. However, more studies using larger sample

sizes, from different ethnic and geographical populations and under different settings and preferably compared with the other reference method are recommended.

Conversion factors to SI units: To convert TG from mg/dL to mmol/L multiply by 0.01129. To convert total cholesterol, LDLc and HDLc from mg/dL to mmol/L multiply by 0.02586.

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