Comparison of LDL-cholesterol estimated by various formulae with directly measured LDL-cholesterol in a tertiary care centre of Maval Taluka

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Abstract

Introduction: Low-density Lipoprotein Cholesterol (LDL-C) concentration in blood gives indication of cardiovascular disease risk. LDL-C may vary at various strata of Triglycerides and in metabolic diseases like Diabetes Mellitus. Measurement of LDL-C directly on laboratory instruments is the best method but is costly. It can also be estimated using various formulae. Our study involves the comparison of LDL-C measured directly with LDL-C estimated by calculations using various formulae.

Materials and Methods: Lipid profile data was collected from Central Clinical Laboratory of MIMER Medical College and hospital, Talegaon Dabhade. Serum LDL-C values were estimated by calculation from Triglycerides (TG), Total Cholesterol (TC) and High-density lipoprotein cholesterol (HDL-C) values, using Friedewald's, Cordova and Cordova, Vujovic, Anandaraja, Puavillai, and Hattori formula. Data of Direct LDL-C and LDL-C estimated by various formulae were compared and correlated at various levels of TG**.**

Result: Puavillai formula correlates the best with Direct LDL-C at TG </= 150 mg/dL. At TG 151-199 mg/dL, Friedewald's formula, while at TG 200-399 mg/dL, Anandaraja formula is the best. At all these TG levels, Puavillai formula correlates the best with Direct LDL-C.

Conclusion: Puavillai formula is the most accurate formula to calculate LDL-C at TG levels up to150 mg/dL and also at all TG levels studied. Friedewald's formula is the best at TG 151 to 199 mg/dL and Anandaraja formula at TG 200 to 399 mg/dL in our study population.

Keywords: LDL-cholesterol, Friedewald's formula, Cordova and cordova formula, Vujovic formula, Anandaraja formula, Puavillai formula, Hattori formula.

Introduction

Lipid profile is a routine blood test done in clinical laboratories. It involves estimation of Triglycerides (TG), Total Cholesterol (TC), High-density Lipoprotein Cholesterol (HDL-C), Low-density Lipoprotein Cholesterol (LDL-C) and Very low density Lipoprotein Cholesterol (VLDL-C) in serum sample. It is useful for screening of abnormalities like hyperlipidemia. LDL-C is an important parameter. Increased LDL-C concentration in blood alarms the risk for cardiovascular disease.[1]

Beta quantification is the method of reference for serum LDL-C measurement. It involves ultracentrifugation technique. The process is not simple to perform. It involves multiple steps. Several direct methods are also available based on selective precipitation. But the sample processing time is long and they are expensive. So the alternative method employed by clinical laboratories is to estimate LDL-C level by calculation. Friedewald's formula is the most commonly used formula.[2] Results of this formula are good when TG is less than 300 mg/dL. But as TG levels increase above 300 mg/dL, calculated LDL-C values are underestimated,[3] and at TG below 150 mg/dL, LDL-C values calculated by using Friedewald's formula are overestimated compared to Directly measured LDL-C values.[4]

LDL Cholesterol can also be calculated using various other formulae.[5] These are Cordova and Cordova,[6] Vujovic,[7] Anandaraja,[8] Puavillai[9] and Hattori.[10] Like Friedewald's formula, these formulae also use TG, TC and HDL-C to calculate LDL-C.

LDL-C may vary at various strata of TG, in metabolic diseases like Diabetes Mellitus, in patients on lipid lowering drugs and also in fasting/ non-fasting samples.

In this study, LDL-C calculated by using different formulae were compared with LDL-C measured directly on biochemistry analyzer over a wide range of TG levels in a population around of MIMER Medical College and hospital-a tertiary care centre in Maval Taluka of Pune district in Maharashtra (India).

Aims and Objectives

The aim of the study was to compare the estimated values of LDL-Cholesterol (LDL-C) calculated by various formulae with LDL-C estimated by direct method. Direct LDL-C assay in clinical laboratory was based on modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol methyl ether (PEGME) coupled classic precipitation method.

The objective was to collect LDL-Cholesterol values measured directly as well as values of Triglycerides (TG), Total Cholesterol (TC) and HDL-

Cholesterol (HDL-C) of same patients from the laboratory registers and to estimate LDL-Cholesterol (calculated) with the help of other analytes of lipid profile namely TC, TG & HDL-C of respective patients by using various formulae: Friedewald's, Cordova and Cordova, Vujovic, Anandaraja, Puavillai and Hattori formula.

Materials and Methods

This was an observational, retrospective data analysis study. The study was approved by the institutional ethical committee. Data of lipid profile values of two months was collected from Biochemistry OPD laboratory registers available in the Central Clinical Laboratory (CCL) of MIMER Medical College and Dr. B.S.T.R. Hospital, Talegaon Dabhade. Data contained Triglycerides (TG), Total Cholesterol (TC), HDL-Cholesterol (HDL-C) and directly measured LDL-C. After collecting lipid profile data, LDL-C values were calculated from respective TG, TC and HDL-C values by using different formulae as follows (Table 1)

Table 1: Formulae for LDL-C calculation

Formula Name	Formula
Friedewald's formula	LDL-C = TC – HDL-C –(TG/5)
Cordova & Cordova formula	LDL-C = 3/4 x (TC–HDL-C)
Vujovic formula	LDL-C = TC– (TG/6.85)–HDL-C
Anandaraja formula	LDL-C =0.9 x TC–(0.9 x TG/5)–28
Puavillai formula	LDL-C =TC –HDL-C –(TG/6)
Hattori formula	LDL-C =0.94 x TC–0.94 x HDL-C –0.19 x TG

Data of Directly measured LDL-Cholesterol (Direct LDL-C) and calculated LDL-Cholesterol using various formulae was compared and analyzed at various TG strata: up to 150 mg/dL, 151 to199 mg/dL, 200 to 399 mg/dL and more than 399 mg/dL using correlation coefficient and coefficient of determination by regression analysis with the help of Microsoft Office Excel 2007 (12.0.6787.5000) version for Windows.

Results

A total 236 lipid profile results were studied. LDL-C values were grouped in different strata of Triglyceride (TG) values according to Adult Treatment Panel III (ATP III) guidelines: </=150 mg/dL (N=182), 151 to 199 mg/dL (N=31), 200 to 399 mg/dL (N=20), >399 mg/dL (N=3). Total number of LDL-C values obtained in these groups is as follows (Table 2)

Table 2: LDL-C values grouped according to different TG levels

Triglycerides (TG)	No. of study subjects	Percentage
</=150 mg/dL	182	77%
151-199 mg/dL	31	13%
200-399 mg/dL	20	09%
>399mg/dL	03	01%
Total	236	100%

In stratum of TG values up to 150 mg/dL, values of Direct LDL-C and calculated LDL-C using various formulae showed good correlation. Mean LDL-C value calculated using Puavillai formula (106 mg/dL) was near to mean Direct LDL-C (99.2 mg/dL).The mean difference observed was -6.8 mg/dL and this estimated LDL-C showed the best statistically significant correlation with Direct LDL-C (r^2 0.8934, p 0.011) (Table 3 & Fig.1):

Table 3: LDL-C results by different formulae at TG </=150 mg/dL

Method	Mean ± SD (mg/dL)	Mean difference (mg/dL)	Coefficient (r^2)	p value
Direct LDL-C	99.2± 28.1			
Friedewald's formula	103 ± 27.9	-3.8	0.8933	0.097
Cordova & Cordova formula	90.6 ± 22.3	8.6	0.8681	0.001
Vujovic formula	107.8 ± 28.3	-8.6	0.8926	0.002
Anandaraja formula	101.5 ± 28.1	-2.3	0.8612	0.222
Puavillai formula	106 ± 28.1	-6.8	0.8934	0.011
Hattori formula	96.7 ± 26.2	2.5	0.8932	0.188

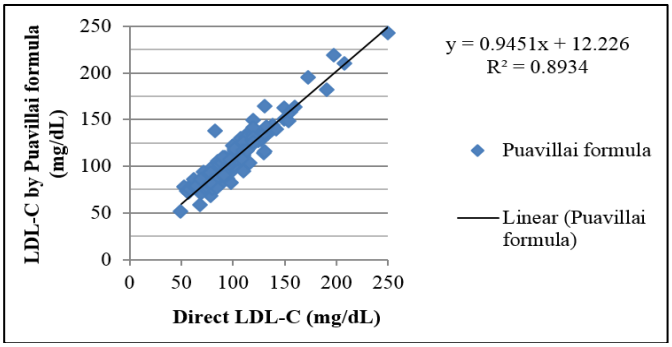

Fig. 1: Comparison of LDL-C estimated by Puavillai formula and direct LDL-C at TG </= 150 mg/dL

At TG stratum of 151 to 199 mg/dL, mean LDL-C calculated using Friedewald's formula showed statistically significant correlation with directly measured LDL-C value, when compared with LDL-C calculated using other formulae. Mean LDL-C estimated by this formula was 106.6 mg/dL. The mean difference between two values was 8 mg/dL with mean Direct LDL-C value (114.6 mg/dL) (r^2 0.7237, p 0.016) (Table 4, Fig. 2)

Table 4: LDL-C results by different formulae at TG 151-199 mg/dL

Method	Mean ± SD (mg/dL)	Mean difference (mg/dL)	Coefficient (r^2)	p value
Direct LDL-C	114.6 ± 31.5			
Friedewald's formula	106.6 ± 33.9	8	0.7237	0.016
Cordova & Cordova formula	106.3 ± 25.6	8.2	0.7048	0.007
Vujovic formula	116.1 ± 33.9	-1.5	0.6886	0.334
Anandaraja formula	103.9 ± 34.1	10.7	0.6123	0.006
Puavillai formula	112.5 ± 33.9	2.1	0.6859	0.277
Hattori formula	99.8 ± 31.9	14.7	0.681	<0.001

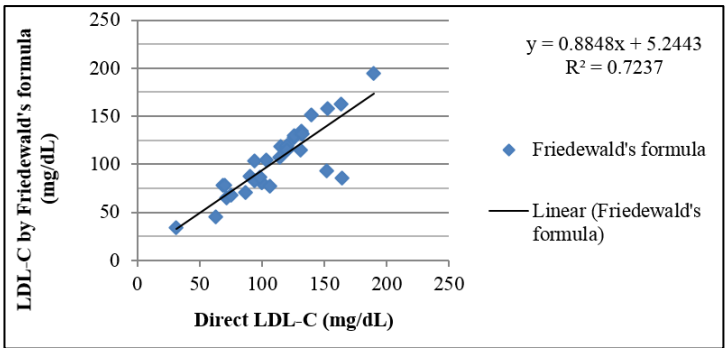

Fig. 2: Comparison of LDL-C estimated by Friedewald's formula and direct LDL-C at TG 151-199 mg/dL

At TG stratum of 200 to 399 mg/dL, mean LDL-C calculated by using Anandaraja formula (117 mg/dL) showed mean difference of 8.1mg/dL when compared with mean LDL-C value measured directly (125.1 mg/dL). It showed statistically significant correlation than other formulae (r^2 0.7992, p 0.028) with mean Direct LDL-C value (Table 5, Fig. 3):

Table 5: LDL-C results by different formulae at TG 200-399 mg/dL

Method	Mean ± SD (mg/dL)	Mean difference (mg/dL)	Coefficient (r^2)	p value
Direct LDL-C	125.1 ± 31.1			
Friedewald's formula	118.4 ±38.1	6.7	0.7635	0.07
Cordova & Cordova formula	125.1 ± 27.8	0	0.6985	0.5
Vujovic formula	131.5 ± 37.7	-6.4	0.7519	0.077
Anandaraja formula	117 ± 37.9	8.1	0.7992	0.028
Puavillai formula	126.5 ± 37.8	-1.4	0.7568	0.371
Hattori formula	110.9 ± 35.8	14.2	0.7638	0.001

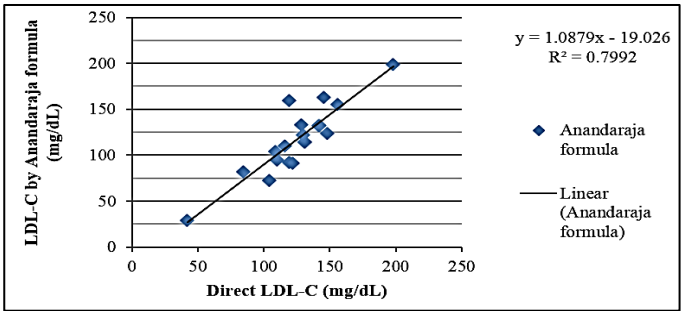

Fig. 3: Comparison of LDL-C estimated by Anandaraja formula and direct LDL-C at TG 200-399 mg/dL

At all above TG levels studied, mean LDL-C calculated by using Puavillai formula (107.9 mg/dL) showed a mean difference of -4.8 mg/dL when compared to mean LDL-C value measured directly (103.1 mg/dL). It showed better statistically significant correlation with mean Direct LDL-C value, as compared to other formulae (r^2 0.8119, p <0.001) (Table 6):

Table 6: LDL-C results by different formulae at all levels of TG (mg/dL)

Method	Mean ± SD (mg/dL)	Mean difference (mg/dL)	Coefficient (r^2)	p value
Direct LDL	103.1 ± 29.8			
Friedewald's formula	104 ± 30.6	-0.9	0.7889	0.175
Cordova & Cordova formula	95.5 ± 25.5	7.6	0.7982	<0.001
Vujovic formula	110.3 ± 30.9	-7.2	0.8215	<0.001
Anandaraja formula	102.3 ± 30.6	0.8	0.7625	0.222
Puavillai formula	107.9 ± 30.7	-4.8	0.8119	<0.001
Hattori formula	97.5 ± 28.7	5.6	0.7871	<0.001

Discussion

LDL-Cholesterol concentration in blood has positive correlation with coronary heart diseases like atherosclerosis. This is due to deposition of LDL-Cholesterol in tissues and endothelial spaces of arteries like coronaries. Hence it is called as bad cholesterol in general terms. As per Adult Treatment Panel III (ATP III), accuracy as well as reproducibility of LDL-C test is very essential.

Direct measurement of LDL-C is accurate but is expensive. For diagnostic laboratories, National Cholesterol Education Programme (NCEP) has recommended widely used Friedewald's formula as a routine method for estimation of LDL-C. But it has certain limitations. At TG level more than 300 mg/dL, Friedewald's formula is not very accurate in calculating LDL-C.[7] In a study, Ahmadi et al. noted that LDL-C calculation by using Friedewald's formula showed lower values compared to Direct LDL-C values at serum TG more than 400 mg/dL. At low TG levels, calculated LDL-C values were higher than Direct LDL-C values. But at TG between 150 and 300 mg/dL, calculated and Directly measured LDL-C values correlated well.[4] In our study population, at TG 151 to 199 mg/dL, Friedewald's formula (mean LDL 106.6 mg/dL) showed statistically significant correlation with Direct LDL-C results (114.6 mg/dL) with a mean difference of 8 mg/dL (r^2 0.7237, p 0.016). But at TG </= 150 mg/dL and 200-399 mg/dL it failed to show statistically significant correlation with Direct LDL-C values. Newer formulae offered few advantages over the Friedewald's formula at different TG levels.

Cordova et al. tested a new formula for estimation of LDL-C. The study was conducted in population of Brazil. LDL-C calculated using the formula showed good accuracy and correlated well with LDL-C measured directly.[6] In our study population, LDL-C calculated using Cordova formula showed fair accuracy with Direct LDL-C values, but did not show statistically significant correlation with mean Direct LDL-C measurements in any of the above studied TG levels.

Vujovic et al. stated in their study that, in Serbian population, formula developed by them for estimation of LDL-C showed better accuracy when compared to Friedewald's formula.[7] In our study population, Vujovic formula performed well but not the best at different TG levels.

Anandaraja et al. stated a formula for estimation of LDL-C. Formula was tested on Indian population data. They observed that LDL-C calculated using this formula showed good accuracy and correlation with LDL-C measured by direct method.[8] Also, there was less over estimation of LDL-C values calculated using Anandaraja formula when compared with the values obtained from Friedewald's formula.[8] Gupta et al. compared results of LDL-C obtained by Friedewald's formula and Anandaraja formula. But they observed that LDL-C estimation error was more when calculated using Anandaraja formula. The results were more erroneous at low total cholesterol and HDL-C values,

whereas results obtained by Friedewald's formula were better.[11] In our study population, at TG stratum of 200 to 399 mg/dL, Anandaraja formula showed mean LDL-C value of 117 mg/dL with a mean difference of 8.1 mg/dL with mean Direct LDL-C values (125.1 mg/dL). At this TG range, the formula showed statistically significant correlation (r^2 0.7992, p 0.028) with Direct LDL-C values.

Puavillai et al. made change in Friedewald's formula. It was observed that the original Friedewald's formula showed good accuracy in LDL-C estimation up to TG level of 200 mg/dL, but not beyond this TG value. As per them, the modified formula developed by them was better in calculating LDL-C in TG stratum of 200 to 499 mg/dL and it showed a better correlation with Direct LDL-C.[9] In our study population, Puavillai formula showed good accuracy with statistically significant correlation (r^2 0.8934, P 0.011) with Direct LDL-C at TG </= 150 mg/dL. Also, at all above TG levels studied, this formula stood the best in terms of accuracy and correlation with Direct LDL-C values (r^2 0.8119, P <0.001)

Formula developed by Hattori et al. does not show better correlation between calculated and directly measured LDL-C values when compared with other formulae in any of the above studied TG groups.[10]

Study conducted by Nishtha Wadhwa et al. concludes that Vujovic formula is more accurate than other formulae in Indian population.[12] But in our study, LDL-C estimated by Puavillai formula was found to be more accurate & correlated with directly measured LDL-C in all TG strata studied.

Limitations

In this study, LDL-C direct assay was used for comparison of LDL-C values instead of reference assay. Sample size is also small; especially at TG more than 399 mg/dL very less data was available. Hence comparison study in this TG stratum was not possible. Besides this, many formulae for LDL-C estimation other than those studied here are not considered.

Competing Interests: None.

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Conclusion

In our study population, Puavillai formula is the best to calculate LDL-C when TG is </= 150 mg/dL. At TG stratum of 151 to 199 mg/dL, Friedewald's formula, while at TG stratum of 200 to 399 mg/dL, Anandaraja formula is the most accurate. At all above TG levels studied, Puavillai formula is the best to calculate LDL-C. However, more studies using larger sample size are recommended.

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