Original Research Article

Current Understanding in the Measurement of Low Density Lipoprotein Cholesterol: A Comparison of Direct Measurement by Homogenous Assays versus Calculations

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Abstract

Background: To compare the results obtained by direct homogenous assay for LDL-C to those obtained by Friedwalds and Anandrajas formulas with the assumption that the results obtained by direct assay are most accurate. This was a comparative study for the estimation of LDL-C using two different types of calculation formulas and direct estimation of LDL-C by homogenous method.

Method: Serum Lipids and lipoproteins were measured in 505 fasting samples. Serum Total cholesterol was measured using CHOD-PAP method first described by Stadman on Siemens Dimensions Clinical Chemistry RXL Analyzer. Triglycerides were measured by Glycerol Phosphate peroxidase - PAP method. Direct LDL-C was measured by a homogenous assay by siemens diagnostics. A- HDL was measured by a homogenous method which uses PEG- cholesterol esterase using kit from siemens diagnostics. This was a comparative study for the estimation of LDL-C using two different types of calculation formulas and direct estimation of LDL-C by homogenous method.

Results: A good correlation was found between D-LDL as compared to both F-LDL and A-LDL. Pearsons coefficient of correlation between F-LDL & D-LDL was 0.891 (p<0.001) which was comparatively better than that between A-LDL & D-LDL which came out to be 0.850.

Conclusion: In conclusion, regarding patients convenience, financial reasons and accuracy we support the reliability of Anandrajas formula as indirect low density lipoprotein estimation – in Punjabi population.

Keywords: Anandraja's LDL, Friedwald's LDL, Beta Quantification, Calculated LDL, Direct LDL-C.

Introduction

The concentration of Low Density Lipoprotein Cholesterol (LDL – C) is one of the strongest markers of atherosclerosis and a very strong predictor of Coronary Artery disease. LDL-C is the basis for treatment and appropriate patients classification according to risk categories.[1] Beta Quantification also called as ultracentrifugation-polyanion precipitation method is the reference method for determining LDL-C. But being an expensive and a time consuming technique and having certain limitations of requiring ultracentrifugation and large sample volumes, it's not a preferred technique for estimating LDL Cholesterol.[2] Therefore this method is not suitable for routine laboratory testing. Friedwalds et al in 1972, validated a landmark formula to estimate LDL-C in the laboratory and compared it with the gold standard of ultracentrifugation.[3] All the parameters in Friedwald's

formula can be measured without ultracentrifugation, making it a very user friendly method. Although this estimation formula highly correlates with beta quantification, it also has certain limitations: it is not valid for samples with Chylomicrons with TG >400 mg/dl or in patients with dysbetalipoproteinemia. This formula overestimates VLDL-C and underestimates LDL- C as result if TG rich chylomicrons and chylomicron remnants are present in the serum sample, the values will not be accurate, hence the requirement for a fasting sample. It is not recommended for type 2 diabetes mellitus, nephrotic syndrome and chronic alcoholic patients, because of accompanying abnormalities in lipoprotein compositions.[4,5]

The homogenous assays, developed in 1998, directly measure LDL- Cholesterol after either blocking or solubilizing other lipoprotein classes.[6] These assays are not or only mildly influenced by the presence of chylomicrons and chylomicron remnants and therefore theoretically should not be influenced by a non-fasting state Although very accurate & certified by cholesterol reference method Laboratory for Disease control and prevention. This method is not routinely used in Indian laboratories as it is expensive, thus increasing the cost of lipid profile estimation.[7,8] Moreover many studies done to compare D-LDL-C and F- LDL-C have shown satisfactory results.

A Recent formula proposed by Anandraja and colleagues for LDL-C estimation still needs to be evaluated before it is extensively applied in diagnosis. This formula uses only two analytes – TG & TC for calculation thus decreasing the total error when compared to Friedwald's, because it does not use HDL-C. This formula has been approved in Brazilian and Greek population.[9,10] There are very less studies reporting the use of this formula in India especially in Punjabi population. The increase in cardiovascular disease in Punjabi adults together with the fact that risk factor needs to be established at an early stage of the disease, underline the necessity to obtain the most precise and reliable formula for LDL-C concentrations. The aim of this study is to compare the results obtained by direct homogeneous assay for LDL-C to those obtained by Friendewald's and Anandraja formulas with the assumption that the results obtained by direct assay are most accurate.

Material & Method

This was a comparative study for the estimation of LDL-C using two different types of calculation formulas and direct estimation of LDL-C by homogenous method. The approval of the ethical committee of the institute was taken. Data was collected retrospectively from patients samples received in clinical Biochemistry Lab, SGRDlMSAR from January 2014 to January 2016. A total of 500 samples were taken. Patients between 18-60 years age group were included and those having $TG \geq 400$ were excluded from the present study.

Before taking blood samples from antecubital vein, patients assumed a sitting posture for 5 min, since postural change alters Serum Cholesterol concentration. Serum was collected after a 12 hr. period of fasting in tubes without an anticoagulant. Serum was separated from the cells within 2 hours from time of collection and specimen was ensured to be free of particulate matter. Calibration was done for every parameter before a lot was started.

Serum Total Cholesterol was measured using CHOD method first described by stadman on Siemens dimensions clinical chemistry analyser. A three level calibration was done using Cholesterol calibration (Cat No DC 16).[11]

Triglycerides were measured by Glycerol phosphateperoxidise- PAP method and 3 level calibrations were done C CHEM II Calibrator Cat No. DC 20 by Siemens RxL Dimensions Analyzer.[12]

Lipoprotein Analysis:

Direct- LDL was measured by a homogeneous assay by Siemens diagnostics. The method is based on synthetic polymer method of Daiichii. Detergent 1 causes release of cholesterol from HDL, VLDL and Chylomicrons so that it can be removed. Reagent 2 contains another detergent which specifically acts on LDL to release cholesterol which can be estimated enzymatically by cholesterol- oxidase peroxidise.[13]

A- HDL was measured by a homogenous method which uses PEG- Cholesterol esterase using Kit from Siemens diagnostics. 3 Level Calibration was done by AHDL Calibrator (Cat No. DC48B).[14]

LDL-C concentrations were also calculated by FF [8| and Anandaraja's formula[17] as follows:

F-LDL-C= TC-HDL-(TG/5)
A-LDL-C= (0.9*TG/5)-28

The mean percentage difference ($\triangle$% LDL) defined as calculated LDL-C minus D-LDL-C compared to the direct measurement was calculated using the formula:

% $\triangle$ Calculated LDL-C = {(Calculated LDL-C)-(D-LDL-C)}/D-LDL-C*100.

The performance of two formulas was compared at different levels of TC, TG and HDL-C.

Statistical Analysis

The statistical analysis was done using Microsoft Excel 2007 and SPSS version 16.0. Paired 't' test and Pearson's correlation coefficient were used for the analysis. Two tailed P value of <0.05 were considered statistically significant. To examine the degree of agreement between the values obtained by the two methods. Bland- Altman graphical plots were used.

Results

A total of 517 patients attending the OPD of SGRDIMSAR, Vallah were assessed. Out of these 14 patients having TGs > 400 mg/dl were excluded from the present study and only 503 samples were analysed. The mean age of the patients was 43 years. The mean TC was 186.01 mg/dl.

A good correlation was found between D-LDL as compared to both F-LDL and A-LDL. Pearsons coefficient of correlation between F-LDL & D-LDL was 0.891 (p<0.001) comparatively better than that between D-LDL & A-LDL which came out to be 0.850 (p<0.01).

Comparison of LDL-C concentrations at different levels of TGs showed statistically significant difference (p<0.05) at all concentrations of TGs as compared to calculated formulas except at low TGs i.e between 1-100 mg/dl.

D-LDL overestimated the values at lower concentrations of TC as compared to calculated values whereas at higher concentrations of TC > 200 mg/dl there was an underestimation by D-LDL as compared to calculated values by both Friedwalds formula and Anandrajas formula. The comparison at all levels of TC was highly significant.

Bland Altmann plots indicated an obvious relationship between the differences and means for both the calculation formulas and the measured LDL-C.

Discussion

The importance of LDL- C to establish the CHD risk profile and manage treatment has long been established. Strategies for treatment of lipid abnormalities are primarily based on LDL-C concentrations. Thus, LDL-C must be accurately determined to establish a personal risk profile in order to initiate dietary adjustments and drug therapy.[15] The limitations of the reference method of Beta-quantification make it unsuitable for use in routine practice. Homogenous direct assays shows clear benefits as they directly measure LDL – cholesterol after either blocking or solubilizing other lipoprotein classes. This method also has limitations – it often includes VLDL and Liproprotein (a) to varying degrees.[7,8]

Friedwalds' formula although well established and used in many Indian laboratories, has not been able to obtain a recommended analytical quality of <12% total error. Anandraja et al described a new formula for calculation in Indian population of 1000 patients and validated its accuracy after applying multiple regression analysis.[16,17] We designed the present study to evaluate the performance of this formula in Punjabi population. We found a correlation of 0.891 between F- LDL and D- LDL and 0.850 between A- LDL and D- LDL, indicating a more strong correlation of F- LDL with D- LDL as compared to A- LDL. Anandraja et al reported the correlation coefficient between A- LDL and D- LDL to be 0.97, which was better as compared to D- LDL and F- LDL C. Shalini et al also reported findings similar to Anandraja contradictory to our findings.

We have found direct LDL to be higher as compared to both the calculated formulas. Shailini et al[18] and Vujoic et al[19] have also reported over – estimation by Direct LDL- C as compared to calculated formulas.

The mean % difference in our study for F- LDL was -0.12% which was much less as compared to the mean difference of -0.89% for Anandrajas LDL-C.

Gasko et al[10] reported Anandrajas formula to be very closer to D-LDL-C showing a mean difference of -1 mg/dL similar to our results.

LDL-C levels of 160,130 and 100 mg/dl are the treatment goals for low, moderate and high risk patients according to NCEP ATP III guidelines.[17] We found a statistically significant difference in risk classification of patients when D- LDL was used in place of calculated LDL-C. There is a statistically significant overestimation of D- LDL at all Total cholesterol levels except at higher TC i.e. TC > 300 mg/dl. There are also no significant variations in LDL by A- LDL method at various levels of HDL-C. Kamazeki et al[20] have reported an underestimation of 5.9 mg/dl by FF compared to the direct method. Some studies have reported opposite trends with higher results by calculated LDL-C by FF as compared to D-LDL-C.[10,21]

Therefore direct measurement leads to more patients being put on lipid lowering drugs. To conclude, D-LDL measurements clearly being more expensive and overestimating LDL as compared to calculated formulas with the mean error percentage of only -0.12% and -0.89% for F-LDL v/s D-LDL and A-LDL v/s D-LDL, calculated LDL can be used in routine setting. Moreover, D-LDL overestimates LDL putting more patients on treatment, calculated LDL can be used in situations where serum triglyceride values are less than 400 mg/dl. Also Anandrajas formula giving more negative bias as compared to Friedwalds assays. It would appear that calculated LDL-C by both Friedwald's and Anandraja's formula give satisfactory results compared with direct homogenous assays. It seems that the main advantage of Anandraja's formula is the requirement of the concentration of only two variables (TC and TG) which reduces the analytical error. We verify the use of Anandrajas assay in cases where HDL estimations are not available and for economic reasons. Still more future trials are required to verify its use in routine settings.

Table and Figures

Table 1: Table depicting the correlation of F-LDL and A-LDL as compared to D-LDL

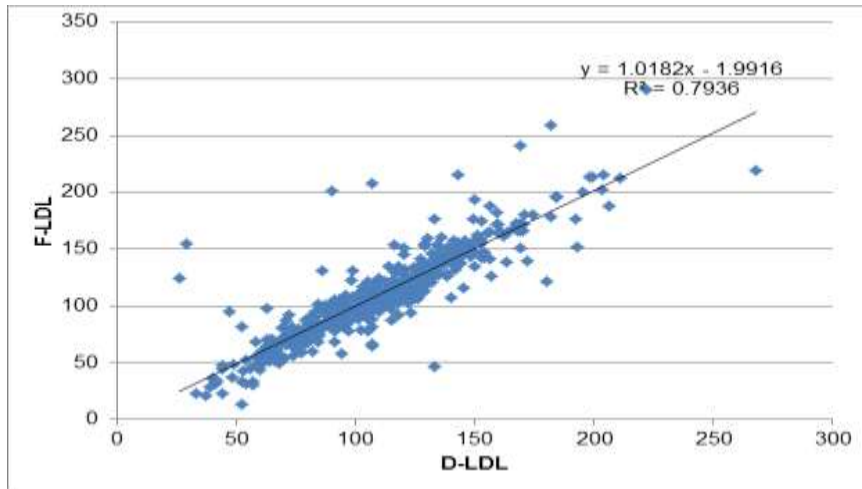|	Mean±SD	Mean difference	Correlation (r)	P value	%Diff
F-LDLC vs D-LDLC	109.96±39.11 110.10±34.28	0.1396	0.891	<0.001	0.3438
A-LDLC vs D-LDLC	109.12±37.78 110.10±34.28	0.9888	0.850	<0.001	0.4018

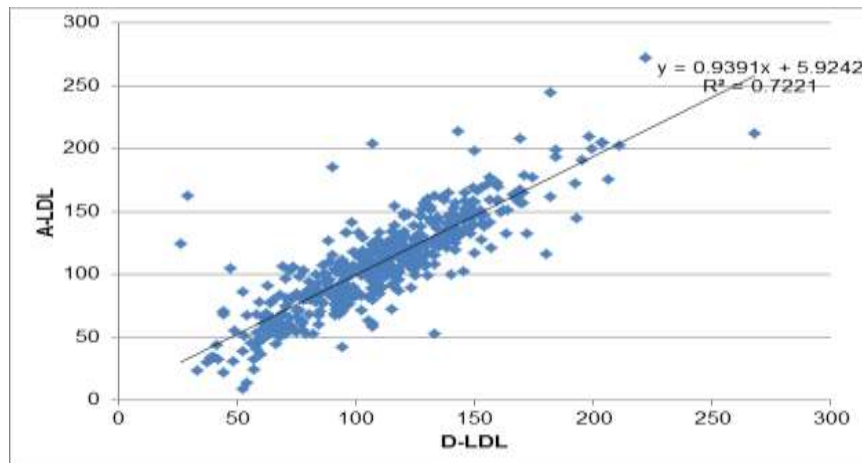**Fig. 1:** *Scatter plot* **of F-LDL-C against directly measured LDL-C**

Fig. 2: *Scatter plot* **of A-LDL-C against directly measured LDL-C**
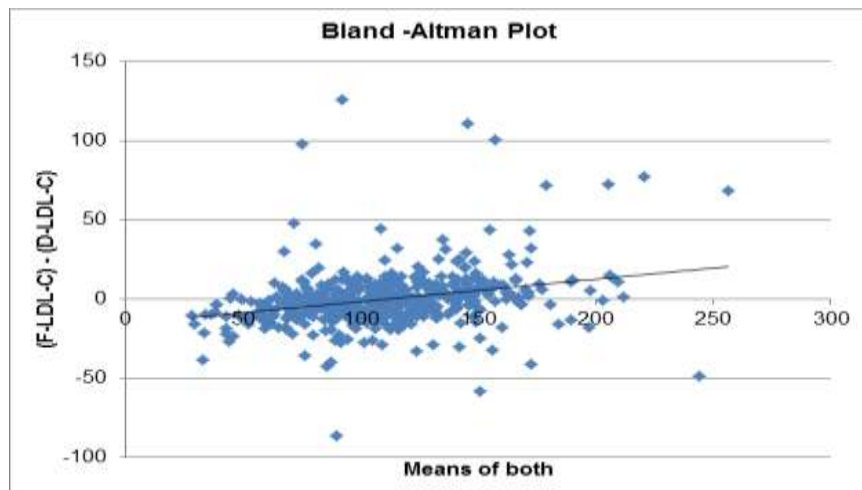

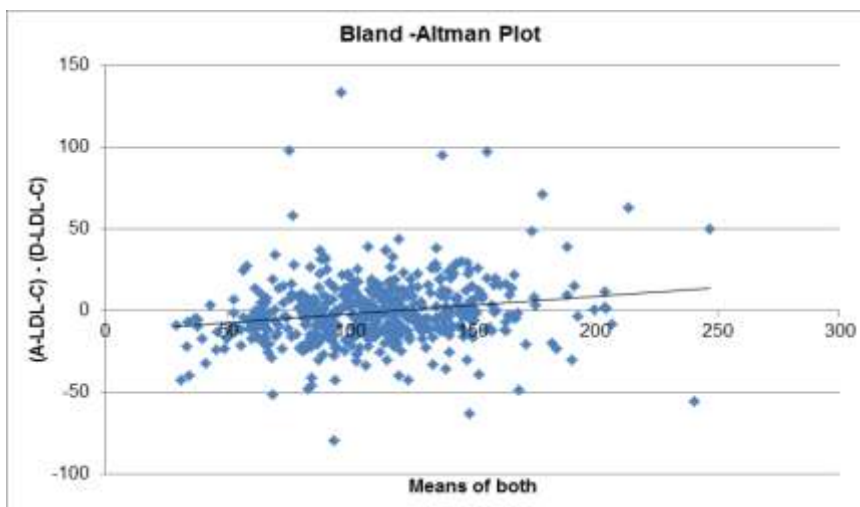**Fig. 3: Bland Altman Plot depicting Difference between F-LDL & D-LDL and the Means of both**

Fig. 4: Bland Altman Plot depicting the Difference between A-LDL & D-LDL and the Means of both

Table 2: Table Showing Comparison of calculated and direct LDL-C values at different levels of TC

	D-LDL	F-LDL	A-LDL
TC (1 – 100 mg/dl) (n=12)	45.42±8.74	33.77±7.45	33.14±8.52
TC (101-200 mg/dl) (n = 323)	95.39±21.29	91.61±22.69	91.47±22.72
TC (201-300 mg/dl) (n=162)	140.56±26.00	147.75±24.69	145.77±22.57
TC (>300 mg/dl) (n=6)	209.17±32.33	230.00±37.22	220.85±31.24
P value	<0.001**	<0.001**	<0.001**

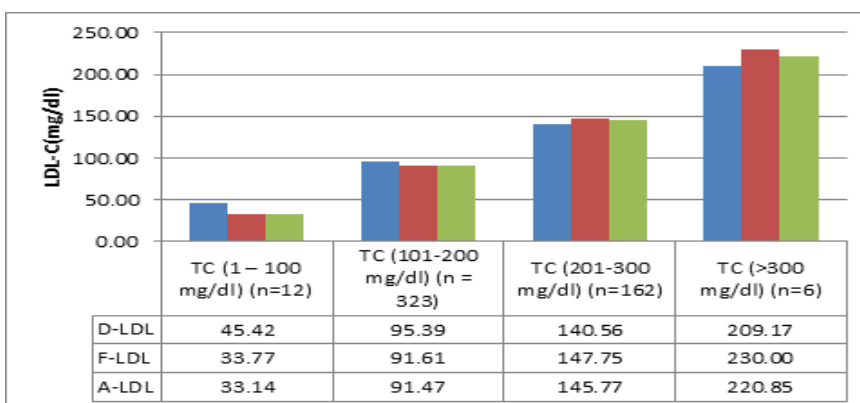**p<0.001; Highly significant

Fig. 5: Figure depicting the Comparison of calculated and direct LDL-C results at different levels of TC

Table 3: Table showing the Comparison of calculated and direct LDL-C results at different levels of TG

	D-LDL	F-LDL	A-LDL
TG (1 – 100 mg/dl) (n=103)	93.18±31.17	94.74±31.05	99.36±31.10
TG (101-200 mg/dl) (n=265)	110.28±33.61	110.77±40.64	110.15±38.84
TG (201-300 mg/dl) (n=100)	121.53±30.35	119.12±35.19	114.52±36.43
TG (>300 mg/dl) (n=35)	125.89±38.49	122.51±46.24	114.51±46.70
P value	<0.001**	<0.001**	0.020*

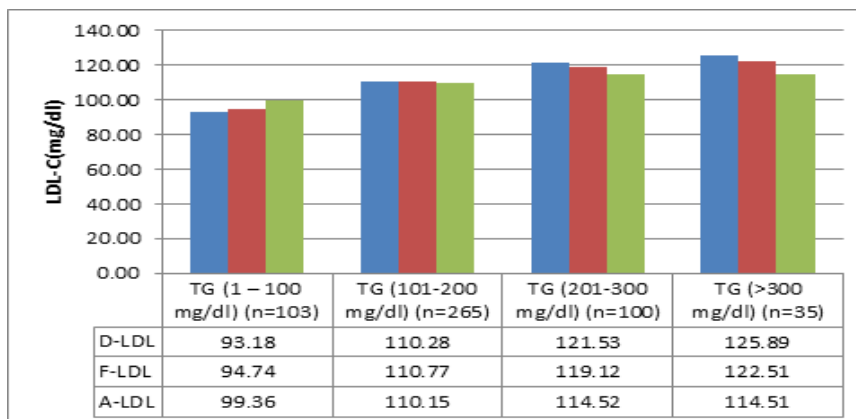*p<0.05; Significant; **p<0.001; Highly significant

Fig. 6: Figure depicting the Comparison of calculated and direct LDL-C results at different levels of TG

Table 4: Table showing the Comparison of calculated and direct LDL-C results at different levels of HDL

	D-LDL	F-LDL	A-LDL
HDL (<40 mg/dl) (n=218)	105.26±32.11	101.99±37.21	93.43±34.34
HDL (40-59 mg/dl) (n=247)	115.14±36.26	117.61±40.78	120.24±37.15
HDL (>=60 mg/dl) (n=38)	105.18±28.74	106.02±28.04	126.74±25.98
P Value	0.005*	<0.001**	<0.001**

*p < 0.05; Significant; **p < 0.001; Highly significant
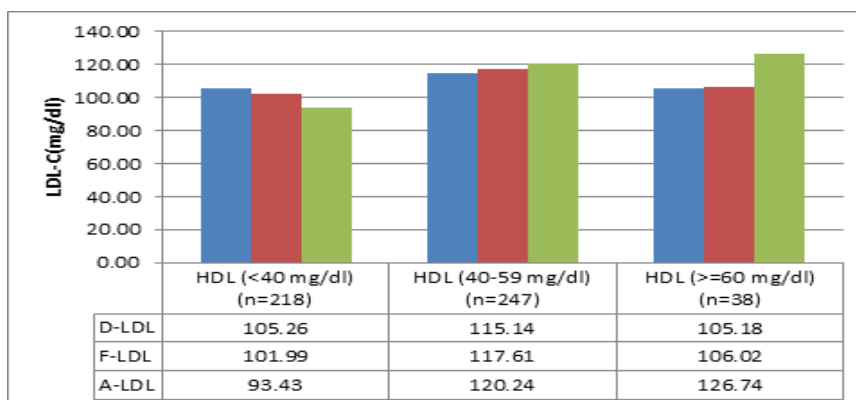

Fig. 7: Comparison of calculated and direct LDL-C results at different levels of HDL

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