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Assessment of various calculation methods for measurement of LDL-Cholesterol

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Abstract

Background: Coronary Artery Disease is the leading cause of death worldwide and LDL has been recommended as the primary lipid subset for prediction of risk of CAD NCEP guidelines. Many assays have been developed for measurement of LDL levels and have shown reasonable accuracy as compared to reference method but still not cost effective and cannot be affordable by majority of laboratories. Laboratories use the cost effective Friedewald's formula for calculating the LDL instead of direct assay which give near to accurate value but has its own limitations. In recent days many newer formulae have come up with lesser limitations and here an attempt is made to evaluate these formulae and to correlate with direct measurement of LDL.

Methodology: It's a cross sectional study. Sampling technique is Census method and involves sample size of 1020 cases. The entire lipid Parameters (LDL, HDL, TC, and TG) were estimated using Kits purchased by Roche /Cobas and then LDL also calculated using various formulae. Data was entered in Excel and analysed by Epi info software. Descriptive statistics like mean, standard deviation, standard error of mean were calculated. Student t test and Pearson's correlation are used to find the correlation between measured LDL and calculated LDL at different intervals of TG, TC and HDL.

Results: A total of 1020 samples were studied. The Cordova Formula correlated well in all the 1000 samples as a whole and in subjects with normal lipid profile and also at all lipid levels except for TG < 200mg/dl, TC < 100mg/dl. At TG < 200mg/dl Anandaraja's formula shows better correlation and at TC < 100mg/dl none of the formulae performed well as all formulae negatively correlated with the direct measurement of LDL.

Conclusion: Even though Cordova formula in our study has outperformed the other formulae, there are lots of factors which will affect the calculation. So it is highly recommended to switch to newer direct assays available in the market which are more precise, accurate, cost effective and also having low total allowable error < 12 and and a CV of <4%.

Keywords: Direct LDL, Calculated LDL, Formulae.

Background

CAD (Coronary Artery Disease) is the leading cause of death worldwide; its incidence is also increasing in India. LDL (Low density lipoprotein) is the major lipid for assessing the risk of CAD[1]. LDL has been recommended as the primary lipid subset for prediction of risk of CAD and therapeutic target by Adult Treatment Panel III (ATP III) of The National Cholesterol Education Programme (NCEP)[2]. This highlights the importance of accuracy and precision of LDL estimation. Ultracentrifugation-poly anion precipitation / Beta Quantification (ßQ), the reference method for measurement of LDL concentration, is expensive, laborious and not available everywhere[3]. During the recent times, direct homogeneous assays have been developed for measurement of LDL levels and have shown reasonable accuracy and precision as compared to reference method[4,5]. But still not cost effective. Commercially available direct LDL kits have been certified by NCEP and Cholesterol Reference Method Laboratory Network of Centre for Disease Control and Prevention for use in routine clinical laboratories.

Majority of rural laboratories where the patients can't afford the cost of the test are not using direct homogeneous assays because of non-accessibility and expensive kits. Laboratories use the Friedewald's formula (FF) for calculating the LDL by using other lipid parameters HDL (high density lipoprotein), TG (triglycerides) and TC (Total cholesterol) instead of direct measurement by homogenous assay as it is cost effective. Friedwald's formula is the cost effective measurement of LDL which also proved to give near to accurate value but has its own limitations[6,7]. In recent years the newer formulae have come up and they claim to be more accurate than Friedwald's formula and with lesser limitations. So here an attempt is made to evaluate newer formulae like, Anandaraja's formula (AF)[8], Vujovic Modified Friedwald formula (VMF)[9], De Cordova formula (Cordova F)[10], Teerakanchana formula (Teer F)[11], Chen formula (Chen F)[12], Hattori formula (Hattori F)[13] and M. Saiedullah formula (MSF)[14] with direct measurement of LDL.

Objectives

1. To evaluate different formulae for LDL (Friedwald's formula, Anandaraja's formula, Vujovic Modified Friedewald formula, De Cordova Formula, Teerakanchana formula, Chen formula, Hattori formula and M. Saiedullah formula or Bangladeshi formula) and correlate with values obtained by direct LDL measurement by homogenous assay.
2. To evaluate the different formulae at different lipid ranges and to correlate with values obtained by direct measurement by homogenous assay.

Methodology

After obtaining ethical committee clearance, the study was conducted at K R Hospital attached to Mysore Medical College and Research Institute for the period of November 2015 to April 2016. It is a cross sectional study. The cases are selected from outpatient and inpatient data base. Sampling technique is Census method and involves sample size of 1020 cases.

All patients attending K R Hospital outpatient and inpatient departments and who were advised to undergo Lipid profile tests in the laboratory, irrespective of health status were included. Subjects of age ranging 18 – 75 years were included in the study. Patients with incomplete Lipid profile were excluded.

Method of data collection: All the registered cases from 2015 November were selected. Data were collected using a predesigned semi-structured questionnaire which includes demographic profile of study subjects. Then the blood was drown under aseptic precautions and analysed for LDL, TG, TC and HDL using Roche 6000 instruments. LDL was measured by Direct method involving Homogeneous enzymatic colorimetric assay, TG by GPO-Trinder method, TC by Homogeneous enzymatic colorimetric assay using CHOD-POD (Cholesterol Oxidase –Peroxidase) method and HDL by Homogeneous enzymatic colorimetric test using PEG modified enzymes and dextran sulfate. All the lipid Parameters were estimated using Kits purchased by Roche/ Cobas and all the assays meet the National Institutes of Health (NIH) National Cholesterol Education Program (NCEP) goals for acceptable performance (LDL - CV $\leq$4%, Bias <4% and Total Error of $\leq$12 percent, for HDL - CV<4%, Bias $\leq\pm$ 5% and total error $\leq$13%, $\leq\pm$ 3% for total cholesterol TC, and $\leq\pm$ 4% for TG).

The quality of reports was assured by Biorad quality control materials. LDL was calculated using different formulae for varies lipid ranges [TG < 200, TG - 200-400, TG > 400, TC < 100, TC- 100-200, TC > 200, HDL > 40, HDL < 40 (values are in mg/dl)]. The formulae are as below,

Friedewald's formula (FF) = TC - HDL - TG/5
Anandaraja's formula (AF) = 0.9TC - 0.9TG/5-28
Vujovic Modified = TC - (HDL + TG/3)
Friedewald formula (VMF)
De Cordova formula (Cordova F = 0.7516 (TC - HDL)
Teerakanchana formula (Teer F) = 0.910TC - 0.634HDL - 0.111TG - 6.755
Chen formula (Chen F) = (TC 2 HDL) x 0.9 2 (TG x 0.1) and
Hattori formula (Hattori F)= 0.94TC - 0.94HDL - 0.19 x TG
M. Saiedullah formula (MSF)
Or Bangladeshi formula = 0.83 x TC – 0.10 x TG – 0.62 x HDL + 5.6

Statistical analysis: Data was entered in Excel and analysed by Epi info software. Descriptive statistics like mean, standard deviation, standard error of mean were calculated. Student t test and Pearson's correlation are used to find the correlation between measured LDL and calculated LDL at different intervals of TG, TC and HDL. Formula which has "r" value near to +1 is considered as the best formula for calculating the LDL.

Results

A total of 1020 samples were studied. Out of 1020 samples for which the analysis was done 556 (54.5%) samples were received from males and 464 (45.5%) from females. The mean age of the study population was 49±14 years. Table 1 shows statistical significance and correlation of Direct LDL and the calculated LDL-C for different formulae. According to Table 1 LDL calculated by all formulae show statistically significant difference when compared with direct method except for Hattori F but LDL calculated by Cordova F shows better correlation with the direct LDL values.

Table 1: Comparison and correlations of Direct LDL with Calculated LDL by different formulae

	N	Mean	SD	t value	p value	r value	p value*
LDL-D	1020	101.44	30.21	-	-	-	-
FF	1020	107.70	48.56	-04.603	0.000	0.471	0.000
AF	1020	108.96	48.55	-05.849	0.000	0.539	0.000
VMF	1020	81.30	54.76	11.88	0.000	0.297	0.000
Cordova F	1020	110.72	37.27	10.39	0.000	0.661	0.000
Chen F	1020	112.77	42.65	10.24	0.000	0.575	0.000
Teer F	1020	117.59	44.54	14.18	0.000	0.585	0.000
Hattori F	1020	100.88	45.70	00.45	0.647	0.468	0.000
MSF	1020	117.40	40.39	-15.23	0.000	0.583	0.000

P<0.001 considered significant.

Table 2-4 depicts comparison and correlation between measured LDL and calculated LDL at different intervals of TG. For TG < 200mg/dl VMF was better than other formulae as there was no statistically significant difference between direct LDL and calculated LDL by VMF. For TG 200-400 mg/dl FF and AF showed better statistical comparison than other formulae. For TG >400 mg/dl FF and AF are not acceptable whereas Cordova F performs better. As seen from the Tables AF Correlates well at TG levels <200mg/dl and Cordova F Correlates well between

200-400 mg/dl with r values of 0.613, 0.725 respectively, whereas none of the formulae correlate well for TG levels > 400 mg/dl.

Table 2: Statistics and correlations of samples having TG < 200 mg/dl with direct measurement and by different formulae

	N	Mean	SD	t value	p value	SEM	r value	p value
LDL-D	630	93.70	26.81	-	-	-	-	-
FF	630	110.81	46.17	-11.39	0.000	1.50	0.578	0.000
AF	630	111.32	46.78	-11.94	0.000	1.47	0.613	0.000
VMF	630	93.65	46.01	00.03	0.975	1.53	0.548	0.000
Cordova F	630	102.63	35.61	-07.80	0.000	1.14	0.610	0.000
Chen F	630	110.02	41.91	-12.12	0.000	1.34	0.594	0.000
Teer F	630	115.36	43.71	-15.63	0.000	1.38	0.606	0.000
Hattori F	630	103.90	43.39	-07.21	0.000	1.41	0.577	0.000
MSF	630	115.30	39.65	-17.08	0.000	1.26	0.604	0.000

Table 3: Statistics and correlations of samples having TG 200-400 mg/dl with direct measurement and by different formulae

	N	Mean	SD	t value	p value	SEM	r value	p value*
LDL-D	335	111.43	29.08	-	-	-	-	-
FF	335	108.85	46.74	01.43	0.151	1.86	0.696	0.000
AF	335	109.62	48.89	01.03	0.301	1.89	0.723	0.000
VMF	335	73.17	47.95	19.23	0.000	2.00	0.657	0.000
Cordova F	335	122.03	35.29	-07.84	0.000	1.35	0.725	0.000
Chen F	335	119.37	41.88	-04.87	0.000	1.62	0.714	0.000
Teer F	335	123.46	44.32	-07.04	0.000	1.70	0.722	0.000
Hattori F	335	101.8	44.19	05.57	0.000	1.74	0.696	0.000
MSF	355	122.93	40.31	-07.40	0.000	1.53	0.721	0.000

P<0.001 considered significant

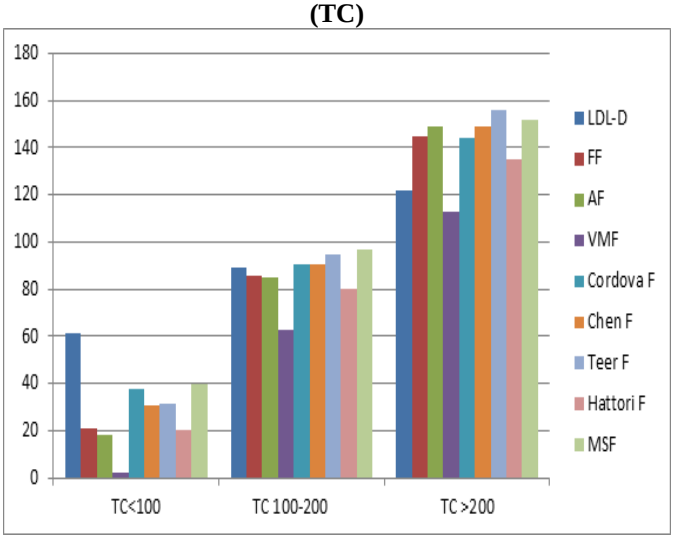
Table 4: Statistics and correlations of samples having TG > 400 mg/dl with direct measurement and by different formulae

	N	Mean	S D	t value	p value	SEM	r value	p value*
LDL-D	55	129.15	38.900	-	-	-	-	-
FF	55	65.025	64.59	06.61	0.000	9.69	0.103	0.45
AF	55	77.81	56.04	06.58	0.000	7.79	0.302	0.02
VMF	55	-10.81	82.90	11.24	0.000	12.44	- 0.021	0.87
Cordova F	55	134.37	40.04	-00.86	0.388	6.01	0.362	0.007
Chen F	55	104.02	50.72	03.27	0.002	7.65	0.218	0.11
Teer F	55	107.30	51.36	02.92	0.005	7.47	0.271	0.04
Hattori F	55	59.98	60.94	07.44	0.000	9.29	0.101	0.464
MSF	55	108.14	46.81	02.96	0.004	7.07	0.262	0.054

P<0.001 considered significant

Chart 1 show Comparison of Direct LDL with Calculated LDL formulae at different levels of Total cholesterol (TC). Cordova F performs well at TC levels of 100-200 mg/dl and also it correlates well with direct measurement of LDL. For TC< 100 mg/dl none of the formulae performed well and all formulae negatively correlated with the direct measurement of LDL.

Chart 1: Comparison of Direct LDL with calculated LDL formulae at different levels of Total cholesterol (TC)



In Chart 2 Comparison of Direct LDL with Calculated LDL formulae at different levels of HDL have been shown. Samples having HDL levels <40mg/dl FF compared statistically better with Direct LDL and Hattori F was Statistically better for samples with HDL>40 mg/dl. At both intervals LDL by Cordova F Correlated well with Direct LDL compared to other formulae.

Chart 2: Comparison of Direct LDL with calculated LDL formulae at different levels of HDL

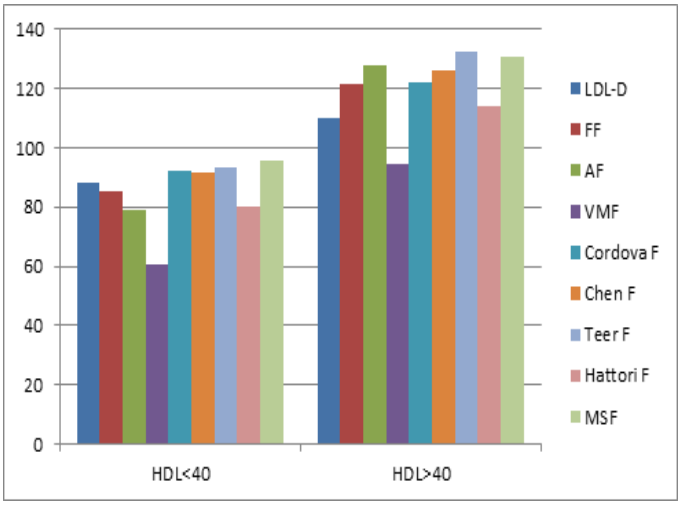


Table 5 shows the statistical comparison of Direct LDL with calculated LDL by different formulae in study subjects having Normal lipid profile. Even here the Cordova F is better correlated with that of direct measurement of LDL and does not show any statistically significant difference with Direct LDL levels.

Table 5: Showing the statistical comparison of Direct LDL with calculated LDL by different formulae in study subjects having Normal lipid profile

	N	Mean	SD	t value	p value	SEM	r value	p value
LDL-D	168	83.46	18.87	-	-	-	-	-
FF	168	92.27	28.18	-04.64	0.000	1.89	0.590	0.000
AF	168	96.99	25.94	-07.70	0.000	1.75	0.592	0.000
VMF	168	78.39	27.95	02.58	0.001	1.95	0.553	0.000
Cordova F	168	84.99	22.02	-01.04	0.299	1.47	0.634	0.000
Chen F	168	91.37	25.71	-04.62	0.000	1.71	0.609	0.000

Teer F	168	97.46	26.02	-08.10	0.000	1.72	0.610	0.000
Hattori F	168	86.46	26.48	-01.71	0.089	1.79	0.589	0.000
MSF	168	86.46	23.72	-09.61	0.000	1.59	0.610	0.000

Fig. 1 is the Bland Altman plot of LDL calculated by Cordova F compared with Direct LDL for all the 1020 samples.

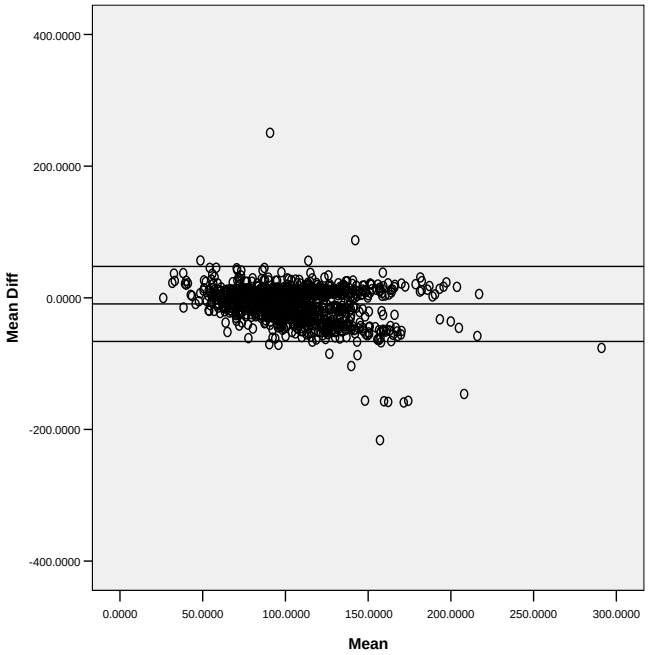


Fig. 1: Bland Altman plot of LDL calculated by Cordova Formula compared with Direct LDL estimation

Discussion

CAD management is most critical and that should be precisely monitored by the levels of LDL[2,15], the measurement of which is most uncertain and inaccurate and remained as an unresolved issue since decades. Ultracentrifugation method[3] being the gold standard method for measurement is laborious and has remained as research importance. That led to development of FF[6] which was the only formula for decades for calculating LDL. But the formula has many limitations like in patients with hypertriglyceridemia, type III hyperlipidemia, renal and liver diseases, and those with diabetes mellitus and other metabolic conditions[16,17] which was also found true in our study where the values are not reliable when triglycerides > 400mg/dl with r = 0.103, MD (Mean Difference) of 64.13 and SEM (Standard Error of Mean) of 9.69. Along with these limitations it also aggregates the errors of HDL, TG and TC measurements; that make it even more unreliable as the formula is based on these 3 basic lipid subsets. To address these limitations many formulae have been designed in the recent years and are under validation process. Hence the present study was undertaken to assess and compare the various methods for calculation of LDL and to correlate the values with Direct LDL values.

An Indian formula AF developed by Anandaraja and colleagues have been shown by many researchers that the formula has no advantage over FF[18,19] which is in contrary to our study where we found AF in better agreement with direct assay than the FF at all lipid ranges except for TC< 100mg/dl. The results of the study on normal healthy individual by Gasko R et al.,[20] and on metabolic syndrome by Gazi IF et al.,[21] support our study. We found the AF as the best correlated (0.613) among all the formulae at TG < 200mg/dl; closely followed by Cordova F (0.610). Even though it was not the best formula at other intervals of TG it managed to be the second choice at TG< 200mg/dl (r=0.723) and TG> 400mg/dl (r=0.302); whereas Cordova becomes the first choice with r= 0.725 & r= 0.362 respectively.

Cordova F has better statistical correlation than others in TC 100-200mg/dl (0.590) and TC > 400mg/dl (0.347). In TC < 100mg/dl category all the formulae show statistically negative correlation and VMF was better correlated among them (-0.864). But the Cordova F has less SEM in all 3 categories of TC which exhibits the less variability and accuracy of the formula. However VMF other than this doesn't perform well in any of the lipid ranges which is similar to the study by Muhammad Anwar et al., explaining the inconsistency

of the formula at different lipid levels[22]. Vujovic A et al., On the other hand clearly unveiled the better performance of the formula[9] and the study done in the Thailand found VMS in better agreement with direct assay than the Cordova method[23].

The Bangladeshi MSF, a recently published formula showed a good correlation (0.533) in the category of HDL< 40mg/dl and follows Cordova F(0.609) and Chen F (0.537) and it follows Cordova F (0.609) and Teer F (0.508) in the category of HDL >40mg/dl with r value of 0.506. In the present study Chen F other than HDL <40mg/dl and TC 100-200mg/dl categories fails to produce good results, whereas a study by Prabhop Dansethakul et al., and another similar study disagrees with our results where Chen F was found to be better than Cordova F at different lipid levels[23,24]; but contrary results of inconsistency of the formula were also observed[10].

MSF and Teer F show good statistical correlation when all 1020 subjects were considered (0.583 and 0.585) and also subjects with normal lipid profile (0.610 and 0.610) when compared to other formulae; however in both the categories again Cordova will be the formula of choice with 'r' value of 0.609 and 0.627 respectively . MSF the most recently proposed formula didn't correlate well in any of the category other than the above mentioned study. Some studies reported MSF as better formula[14,25,26], but needs to be evaluated in different populations worldwide.

Whereas Teer F demonstrate good correlation in normal lipid profile subjects (0.610) next to Cordova F (0.627) and MSF (0.610). Also correlates well in all 1020 subjects as a whole (0.585) and TC > 200mg/dl (0.212) next to Cordova F with "r" value of 0.661 and 0.347. Teer F didn't have a good overall performance when interpreted the formula as a whole at different stages but found to be better than FF at all lipid intervals[27].

To sum up, Cordova F is best correlated and the formula of choice in all categories of lipids except for the TG < 200mg/dl where AF stands best. Our study results were in concordance with the other studies where they witnessed better agreement of Cordova F in terms of correlation of the formula with direct assay as compared to other formulae[10]. Similar to a study by J. Martins et al., Cordova F also showed good accuracy at low triglyceride levels (using Daiichi method for LDL assay)[24]. This formula has been shown to be suitable for both fasting and non-fasting samples[28,29] as it doesn't depend on TG levels. Whereas contrary results are shown by Onyenekwu et al., and others[23,30]. And also Cordova found to have good correlation in both male (0.674) and female (0.647) population than other formulae. Present study shows Cordova F to be the formula of choice at different lipid ranges in subjects with normal lipid profile as well as in hospitalized patients. However, further research with thorough validation needs to be done in larger sample size.

Conclusions

There are many factors that affect the estimations of LDL by calculation, like the assay used for HDL calculation, lot to lot variations, ethnicity, racial origin, subject selection, pathological conditions of subjects which will affect the calculation[31,32]. According to the present study Cordova F is the formula of choice as it performed well in normal lipid profile as well as in Hospitalized Subjects. More than formulae, it is always better to switch to newer direct assays available in the market which are more precise, accurate, cost effective and also having low total allowable error <12[33] and a CV of < 4%[34] as per the guidelines of NCEP ATP III[2].

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