

Comparison of Calculated LDL-Cholesterol Using Various Formulae with Directly Measured LDL-Cholesterol: A Retrospective Study

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Abstract Background: Among the various risk factors for developing dyslipidemia and Cardiovascular Disease (CVD), elevated Low-Density Lipoprotein Cholesterol (LDL-C) has been recognized as an independent and widely accepted risk factor for CVD. Therefore the objective of the study was to calculate LDL-C using various formulae and to compare the various formulae to calculate LDL-C with direct LDL-C measurement. **Materials and methods:** It was a retrospective observational study and study was conducted for a period of 3 Months. During this period, 400 consecutive serum samples were included, out of which 37 had TG value more than 400 mg/dl and were excluded from the study. Finally 363 samples data were included for analysis and data were obtained from the investigation ledger of clinical Biochemistry section and same sample Triglyceride, Total Cholesterol and High Density Lipoprotein Cholesterol was used for calculating LDL-C using various formulae. **Results:** A total of 400 lipid profile reports were collected, of these, 37 reports had triglyceride level ≥ 400 mg/dl and hence 363 samples were included. There is no statistical difference between the direct LDL-C (131.64 ± 29.34 mg/dl) and LDL-C level calculated by Friedewald formula (133.85 ± 35.97 mg/dl, $p > 0.05$). There is a highly significant statistical difference between the direct LDL-C and the LDL-C level calculated using all other formulae ($p = 0.0001$) Cordova and Cordova formula (88.06 ± 28.40 mg/dl), Vujovic formula ($80.44 \pm$

15.52 mg/dl), Ahmadi formula (186.34 ± 63.26 mg/dl), Anandaraja formula (92.46 ± 32.37 mg/dl), Puavillai formula (88.54 ± 32.78 mg/dl) and Hattori formula (77.45 ± 30.31 mg/dl). **Conclusion:** The study established Friedewald formula as the most suitable method for calculating LDL-C in the absence of direct LDL-C measurement facilities. A large sample size and multicentric study is warranted to confirm which formula is most suitable for measuring the LDL-C in the absence of direct LDL-C measurement.

Keywords Cardiovascular Disease, Direct Low-Density Lipoprotein Cholesterol, Calculated Low-Density Lipoprotein Cholesterol, Friedewald Formula

1. Introduction

Cardiovascular disease (CVD) is a group of disease of heart and blood vessels and it is one of the most common causes of morbidity and mortality worldwide. To identify the risk factors of CVD, lipid profile is done routinely as a screening test. It includes the 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).[1]

Among the various risk factors for developing dyslipidemia and CVD, elevated LDL-C has been recognized as an independent and widely accepted risk factor for CVD.[1]

The National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) recommends a goal of maintaining serum LDL-C concentration < 100 mg/dl as optimal. Depending on the value of serum LDL-C, adults are grouped into various risk of developing heart disease if: LDL-C value is 100-129 mg/dl - Desirable Risk, LDL-C value is 130-159 mg/dL -Borderline high-risk and LDL-C value is ≥ 160 mg/dL considered as High risk.[2]

In 1983 homogenous assays was developed for direct measurement of LDL-C. [3] In India this method is not routinely practiced in most of the laboratories because direct LDL-C estimation is expensive and time consuming. Hence, majority of the clinical laboratories measure LDL-C concentration in serum using Friedewalds formula by taking the concentration of TC, HDL-C and TG, with the help of formula $LDL-C = TC - (HDL-C + TG/5)$. [4]

There are several shortcomings of Friedewalds formula, it cannot be used for LDL-C calculation when the patient is not in fasting, if serum TG > 400 mg/dL and in patients of hyperlipoproteinemia.[5] Despite these limitations, Friedewalds formula is still the most commonly used formula in clinical laboratories for measuring LDL-C concentration.[6]

Several other formulae have been proposed over the years for calculation of LDL-C. These are Cordova and Cordova [7] et al., Vujovic [8] et al., Ahmadi [9] et al., Anandaraja [10] et al., Puavillai [11] et al., and Hattori [12] et al., Similar to Friedewald's formula, these formulae also use TG, TC and HDL-C to calculate LDL-C. These formulae are not without the limitation. By considering this ambiguity in reporting, the present study was undertaken with the objectives to calculate LDL-C using various formulae and to compare with direct LDL-C assay, so as to obtain an acceptable formula at our setting in replacement for the direct measurement hence making lipid profile estimation cost effective.

2. Material & Method

This was a retrospective observational study and study was initiated after obtaining approval from the Institutional Ethics Committee of Adichunchanagiri Institute of Medical Sciences (AIMS), B G Nagara. The fasting serum sample received at Biochemistry section of clinical Laboratory for estimating the lipid profile was analyzed using various enzymatic methods.

The data required for the present study was obtained from the investigation ledger of Biochemistry section of clinical Laboratory and same sample TG, TC and HDL-C was used for calculating LDL-C using various formulae.

The present study was carried out for a period of 3 Months. During this period a total 400 consecutive serum

samples that were in the age group between 18-70 years, received in the clinical laboratory for estimating lipid profile were considered. Out of 400 samples, 37 had TG value more than 400 mg/dl and were excluded from the study. Finally 363 samples data were included for analysis.

Lipid profile was analyzed using standard methods such as TC was analyzed by cholesterol oxidase peroxidase method. TG was estimated by glycerol peroxidase - peroxidase (GPO-PAP) method. HDL-C was analyzed by PEG - cholesterol esterase (direct enzymatic) method. The LDL-C assay was analyzed by cholesterol esterase - cholesterol oxidase (homogeneous) method (Direct LDL-C).

LDL-C was also calculated by using various formulae by considering the values of TC, serum TG, HDL-C data from the investigation ledger.

Various Formulae Used for Calculating LDL-C

1. Friedewald [4] et al., $LDL-C = TC - HDL-C - (TG/5)$
2. Cordova and Cordova [7] $LDL-C = 3/4 \times (TC - HDL-C)$
3. Vujovic [8] et al., $LDL-C = TC - (TG/6.85) - HDL-C$
4. Ahmadi [9] et al.,
 $LDL-C = TC/1.19 + TG/1.9 - HDL-C/1.1$
5. Anandaraja [10] et al.,
 $LDL-C = 0.9 \times TC - (0.9 \times TG/5) - 28$
6. Puavillai [11] et al., $LDL-C = TC - HDL-C - (TG/6)$
7. Hattori [12] et al.,
 $LDL-C = 0.94 \times TC - 0.94 \times HDL-C - 0.19 \times TG$

The factor $[TG] / 5$ is an estimate of VLDL-C concentration, and is based on average ratio of TG to cholesterol in VLDL-C.

Statistical Analysis

Data were entered into Microsoft Excel sheet and analyzed using SPSSv15 software. Results are represented as mean \pm standard deviation. The statistical significance was evaluated at 95% confidence level and probability value (p) of < 0.05 was considered as statistically significant.

3. Results

Lipid profile data including direct LDL-C were obtained from the investigation ledger of Biochemistry section of clinical Laboratory retrospectively from past 3 months. A total of 400 lipid profile reports were included. Of these, 37 reports had triglyceride level ≥ 400 mg/dl, and hence were rejected before final analysis.

The mean age distribution of the subject was 50.38 ± 11.92 years with male as 50.23 ± 11.44 years and female as 50.67 ± 12.28 years (p value > 0.05). Figure 1 shows the distribution of male and female study population and the male to female ratio was 1.8:1.

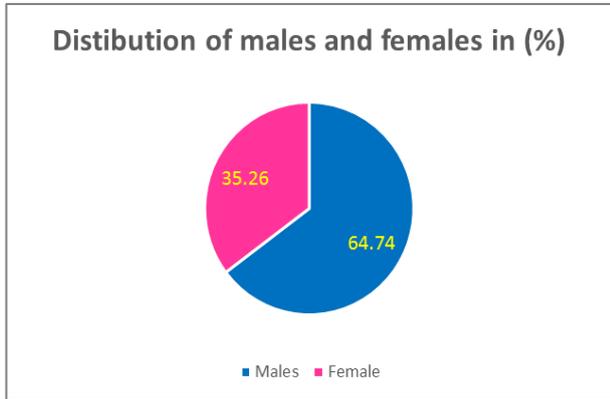


Figure 1. Male (n= 235) and female (n=128) distribution of study population

The estimated lipid profile is as shown in table 1. The mean values of all parameters except triglycerides are within normal limits. However, the triglycerides mean values are borderline high.

Table 1. Lipid profile of the study population (n=263)

Parameters	Mean	SD
TC (mg/dl)	168.50	41.78
TG (mg/dl)	173.25	76.58
HDL-C (mg/dl)	51.09	9.89
LDL-C (mg/dl)	131.64	29.34

Table 2 shows distribution of study Population having the normal and abnormal values of lipid profile. Majority of the study population were having total cholesterol (74.38%) and HDL-C (85.39%) in reference range. However more than half of the study population was having higher triglycerides (55.37%) and another 78.78% of them were having LDL-C above reference range.

Table 2. Distribution of Proportion of lipid profile in the Study Population

Parameters		Number (n=363)	Percentage (%)
TC (mg/dl)	<200mg/dL	270	74.38
	≥200mg/dL	93	25.62
TG (mg/dl)	<150mg/dL	162	44.62
	≥150mg/dL	201	55.37
HDL-C (mg/dl)	>40mg/dL	310	85.39
	≤40mg/dL	53	14.60
LDL-C (mg/dl)	<100mg/dL	67	18.45
	≥100mg/dL	296	78.78

Table 3 & figure 2 shows the comparison of the estimated LDL-C (Direct LDL-C) levels with the calculated LDL-C values by various formulas. There is no statistical difference between the direct LDL-C and LDL-C levels calculated by Friedewald formula (p>0.05). There is a highly significant statistical difference between the direct LDL-C and the LDL-C levels calculated using all other formulae (p=0.0001) except Friedewald formula. The LDL-C levels are overestimated when Ahmadi formula is applied whereas the values are underestimated by using the other formulae.

Table 3. Comparison of Direct LDL-C levels with the calculated LDL-C values by various formulas (n=363)

Formulas to calculate LDL-C values	Mean LDL-C levels (mg/dl)	SD	p value
Direct LDL-C	131.64	29.34	--
Friedewald formula	133.85	35.97	>0.05
Cordova and Cordova formula	88.06	28.40	0.0001
Vujovic formula	80.44	15.52	0.0001
Ahmadi formula	186.34	63.26	0.0001
Anandaraja formula	92.46	32.37	0.0001
Puavillai formula	88.54	32.78	0.0001
Hattori formula	77.45	30.31	0.0001

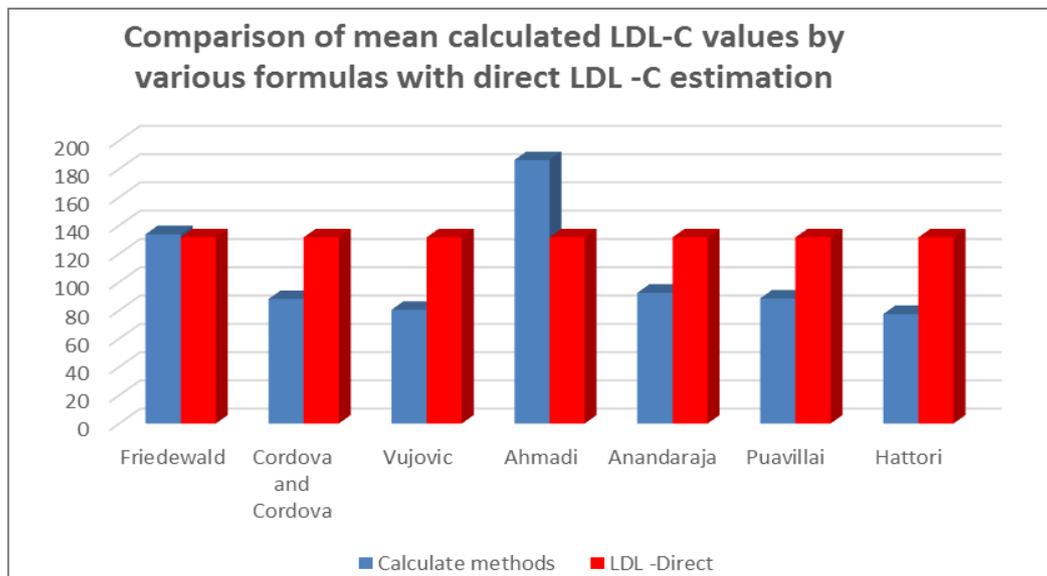


Figure 2. Comparison of Direct LDL-C levels with the calculated LDL-C values by various formulas (n=363)

A similar significance was also observed when comparison was done between the direct LDL-C and calculated LDL-C levels at different LDL-C ranges as shown in table 4.

Table 4. Comparison of Direct LDL-C with calculated LDL-C values at different LDL-C levels

	LDL-C <100 mg% (Optimal range)		LDL-C between 130 - 129 mg% (Desirable range)		LDL-C between 130 - 159 mg% (Borderline risk)		LDL-C ≥ 160 mg% (High risk)	
	n=68		n= 79		n= 171		n= 46	
	Mean ±SD	p value	Mean ±SD	p value	Mean ±SD	p value	Mean ±SD	p value
LDL-C Direct	84.87 ± 13.11	--	113.27 ± 8.84	--	148.7 ± 5.94	--	167.89 ± 10.18	--
Friedewald	91.07 ± 25.48	0.0767	116.76 ± 26.58	0.2698	147.4 ± 23.26	0.4964	174.94 ± 22.81	0.0588
Cordova & Cordova	57.78 ± 18.83	<0.0001	76.63 ± 23.67	<0.0001	95.85 ± 19.46	<0.0001	122.82 ± 22.56	<0.0001
Vujovic	61.68 ± 10.79	<0.0001	74.28 ± 12.31	<0.0001	86.55 ± 10.87	<0.0001	95.66 ± 9.41	<0.0001
Ahmadi	136.56 ± 47.83	<0.0001	172.39 ± 59.88	<0.0001	195.59 ± 53.40	<0.0001	248.42 ± 59.63	<0.0001
Anandaraja	53.96 ± 22.93	<0.0001	77.08 ± 23.92	<0.0001	104.71 ± 20.94	<0.0001	129.45 ± 20.52	<0.0001
Puavillai	53.38 ± 23.47	<0.0001	73.72 ± 25.57	<0.0001	98.68 ± 22.69	<0.0001	127.48 ± 24.27	<0.0001
Hattori	45.44 ± 22.38	<0.0001	63.60 ± 23.43	<0.0001	86.93 ± 21.46	<0.0001	112.58 ± 22.33	<0.0001

Mean value of lipid profile and calculated LDL-C using various formulae did not show gender wise difference and it was found to be statistically insignificant as shown in table 5.

Table 5. Gender wise comparison of lipid profile

	Male (n=235)		Female (n= 128)		p value
	Mean	SD	Mean	SD	
Age	50.23	11.44	50.67	12.78	0.73
TC	167.38	42.51	170.55	40.50	0.50
TG	175.61	76.85	168.92	76.20	0.43
HDL-C	50.60	9.85	51.98	9.94	0.20
LDL-C	131.09	29.28	132.65	29.53	0.63
TG/5	35.12	15.37	33.78	15.24	0.43
Friedewald	132.26	37.11	136.76	33.73	0.26
Cordova and Cordova	87.59	28.82	88.92	27.71	0.67
Vujovic	79.80	15.74	81.63	15.10	0.23
Ahmadi	187.08	63.44	184.96	63.16	0.76
Anandaraja	91.04	33.40	95.09	30.36	0.26
Puavillai	87.51	33.54	90.41	31.36	0.42
Hattori	76.41	31.07	79.35	28.88	0.38

4. Discussion

The study compared several formulae including Friedewald formula with direct LDL-C measurement. The measurement of LDL-C level is considered as a predictor for assessing the risk of various cardiac diseases and for treatment of dyslipidemias. Even though various formulae have been developed to calculate the LDL-C, most of the laboratories use Friedewald formula as the choice of formula to calculate LDL-C but many studies have shown its limitation and some have shown that other equations perform better for certain groups of populations.

The important finding of present study is that LDL-C level calculated using Friedewald formula did not show statistical significant difference with the directly measured LDL-C. But the LDL-C levels are overestimated when Ahmadi formula is applied whereas the values are underestimated by using the other formulae except Friedewald formula as seen in Table 3 & Graph 2.

The present study results are in concurrence with the study done by Niranjana et al. In this study on comparison of Anandaraja Formula and Friedewald Formula with Direct LDL-C measurement, they concluded that Friedewald formula showed a strong correlation (0.876) in comparison with Anandaraja formula (0.844). [13]

In another study by Nishtha Wadhwa [14] et al., it was concluded that in Indian Population, Vujovic formula appears to be more accurate than any other formula. Whereas in another study conducted by Mugdha Dilip Garule [15] et al, it was reported that, Puavillai formula is the most accurate formula to calculate LDL-C at TG levels up to 150 mg/dL and also at all TG levels studied whereas Friedewald's formula is the best at TG levels between 151 to 199 mg/dL and Anandaraja formula at TG 200 to 399 mg/dL.

However in the present when the comparison was done even at different levels of LDL-C (ie optimal level, desirable level, borderline and high-risk level), calculated LDL-C using Friedewald formula did not show significant difference whereas the other formulae showed significant difference with Ahmadi formula overestimating and others formulae the underestimating the LDL-C values at all levels of LDL-C as shown in table 4.

Farideh Razi [16] et al in a study suggested calculation of LDL-C based on Friedewald and Chen formula can be a good alternative for direct measurement especially in regions with limited resources. This study results are in concurrence with our study results. In our study we found that compared to other formulas, LDL-C calculated using Friedewald formula correlates better with direct LDL-C.

5. Conclusions

To conclude, the study established Friedewald formula as the most suitable method for calculating LDL-C in the absence of direct LDL-C measurement facilities. The

limitation of the present study is small sample size and comparison was not done at different levels of triglyceride including when triglyceride levels were greater than ≥ 400 mg/dl. A large sample size and multicentric study is warranted to confirm which formula is most suitable for measuring the LDL-C in the absence of direct LDL-C measurement.

Further the authors propose to continue future studies comparing the usefulness of these formulae in calculating LDL-C when triglycerides are ≥ 400 mg/dl which is considered as a limitation when Friedewald formula is applied.

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