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Original Research Article

Comparison of Friedewald's formula, modified Friedewald's formula and Anandaraja's formula with direct homogenous serum LDL cholesterol method in CHD patients

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ABSTRACT

Background: Elevated serum Low-Density Lipoprotein Cholesterol (LDL-C) concentration is a well-known atherogenic risk factor with a high predictive value for coronary heart disease. An important aspect of the assessment of coronary heart disease risk for a dyslipidemic subject is the estimation of serum Low-Density Lipoprotein Cholesterol (LDL-C). There are many homogenous assays currently available for the estimation of serum LDL-C. Most clinical laboratories determine LDL-C (mg/dl) by Friedewald's formula (FF), LD-=(TC)-HDL-C)-(TG/5), Modified Friedewald's formula (MFF), LDL-C=(TC)-(HDL-C)-(TG/6), Recently Anandaraja and colleagues have derived a new formula for calculating LDL-C, AR-LDL-C=0.9 TC-(0.9 TG/5)-28.
Methods: It is cross-sectional study. Lipid profile data was collected from known of CHD patients, who had come for lipid profile investigation to the Central Biochemistry laboratory of ACPM Medical College and hospital. LDL-C estimation was done by direct homogenous assay and also calculated using the Friedewald's Formula, Modified Friedewald's Formula and Anandaraja's Formula for assessing and validity of the LDL cholesterol.
Results: From the present study, The LDL-FF, MFW and AR are increased with levels of TGL > 200 mg/dl and decreased level of TC < 200 mg/dl seem to interfere with the estimation of Direct LDL cholesterol
Conclusions: Authors conclude that, LDL-C by direct method is most reliable and sensitive in CHD patients compare with FF, MFW, and ARF.

Keywords: Anandaraja formula, Coronary heart disease, Friedewald's formula, Low density lipoprotein, Modified Friedewald's formula

INTRODUCTION

The concentration of low-density lipoprotein cholesterol (LDL-C) is one of the strongest markers of atherosclerosis and predictor for assessing coronary heart disease (CHD) risk. Strong positive association between increased LDL-C and CHD has been well documented.[1-3] The National Cholesterol Education Programme's

(NCEP) Adult Treatment Panel III (ATP III) deemed that LDL-C concentration was the primary basis for treatment and appropriate patients' classification in risk categories.[4] Homogenous assays for direct LDL cholesterol (D-LDL-C) estimation were developed in 1998. The Cholesterol Reference Method Laboratory Network of the Centres for Disease Control and Prevention has approved the use of five commercially available homogenous assays for LDL-C estimation.[5]

In routine practice, most clinical laboratories estimate LDL-C concentrations in serum by Friedewald formula from the concentrations of Total Cholesterol (TC), Triglyceride (TG), and High- Density Lipoprotein Cholesterol (HDL-C).[6]

Calculated low-density lipoprotein cholesterol estimation Apart from above method, LDL cholesterol was calculated by following formulae: Friedewald: Friedewald low-density lipoprotein cholesterol (F-LDL-C)=TC−(TG/5+HDL-C). Modified Friedewald: Modified Friedewald's low-density lipoprotein cholesterol (MF-LDL-C)=TC−(TG/6+HDL-C). Anandaraja: Anandaraja low-density lipoprotein cholesterol (A-LDL-C)=(0.9×TC)−(0.9×TG/5)–28.[7]

The LDL-C calculated using Friedewald's formula correlates well with LDL-C measured by beta quantification, but doesn't come without any limitations.

The Friedewald's formula cannot be used for LDL-C calculation when the subject is not fasting, when serum TG >400 mg/dl or < 100 mg/dl.[8]

The accuracy and targets to be achieved regarding the analytical performance of LDL cholesterol were issued by National Cholesterol Education Program (NCEP) panel. As per NCEP guidelines, precision should be <4%, bias < 4% and total analytical error should be < 12%.[9]

Limited study results from India have reached discordant conclusions on this topic. So this present study was examined correlations and concentration differences obtained by the different calculation methods with the direct method.

METHODS

Lipid profile reports was collected from known CHD patients, who had come for lipid profile investigation to the Central Biochemistry laboratory of ACPM Medical College and Hospital, Dhule. LDL-C estimation was done by direct homogenous assay and also calculated using the Friedewald's Formula, Modified Friedewald's Formula and Anandaraja's Formula for assessing and validity of the LDL cholesterol.

Total cholesterol (TC) and TG levels were measured enzymatically by CHOD-PAP and GPO-PAP methods (Roche Diagnostics GmbH, Mannheim, Germany), respectively according to the manufacturer's specifications. High-density lipoprotein cholesterol (HDL-C) was measured using a homogeneous assay without precipitation (Roche Diagnostics GmbH, Mannheim, Germany).

A homogenous enzymatic colorimetric assay offered by Kyowa Medex and distributed by Roche Diagnostics, was used to measure LDL directly.[10]

RESULTS

From the present study, The LDL-FF, MFW and AR are increased with levels of TGL >200 mg/dl and decreased level of TC <200 mg/dl seem to interfere with the estimation of Direct LDL cholesterol (Table 1).

DISCUSSION

Strategies for treatment of lipid abnormalities are primarily based on LDL-C concentration. Therefore, LDLC must be accurately determined to establish a personal CHD risk profile in order to initiate dietary adjustments, drug therapy and to monitor their effects.[11]

Anandaraja and colleagues described a new formula for LDL-C calculation in an Indian population of 1000 patients by applying multiple linear regression analysis and validated its accuracy in 1008 patients. In their study the mean LDL-C concentrations measured by a precipitation method and by their formula were 3.04±1.04mmol/L and 2.96±0.96 mmol/L, respectively. The mean absolute difference between both methods was 0.1±0.24 mmol/L and good correlation was found (r = 0.97).[12]

In addition, they confirmed a reduction in the false overestimation of LDL-C compared with Friedewald's formula. Anandaraja and colleagues called for the reliability of their formula to be tested in other populations. In the past few decades attempts have been made to derive more accurate formulas for LDL-C calculation than the widely used Friedewald's formula on the other hand, Friedewald's formula has been shown to be relatively reliable and recommended by the NCEP as a routine method for estimation of LDL-C despite it having several well -established constraints.[5]

It cannot be applied to samples containing TG levels >4.52 mmol/l (400 mg/dl), to non-fasting samples and to samples of patients with dysbetalipoproteinemia (Fredrickson Type III).[12-14]

Although the newer formulas offered few advantages over the Friedewald's, they have performed only marginally better, possibly due to diversity in terms of study populations and/or pathologies.[15-17]

The use of only two variables- TG and TC in this formula is more likely to reduce analytical errors that are expected

when Friedewald's Formula is used. However, the study by Gupta et al., reported underestimation of LDL by FF at all levels of TG (ranging from 45 to 635 mg/dl).18 Demonstrating that both accuracy and precision of LDL-C analysis are critically important. Low-density lipoprotein (LDL)-cholesterol, as estimated by the Friedewald formula (FF) in routine patient care, is a central focus of clinical practice guidelines throughout the world. LDL can be calculated by FF (total cholesterol (TC) minus high-density lipoprotein (HDL)-cholesterol minus triglycerides (TGs)/5 in mg/dl) or measured directly in the laboratory.

Table 1: The significance in the result and also it the mean and standard deviation of direct LDL versus Friedewald, modified Friedewald and Anandaraja formula in TGL levels in different ranges on CHD patients.

TGL level < 100 mg/dl				
Parameters	**No. of Patients**	**Mean**	**Std. Deviation**	**Significance**
D - LDL	40	70.2500	6.99359	
FW - LDL	40	68.9750	6.71961	
MFW - LDL	40	71.3250	7.10142	0.009
AR - LDL	40	65.6250	9.93230	
Total	160	69.0438	8.01598	
TGL level - 101 - 200 mg/dl				
D - LDL	40	93.3500	11.66751	
FW - LDL	40	90.4250	11.24981	
MFW - LDL	40	95.3250	11.67155	0.003
AR - LDL	40	100.4000	13.21576	
Total	160	94.8750	12.40904	
TGL values - 201 - 300 mg/dl				
D - LDL	40	127.3500	18.97306	
FW - LDL	40	121.7750	18.21452	
MFW - LDL	40	130.1250	19.15683	0.000
AR - LDL	40	144.1250	21.41074	
Total	160	130.8438	20.98563	
TGL values - 301 -400 mg/dl				
D - LDL	40	169.1750	9.99202	
FW - LDL	40	146.1250	8.76820	
MFW - LDL	40	157.9250	9.44726	0.000
AR - LDL	40	183.4500	12.24106	
Total	160	164.1688	17.13293	
TGL values - Above 400 mg/dl				
D - LDL	40	188.3250	14.79499	
FW - LDL	40	159.4750	5.56540	
MFW - LDL	40	173.6500	5.87716	0.000
AR - LDL	40	209.7500	7.91218	
Total	160	182.8000	20.81932	

The FF is not valid for patients with TGs >400 and in patients for type 3 dyslipoproteinemia.

A number of studies have studied the impact of TG on the FF.

A study by Sahuet al, noted that the mean LDL calculated by FF was significantly higher than the direct LDL measurement at TG between 1 and 300 mg/dl. Recently, a new formula for calculation of LDL-C has been proposed by Anandaraja et al. The calculation of LDL-C proposed by Anandaraja et al, (AR-LDL-C) is AR-LDL-C = 0.9 TC- (0.9 TG/5)-28.

LDL was measured using direct homogenous assay (Daiichi Pure Chemicals Co. Ltd, Tokyo, Japan) in both the above studies.

Anandaraja et al, noted that FF overestimated LDL in subjects with TG <350 mg/dl (LDL was measured using heparin precipitation method in their study).[19]

In this study, there is not much significant difference in >100 mg/dl and 101 to 200 mg/dl. And also authors got more significant differences in above 200mg/dl of triglyceride values.

CONCLUSION

Authors conclude that, the LDL-C by direct method is most reliable and sensitive in CHD patients compare with FF, MFW, and ARF.

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