

Research Article



Correlation of Direct LDL Versus Friedewald's, Modified Friedewald's and Anandaraaja's Formula for Estimating LDL Cholesterol Levels along with LDH and Troponin I in Coronary Artery Disease Patients.

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ABSTRACT

The objective of the study is to compare the Friedewald formula, Modified Friedewald formula, and Anandaraja formula with direct homogeneous assay for low-density lipoprotein cholesterol (LDL-C) levels in CAD patients. Study Design is Cross-sectional study. Healthy subjects of both the gender, aged 18 - 75 years were included in this study. Lipid profile, LDH and Troponin I of both the controls and patients sample were collected from OPD of Central Hospital laboratory of Gayatri Vidhya Parishad Institute of Health Care and Medical Technology, Visakhapatnam. LDL-C estimation was done by direct homogeneous assay and also calculated by using the Friedewald's Formula, Modified Friedewald's Formula and Anandaraja's Formula for assessing and validity of the LDL cholesterol. LDH were measured kinetically UV method by Chem Ultra reagent system Pack and Cardiac Troponin I by JusCheck Rapid card test. One hundred samples were analyzed in this study, Out of that 50 are Known CAD patient samples and 50 are controls. The comparison of Lipid profile of Variable versus group. The P value also > 0.05 statistically significant. The Mean value for LDH in Case (Patients) is 486.2, whereas for control, it was 328.3. It shows highly significant for this parameter. The association between Case (Patients) and Control for Troponin I was highly significant in this study. In our study, we conclude that, modified friedewald's formula was closure to the direct method. The performance of calculated methods was not uniform at different TG levels. Even though, Modified Friedewald's formula is closure to Direct LDL, For laboratory setup, Novel and Innovative direct homogeneous assays are most reliable and accurate for the analysis of LDL cholesterol.

Keywords: CAD - Coronary Artery Disease, LDL – Low Density Lipoprotein, FF - Friedewald's Formula, MFF – Modified Friedewald's Formula, ARF – Anandaraja Formula, LDH – Lactate Dehydrogenase.

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INTRODUCTION

Coronary Artery Disease (CAD) is the leading cause of death worldwide.¹ 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.²⁻⁴ The National Cholesterol Education Programme's (NCEP) Adult Treatment Panel III (ATP III) recommended low density lipoprotein cholesterol (LDL-C) as the primary lipid agent for CAD risk prediction and therapeutic target, emphasizing the importance of

accuracy and precision of LDL-C estimation. The reference method for measurement of LDL-C concentration, ultracentrifugation-polianion precipitation / Beta Quantification (β Q), is expensive, laborious and not available everywhere. During the last decade, direct homogeneous assays have been developed for measurement of LDL-C levels and have shown reasonable accuracy and precision as compared to reference method. Commercially available direct LDL- C 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 (labs).^{5,6}

Friedewald Formula (FF) is the most commonly used method to calculate LDL-C in routine clinical labs. FF has several limitations including requirement for fasting, analytical variability and invalidity in samples with triglyceride (TG) > 4.52 mmol/l and certain type of hyperlipidemias. Studies have shown that the accuracy of FF declines as TG increases beyond 2.00 mmol/l, because assumption that Very Low Density Lipoprotein Cholesterol (VLDL-C) = TG/2.2 is not always true.⁷



Another modification in original Friedewald's formula for calculation of LDL was given by Puavilai and Laoragpongse⁸ which assumes that VLDL constitutes one-sixth of total TGs and it is costly for serum LDL test from direct measurement, especially if it has to be tested several times in a year. The authors found modified formula to be more accurate than the original formula in estimation of LDL-C. It also partially overcame the problems of fasting, presence of diabetes, obesity and familial hypertriglyceridemia unlike seen with original Friedewald formula. The literature on the use of this modified Friedewald formula among Indian population is not available.

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 $AR-LDL-C = 0.9 TC - (0.9 TG/5) - 28$.⁹ 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. Many studies done to compare the direct methods of estimation of serum LDL cholesterol with LDL cholesterol calculation by Friedewald's and Anandaraja's formulas have shown conflicting results.¹⁰⁻¹²

Limited study results from India have reached discordant conclusions on this topic. So this present study was contacted for correlations difference obtained by the different calculation methods with the direct method.

MATERIALS AND METHODS

Healthy subjects of either gender, from Visakhapatnam, aged 18 - 75 years were included in this study. Lipid profile, LDH and Troponin I, both controls and patients sample were collected from OPD of Central Hospital laboratory of

Gayatri Vidhya Parishad Institute of Health Care and Medical Technology, Visakhapatnam. 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.¹³

LDL cholesterol was calculated by following formulae: Friedewald formula: $(F-LDL-C) = TC - (TG/5 + HDL-C)$; Modified Friedewald: $(MF-LDL-C) = TC - (TG/6 + HDL-C)$. Anandaraja: $(A-LDL-C) = (0.9 \times TC) - (0.9 \times TG/5) - 28.7$.

LDH were measured kinetically UV method by Chem Ultra reagent system Pack and Cardiac Troponin I by JusCheck Rapid card test.

RESULT

One hundred samples were analyzed in this study, Out of that 50 were Known CAD patient samples and 50 were controls. Table: 1 shows the comparison of Lipid profile of Variable versus group. The P value for this comparison is > 0.05 - statistically significant.

Table 1: Shows the comparison of Lipid profile between Variable with group

Variable	Group				P value
	Case		Control		
	Mean	Std. Deviation	Mean	Std. Deviation	
DLDL	157.8	27.9	160.8	40.9	0.5245
FWLDL	154.8	27.9	160.8	40.6	0.3894
MFWLDL	158.6	28.7	163.3	38.9	0.4917
ALDL	147.5	27.7	151.4	37.7	0.5609

Mean \pm SD; P >0.05 statistically significant

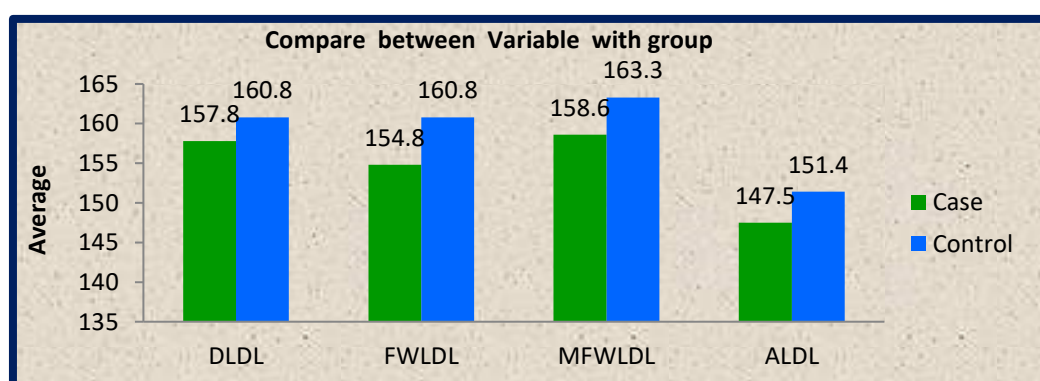


Figure 1: Shows the Comparison of Lipid Profile between Variable with group



Fig: 1 Shows the Bar diagram representation of comparison of Variable with group. The Mean value of DLDL in patients (Case) is 157.8, whereas for control 160.8. The Mean value of FWLDL in patients (Case) is 154.8, whereas for control, it was 160.8. The Mean value of MFWLDL in patients (Case) is 158.6, whereas for control, it was 158.6, whereas for patients, it was 163.3. For ALDL case it was 147.5, whereas for control, it was 151.4.

Table: 2 Shows the difference between Case (Patients) and Controls for LDH.

The Mean value for LDH in Case (Patients) is 486.2, whereas for control, it was 328.3. It shows highly significant for this parameter.

Table 2: Comparison of LDH between the groups.

Variable	Group				P value
	Case		Control		
	Mean	Std. Deviation	Mean	Std. Deviation	
LDH	486.2	19.8	328.3	65.3	*<0.001

Mean ± SD *P<0.05 statistically significant

Figure 2 Bar diagram clearly shows the Comparison of Case (Patients) and Control of LDH parameter.

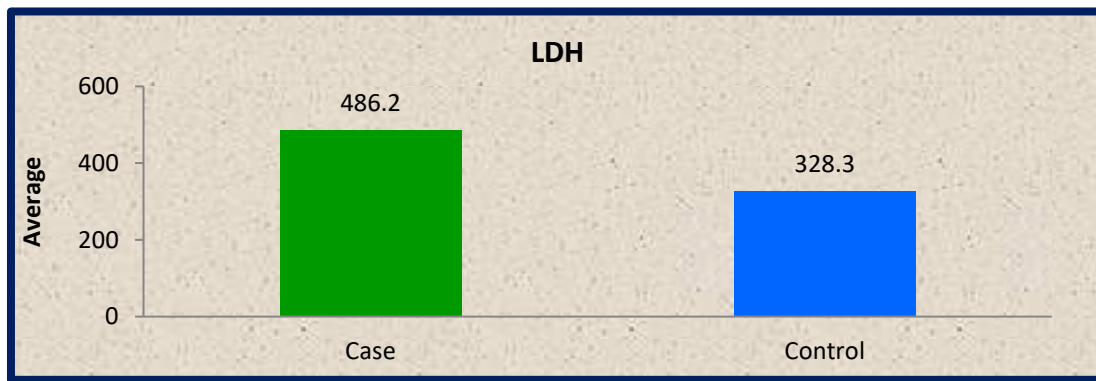


Figure 2: Comparison between Case and Control for LDH

Table 3 shows the association of Case and control for Troponin I

Table 3: The association between Case (Patients) and Control for Troponin I was highly significant in this study.

Troponin I	Group				P value
	Case		Control		
	n	%	n	%	
POSITIVE	0	0%	50	100%	*<0.001
NEGATIVE	50	100%	0	0%	

N=Frequency %= Percentage *P<0.05 statistically significant

Figure 3 Shows the different between Case and Control for LDH, and also it shows highly significant for this study.

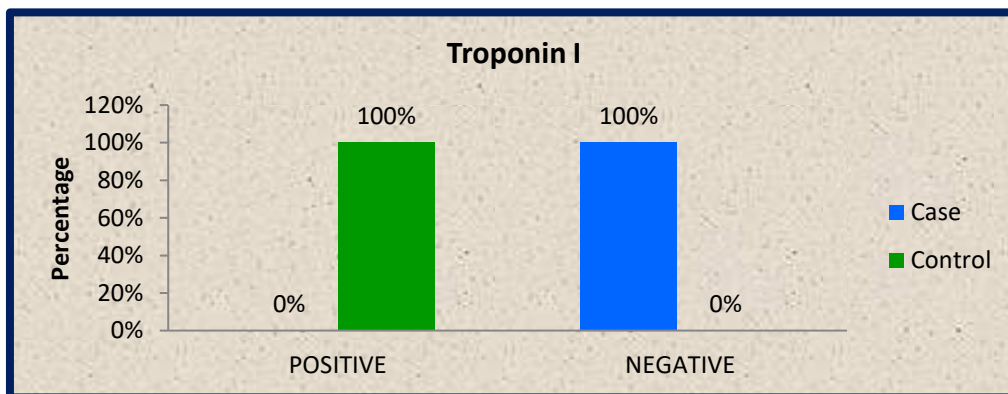


Figure 3: Comparison between Case and Control for LDH

DISCUSSION

Treatment strategies for lipid disorders are primarily based on low density lipoprotein cholesterol (LDL-C) levels. Therefore, to establish personal coronary artery diseases (CAD) risk for initiation of dietary adjustments, drug intervention and monitoring, LDL-C should be estimated accurately. Despite several limitations Friedewald formula (FF) is most commonly used method in routine clinical laboratories to estimate LDL-C. In order to improve the accuracy of FF, many modifications of original formula have been proposed.^{14, 15} but none of these modifications have provided sufficient evidence to replace original formula.¹⁶

After the recommendations of National Cholesterol Education Program's (NCEP) working group on lipoprotein measurements, many direct assays have been developed. These assays are precise, accurate, easily automated and have shown good correlation with β -quantification (β Q) method.¹⁷

Anandaraja's team did not propose any limitations to their formula. Comparing the mean value of the direct LDL-C obtained in the first 1000 patients and that in the validation group of 1008 patients it seemed they did not exclude samples with high TG levels.¹⁸ In a study of over 10000 Brazilian patients Gasko and colleagues¹⁹ supported Anandaraja's formula. The mean LDL-C level measured by a direct method and that estimated by the new formula were similar to the Indian population (2.99 ± 0.57 mmol/L and 2.97 ± 0.59 mmol/L, respectively). The correlation coefficient between both methods was $r = 0.97$. Anandaraja's formula was also checked in 230 Greek patients (118 had metabolic syndrome and 112 were healthy) by Gazi and Elisaf.²⁰ Friedewald's and Anandaraja's formulas gave similar results in the examined Greek population. The latter was approved for use in their laboratories. In our study we investigated if Anandaraja's formula could be applied in the Serbian population by comparing the value obtained with that of the homogenous direct method for LDL-C determination. This is the first study of its kind where the reliability and accuracy of Friedewald's formula were tested in the Serbian population. In our initial group LDL-C values from the direct measurement and from Anandaraja's formula were both higher than the values in Indian.

Mora et al. compared FF and direct assay in specimens from healthy female subjects. They reported that FF LDL-C were significantly higher than LDL-C, although both methods were highly correlated ($r = 0.976$) and the association of CAD with LDL-C levels estimated by both methods was almost identical in fasting specimens.²¹

Direct LDL homogeneous assays are not free from limitations. They exhibit a negative bias as observed in studies done by Rifai et al. and this may result in placing a patient into low risk who actually belongs to high-risk hypercholesterolemia.^{22, 23} Nauck et al. in their study observed, direct LDL method has no advantage when

compared to calculated LDL method and recommended further validation for direct homogeneous methods. Gasko observed Anandaraja's calculated LDL correlated better than Friedewald's calculated LDL with direct LDL in a Brazilian population.²⁴ Nakanishi et al. observed the original Friedewald's calculated LDL correlated best with direct LDL levels in comparison to modified Friedewald's formula and they suggested the chances of error in Calculated LDL increases with increase in TGs.²⁵

CONCLUSION

In our study, we conclude that, Modified Friedewald's formula are comes closure to the Direct method. The performance of calculated methods was not uniform at different TG levels. Novel and innovative direct homogeneous assays are most reliable and accurate. Therefore, for correct cardiac risk classification, direct homogeneous assay should be the method of choice to estimate LDL-C in routine clinical laboratories.

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