Anandaraja Formula or Friedewald Formula, which is a Better Formula for Calculating LDL Cholesterol in Comparision with Direct LDL - Measurement by Homogenous Assay Method

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ABSTRACT

Introduction: LDL Cholesterol is very important marker of atherosclerosis and is used as a predictor to assess coronary heart disease. This study was done to compare the level of LDL by direct method, Friedewald formula and Anandaraja formula.

Material and Methods: A cross-sectional study of 156 subjects, lipid profile with TG<400mg/dl in the department of Biochemistry, Varun arjun Medical College and Rohilkhand hospital, Shahjahanpur(UP) from September 2016 to November 2016. Lipid profile were measured by ERBA EM 200 fully autoanalyser as per laboratory standard practices. LDL-C was estimated by direct LDL, Friedwald Formula and Anandaraja Formula.

Results: Out of 156 subjects, 58.3% were male and 41.7% were female. The mean age of the patients were 47.8±14.6yrs. Lipid profile were assayed by fully autoanalyzer. The mean LDL were 102.1±37.7, 95.4±36.4 and 104.8±14.6 for direct LDL, Friedewald LDL and Anandaraja LDL. A good correlation was found between FLDL-C and DLDL-C(r=0.876) and also ALDL-C and DLDL-C(r=0.844)

Conclusion: Friedewald formula shows a strong correlation(0.876) in comparison with Anandaraja formula(0.844) when both these methods are correlated with Direct homogenous method for calculating LDL-Cholesterol.

Keywords: Anandaraja formula, ALDL, Direct LDL-C, Friedewald Formula, FLDL

INTRODUCTION

The level of Low density lipoprotein (LDL) cholesterol is one of the strongest markers of atherosclerosis and predictor for assessing the risk of coronary heart disease. LDL- cholesterol is used in clinical decision making guidelines to reduce cardiovascular risk events.[1] According to National Cholesterol Education Programme (NCEP) adult treatment panel, LDL-C concentration is the primary basis for treatment and appropriate patient classification in risk categories.[2] Homogenous assays for direct LDL-cholesterol estimation were developed in 1998.[3] These homogenous direct methods uses various combinations of surfactants, polymeric complexes and specific binding molecules to selectively measure cholesterol from LDL fraction.[4] But this method is not used routinely in most laboratories because of it's high cost. In routine practice many laboratories estimate LDL-C concentration in serum by Friedewalds formula from concentration of Total cholesterol (TC), High density lipoprotein (HDL) cholesterol and Triglyceride (TG), with the help of formula (LDL=TC-HDL-TG/5), but this formula has certain limitations, it cannot be used for LDL measurement when the patient is not in fasting condition, serum TG>400 mg/dL and in patients of hyperlipoproteinemia.[6] Despite these limitations, Friedewalds formula is still the most commonly used procedure in clinical laboratories for estimating LDL- cholesterol concentration.[7] As seen in many reports that direct homogenous methods for calculating LDL and by Friedewalds formula are not giving similar results.[8,9] An another formula for LDL-C estimation was given by Anandaraja and colleagues[10] as a substitute for Friedewalds formula in Indian population. The calculation of LDL-C proposed by Anandaraja et. al[10] is LDL-C=0.9TC-(0.9 TG/5)-28. This study was aimed to assess validity of LDL-C values as calculated by Friedewald formula and those calculated by Anandaraja formula and to compare both these values obtained by the direct method, with the assumption that results obtained by direct homogenous assays are most accurate and in order to see out of both these formula methods, which method is most likely to give results similar to values obtained by direct method.

MATERIAL AND METHODS

A cross sectional study of 156 subjects in the Department of Biochemistry, Varunarjun Medical College, Banthra, Shahjahanpur (UP) was conducted. It was performed in the Clinical Biochemistry Laboratory during September 2016 to November 2016. Blood sample were obtained in the morning after an overnight fast (10-12 hrs) from all subjects and were analysed on the day of blood collection. All the subjects were above 18 yrs of age. Subjects with Triglycerides level >400 mg/dl were excluded. Serum was separated by centrifugation and analyzed on ERBA EM-200 as per standard laboratory practices. Total cholesterol and triglycerides was measured by CHOD-PAP and GPO-PAP method. HDL-C was measured using a homogenous assay with precipitation. Direct LDL-C measurement was done by homogenous assay based on modified PVS and PEGME coupled classic precipitation method. LDL-C was also estimated by Friedewalds formula and Anandaraja formula.

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STATISTICAL ANALYSIS

SPSS version 15.0 was used for the statistical analysis. Descriptive statistics like mean and percentages were used for the data analysis.

RESULTS

Table 1 shows that the total number of patients, 58.3% was male and 41.7% were females. The mean age of the patient was 47.8±14.6yrs. The mean and SD values of Total cholesterol, Triglycerides, HDL, Direct LDL, Friedwald formula of LDL and Anandaraja LDL are 178.8±45, 156.7±68.5, 52.10±11.7, 102.1±37.7, 95.4±36.4 and 104.8±36.2 respectively. The correlation value between Freidewald formula of LDL Vs Direct LDL is 0.876 and Anandaraja formula of LDL Vs Direct LDL cholesterol was 0.844. Both are good correlation. The calculated formula underestimates LDL by -6.7mg/dl by Friedewald formula and overestimates LDL by 2.7 mg/dl by Anandaraja formula in comparison to Direct Method as shown in Table - 2. In Table - 3 shows that the comparison of measured LDL-C vs calculated LDL-C by Friedewald formula and Anandaraja formula at different level of Triglycerides level. No statistical significant difference was observed at different levels of Triglycerides. There was underestimation of calculated LDL at all levels of triglycerides with Friedewald formula method, and this underestimation was measured maximum at TG levels of 201-400 mg/dl. Mean difference between FLDL and DLDL was highest (24.3mg/dl) at TG level of 201-400 mg/dl and least (0.96mg/dl) at TG level 101-150. But an overestimation of LDL-C was seen while using Anandaraja formula method except to underestimation of 15.82 at TG level of 201-400mg/dl while at all other TG levels overestimated.

Total number of patients	156
Number of Male	91
Number of Female	65
Mean age of patients	47.8 ± 14.6
Mean ± SD serum TC(mg/dL)	178.8 ± 45
Mean ± SD serum TG(mg/dL)	156.7 ± 68.5
Mean ± SD serum HDL(mg/dL)	52.10 ± 11.7
Mean ± SD serum D-LDL(mg/dL)	102.1 ± 37.7
Mean ± SD serum F-LDL(mg/dL)	95.4 ± 36.4
Mean ± SD serum A-LDL(mg/dL)	104.8 ± 36.2

Table-1: Various parameters of Study Subjects (Mean ± SD)

DISCUSSION

The important finding of our present study is that Friedewald formula tends to underestimate LDL-C all levels of Triglyceride while Anandaraja formula overestimates LDL-C at triglyceride level upto 200mg/dl while it underestimates LDL-C at TG level 201-400mg/dl. LDL-C estimation is essential for assessing the risk of various cardiac diseases and for treatment of dyslipidemias. Vander Heul-Nieuwenhuijsen et al and Tighe DA, Ockene IS et al found that LDL-C calculated by Friedewald

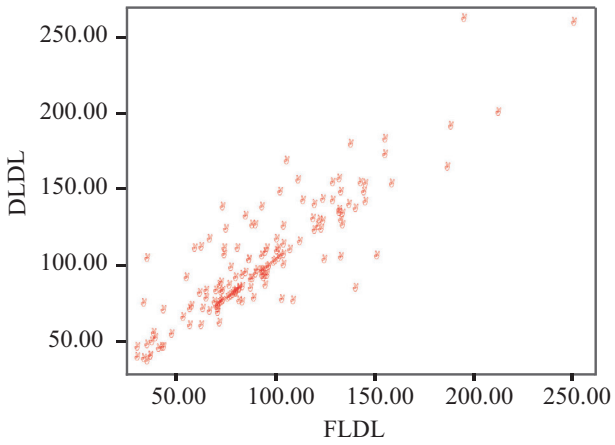

Figure-1: Scatter plot between DLDL and FLDL

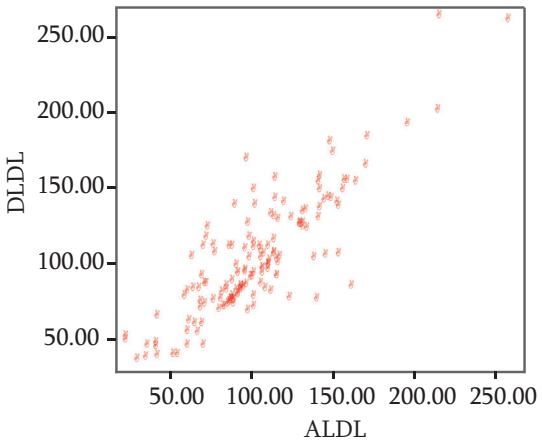

Figure-2: Scatter plot between DLDL and ALDL

	Mean± SD (mg/dL)	Mean difference (mg/dL)	Correlation (r)	p value
FLDL-C vs DLDL-C	95.4 ±36.4 vs 102.1 ±37.7	-6.7	0.876	.111
ALDL-C vs DLDL-C	104.8 ±36.2 vs 102.1± 37.7	2.7	0.844	.525

Table-2: Paired sample statistics and correlation

TG level(mg/dL)	FLDL vs DLDL mean difference(mg/dL)	FLDL vs DLDL p value	ALDL vs DLDL mean difference(mg/dL)	ALDL vs DLDL p value
TG< 100	-5.5	.388	6.2	.320
TG 101-150	-0.96	.848	8.8	.09
TG 150-200	-4.03	.621	3.07	.711
TG 201-400	-24.3	.09	-15.82	.263

Table-3: Mean difference and p value at various Triglyceride levels

formula tend to underestimate LDL-C level when compared to directly measured LDL-C levels.[11,12] There was no statistical significant difference in calculated (both FF andAR) and measured LDL-C at all levels of Triglycerides. The calculated method for LDL estimation doesn't show any uniformity for LDL estimation at different TG level but at Triglyceride level 101-150 mg/dL LDL calculated by Friedewald formula show very minimal difference i.e., (- 0.96mg/dL) showing that at this level of triglyceride calculated LDL by Friedewald formula is very much similar to values obtained by Direct homogenous assay method however seeing at calculated LDL by Anandaraja formula method no such calculated LDL values corresponds maximum to Directly measured LDL. We have found a correlation of 0.844 between ALDL and DLDL in our study,while a correlation of 0.89 between ALDL and DLDL was seen by Vujovic et. al[13] in the study done in Serbian population. We have measured LDL to be higher than that obtained by Friedewald formula, but a higher ALDL-C level was compared to measured LDL-C, but this overestimation was upto TG level<200mg/dl at TG level between 201-400mg/dl underestimation. The mean level of FLDL was approximately 6.7mg/dl less than that of DLDL while mean level of ALDL was approximately 2.7mg/dLl higher than DLDL. Kamazeki et.al reported an underestimation of 5.9mg/dl by Friedewald formula compared to measured Direct LDL-C.[14] Schanagl et.al reported lower level of FLDL-C than DLDL-C.[15]

CONCLUSION

It is concluded on the basis of the present study that Friedewald calculated LDL underestimates measured LDL by direct method at all levels of triglyceride while LDL calculated by Anandaraja formula overestimates measured LDL upto TG level of 200mg/dl while it underestimates LDL at TG level between 201-400mg/dl. Calculated LDL depend on TG level hence LDL-C should be measured by homogenous direct assay methods. But if have to use a formula method when direct homogenous methods are not available, Friedewalds formula seems to give values much closer to values obtained by Direct methods as at TG levels of 101-150 mg/dl, a mean difference of only -0.96 is seen, while at higher triglyceride level >200mg/dl both the formula methods (FF andAR) shows mean difference of -24.3 and -15.82 mg/dl respectively. Also when we calculate the correlation values, Friedewalds formula shows a strong correlation (0.876) in comparision with Anandaraja formula (0.844), when both these methods are correlated with Direct homogenous assay methods.

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