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Low Density Lipoprotein (LDL) Measurement By Direct Method Vs Formula Method(Friedewald's Equation)

Biochemistry

KEYWORDS : F-LDL, D-LDL, cholesterol, Friedewald's formula, Direct method

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ABSTRACT

Estimating LDL-C is an important aspect in assessing the Cardiovascular status of dyslipidemic subjects. Many methods are available for estimating LDL-C concentration. Most laboratories determine LDL-C by Friedewald's formula. The aim of this study was to compare calculated LDL-C with direct homogenous assay, and to determine to what extent LDL-C level was under or overestimated when it was calculated using the formula compared with direct measurement of LDL-C. Our study included 156 patients with TG<400 mg/dL. LDL-C estimation was done by homogenous assay & also calculated using the Friedewald's formula. The mean LDL-C levels were 102. ± 37.7 and 94.4 ± 36.7 for Direct LDL-C and Friedewald's calculated LDL-C. A good correlation was found between the calculated LDL-C method and direct LDL-C method assay, i.e. F-LDL v/s D-LDL (r = 0.867). F-LDL correlated with D-LDL estimation at both TG level of <150mg/dL and TG level between 150-400mg/dL

Introduction:

Level of LDL is one of the main predictor of atherosclerosis and coronary heart disease risk[1-3]. A new method of direct homogenous assays[4,5] for LDL-C determination in serum has been developed with a satisfactory degree of accuracy but they are expensive. The National Cholesterol Education Programme(NCEP) Adult treatment Panel III recommends a goal of maintaining serum LDL-C concentration <100mg/dL. As measurement of LDL-C by direct method is costly, so in many clinical and research laboratories it is calculated using Friedewald's equation[1,2]. The calculation by Friedewald's equation is : FLDL-C =Total cholesterol – HDLC – TG/5 , but it has certain limitations, it is not valid in patients having Triglyceride level more than 400mg/dL or in patients with dysbetalipoproteinemia. This study was undertaken to see that whether the levels of LDL-C was underestimated or overestimated when it was calculated using the Formula method and by direct method. We also made correlations and concentration differences obtained by the formula method and direct method.

Patient & Methods:

This is a comparative study for the estimation of LDL-C using Formula method & direct estimation by a homogenous assay. Data was collected from lipid profile reports, after analysis of serum samples that we got from the patient who came to Clinical Biochemistry Laboratory of Varunarjun Medical College, Banthra, Shahjahanpur (U.P.). This study was undertaken in 156 patients from September 2016 to November 2016. All patients above 18 years of age who came to our Clinical Biochemistry Laboratory for lipid profile investigation was included in the study. Patients with Triglyceride level > 400mg/dL were exluded from the study. The serum samples were obtained by taking 5 mL of venous blood after 10-12 hrs of overnight fasting and collected in plain vials. Serum was separated by centrifugation & analysed on Erba EM-200 as per standard laboratory practices. Total cholesterol and Triglyceride levels were measured enzymatically by CHOD-PAP and GPO-PAP methods. High density Lipoprotein Cholesterol was measured using a homogenous assay with precipitation. Low density Lipoprotein Cholesterol(LDL-C) measurement is based on modified polyvinyl sulfonic acid(PVS) and polyethylene- glycol methyl ether(PEGME) coupled classic precipitation method. LDL-C was also calculated by Friedewald's formula, FLDL-C = Total cholesterol – HDLC – TG/5.

Statistical analysis:

Statistical analysis was performed on statistical package for social sciences(SPSS) version 15.0. All the continuous data was presented as Mean ± SD. Student t test and Pearson's correlation was used for comparing the differences in LDL-C concentrations. The level of significance was taken as $p<0.05$.

Results:

The comparative study was done on lipid profile of 156 patients, out of these 58.3% were male and 41.7% were females. The mean age of the patients was 47.8 ± 14.6 year. The mean Total cholesterol was 178.8 ± 45mg/dL. The mean LDL-C levels were 102.1 ± 37.7 and, 94.4 ± 36.7mg/dL for direct LDL & FLDL-C respectively. Paired t test didn't show any statistical significance between measured and the calculated LDL-C. A good correlation was found between DLDL-C and FLDL-C (r = 0.867). Comparision of measured LDL-C and calculated LDL-C at different triglyceride levels are shown in **Table III**. At low TG level < 150mg/dL measured LDL-C and calculated LDL-C level were 88.8 ± 26.7mg/dL and 84.6 ± 27.6mg/dL having no statistical significance. But when TG level ranges from 150 to 400mg/dL a significant increase in mean difference between measured LDL-C (121.2 ± 42.7mg/dL) and calculated LDL-C level (108.6 ± 43.2mg/dL) was found to be 12.6mg/dL , though no statistical significance was measured at this level also.

Table I: Demographic detail of subjects

Total number of patients included in study	156
No. of Male	91
No. of Females	65
Mean age of subjects in years	47.8 ± 14.6
Mean ± SD serum Total Cholesterol (mg/dL)	178.8 ± 45
Mean ± SD serum Triglyceride (mg/dL)	156.7 ± 68.5
Mean ± SD serum HDL(mg/dL)	52.10 ± 11.7
Mean ± SD serum DLDL-C (mg/dL)	102.1 ± 37.7
Mean ± SD serum FLDL-C (mg/dL)	94.4 ± 36.7

Table II: Paired sample statistics and correlation

Parameters	Mean ± SD(mg/dL)	Mean difference (mg/dL)	Correlation(r)	P value	% Δ LDL-C
DLDL-C vs FLDL-C	102.1 ± 37.7 94.4 ± 36.7	7.7	0.867	0.07	-7.5%

Table III: Comparision of measured & calculated LDL-C

	Triglyceride (mg/dL) (< 150mg/dL)	(150-400mg/dL)
LDL-C (measured)	88.8 ± 26.7	121.2 ± 42.7
LDL-C (calculated)	84.6 ± 27.6	108.6 ± 43.2
Mean difference	4.2	12.6
P value	0.293	0.100

Discussion:

Serum LDL-C is a well known risk factor for atherogenesis, so it's estimation has to be precise and accurate. This study was undertaken to compare the calculated method of estimation of LDL cholesterol, by Friedewald's equation and by direct method. A good correlation was observed between the calculated parameters and directly measured LDL-C levels. The correlation was 0.867 between FLDL-C and DLDL-C. Our finding correlates well with the study done by Kapoor et.al & other studies where correlation ranged between 0.78 to 0.93[6-8]. At different levels of TG our study found that calculated LDL was always lower than the directly measured LDL-C. This difference increases at higher levels of Triglycerides. It is similar to other studies[6]. The mean difference between DLDL-C and FLDL-C was highest (12.6mg/dL) at TG levels 150-400mg/dL and least (4.2mg/dL) at TG levels <150mg/dL respectively. The direct assays are precise, accurate and not affected by triglyceride levels, therefore should be used to measure LDL-C.

Conclusion:

The findings of our study suggested that calculated LDL-C underestimates measured LDL-C, which might lead to inaccurate treatment decisions. Calculated LDL-C may depend on triglyceride level hence LDL-C should be measured by direct assays in clinical laboratories.

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