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Original Research Article**Effect of serum triglycerides on LDL estimation by Friedewald formula and direct assay: A laboratory based study****Sudha K**, Ashok Prabhu K*, Anupama Hegde, Aradhana Marathe and Kiran Kumar A M*Department of Biochemistry, Kasturba Medical College, Mangaluru, Manipal University, Manipal, India****Correspondence Info:**

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E-mail: sudha.k@manipal.edu**Abstract**

Introduction: A long standing association exists between elevated serum LDL and cardiovascular disease. Studies have suggested that, increased LDL in serum is the major contributor to vascular complications of other diseases like diabetes mellitus, its measurement is recommended in routine clinical practice. Currently, estimation of LDL cholesterol is done in the clinical laboratories using Friedewald equation to make the lipid profile cost effective. However, it has been highlighted that calculated LDL is not reliable when serum triglyceride (TG) levels exceed 400mg/dl.

Objective of the study: To compare estimated LDL by homogenous method with calculated LDL by Friedewald equation in lipid profile requests and to compare the same in different groups of triglyceride levels.

Materials and Methods: About 260 lipid profile requests of both the genders aged between 25-75 years were considered for the study. Total cholesterol, triglycerides, HDL-cholesterol, LDL -cholesterol was estimated in the serum spectrophotometrically. LDL-C was also calculated using Friedewald formula. The Data thus collected was segregated based on triglycerides into three groups, Group I (TG \leq 150mg/dl) Group II (TG 151-399 mg/dl) and Group III (TG \geq 400 mg/dl).

Results: LDL determined by direct assay correlated highly with calculated LDL in all subjects irrespective of the TG levels. Correlation coefficients being 0.96, 0.95 and 0.81 in group I, II and III respectively. Estimated LDL was significantly higher than the calculated LDL in group II and group III, suggestive of the fact that calculated LDL underestimates the true LDL levels in cases with TG levels above the normal range. Further, the differences in the means were significantly higher in hypertriglyceridemic groups ($p < 0.001$).

Conclusion: it can be concluded that estimation of LDL needs a better accurate measurement technique than following calculations, considering the importance of patient care in management of life style disorders, aimed at lowering serum LDL levels.

Keywords: Friedewald formula, LDL-estimated, LDL-Calculated, Triglycerides, Total Cholesterol

1.Introduction

Hypercholesterolemia is a lipid abnormality commonly related to atherosclerosis. Extensive studies have suggested that LDL is the major lipoprotein associated with Coronary Artery Disease (CAD) and important contributor to vascular complications in diabetes mellitus [1,2]. The physiological level of LDL that is sufficient for cardio metabolic health range from 25 to 60mg/dl, and LDL is more atherogenic when it exceeds 100 mg/dl [3]. Further, LDL particles that are smaller and denser are considered to be more atherogenic. Since treatment of CAD is targeted at lowering serum LDL

levels its measurement technique requires standardization and good accuracy [4]. The Friedewald formula (FF) is used in the estimation of LDL level that uses the serum levels of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL). $LDL (mg/dl) = TC (mg/dl) - HDL (mg/dl) - TG (mg/dl)/5$ [5,6]. The FF became the standard method for LDL assessment because of its simplicity and cost effectiveness compared to direct assays. FF has limitations under certain conditions like hypertriglyceridemias, which

may alter the relationship between very-low-density lipoprotein cholesterol (VLDL) and TG[7].

Hence this study was conducted to compare LDL by the calculated method and by direct method and establish the effect of serum triglyceride in calculation of LDL by FF.

2. Methodology

2.1 Study design: Case control study

2.2 Inclusion criteria

260 subjects of both genders between age groups of 25-75 years were enrolled for the study.

2.3 Exclusion criteria

Subjects who were on statins, with hypertension, kidney diseases and cardiac disorders were excluded from the study.

2.4 Methods

The Serum samples were obtained by withdrawing venous blood after 10-12 hours of overnight fast and following parameters were estimated:

1. Total Cholesterol (TC) by enzymatic end point CHOD-PAP method[8].
2. Triglycerides (TG) by enzymatic Glycerol Phosphate Oxidase/Peroxidase Method [9,10].
3. HDL-Cholesterol (HDL) by Homogenous Enzymatic Direct assay[11].
4. LDL-Cholesterol (LDL) by Homogenous Enzymatic assay[12].
5. LDL-Cholesterol (LDL) by Friedewald calculation.

Data thus collected was categorized into 3 groups based on the serum TG levels. Group I (TG \leq 150 mg/dl), Group II (TG 151-399 mg/dl) and Group III (TG \geq 400 mg/dl).

Statistical analysis was done using student *t* test and Pearson's correlation coefficient. *p* values $<$ 0.05 were considered to be statistically significant. Data was analyzed using statistical package for social Sciences V 16.0.

3. Results

Table 1 indicates that Group I patients with TG \leq 150mg/dl (n =100), showed no significant difference between the methods of LDL estimation. In the Group II patients with TG $>$ 150mg/dl (n=85), there was a significant difference between the two methods (*p* $<$ 0.05). Further, Group III subjects, with TG \geq 400 (n=75), also showed significant difference between the calculated LDL and estimated LDL (*p* $<$ 0.05). In group II and III with higher TG levels estimated LDL was more than the calculated LDL, showing that calculated LDL underestimates the true LDL concentration.

The differences in means of estimated and calculated LDL were significantly higher in group II and group III (*p* $<$ 0.001) with paired student's *t* test. However, in Group I difference was not statistically significant (Table 2). Comparison of calculated LDL and direct LDL in the groups I, II, III yielded correlation coefficients of 0.963, 0.953 and 0.811 respectively, emphasizing the fact that LDL determined by both the methods correlated significantly in all subjects irrespective of their TG levels (Table 3).

Table 1: Comparison of LDL-estimated with that of LDL-Calculated (in MEAN \pm SD)

Groups	TG Range (mg/dl)	LDL (estimated)	LDL (calculated)
I	\leq 150 (n=100)	120.70 \pm 38.59	121.05 \pm 41.53
II	151-399 (n=85)	136.34 \pm 40.19	125.63 \pm 41.85*
III	\geq 400 (n=75)	165.32 \pm 33.71	134.98 \pm 33.44*

**p* $<$ 0.05, n=number of subjects

Table 2: Paired differences between LDL-estimated and LDL-calculated in different TG groups

Groups	TG (mg/dl)	MEAN \pm SD	P Value
I	\leq 150	0.34 \pm 11.30	0.683
II	151	-39910.17 \pm 12.72	$<$ 0.001*
III	\geq 400	30.34 \pm 20.63	$<$ 0.001*

*significant

Table3: Correlation between calculated and estimated LDL in different TG groups

TG Ranges (mg/dl)	r Value	P Value
\leq 150	0.963	0.000
151-399	0.953	0.000
\geq 400	0.811	0.000

4. Discussion

As the relationship between serum LDL and Cardio vascular disease is well established, reliable methods of measuring the lipid profile are needed to monitor cardiac risk and patient care. Recently, many studies have demonstrated limitations to the most widely used method for serum LDL estimation by the Friedewald formula. Despite the classical indication for direct measurement of LDL as TG $>$ 400mg/dl, some studies have shown that, even for lower TG values, the FF is not as reliable as it was thought to be. In the present study, estimated LDL was significantly higher than the calculated LDL in patients with TG levels more than 150 mg/dl. The outcome of the present study supports some of the earlier studies which showed that LDL got from FF with TG more than 180mg/dl, showed significant differences underestimating the true value, when compared to direct measurement methods. The study conducted by Charuruksand Milintagas[13], found that the direct method was more precise and accurate than FF, even for TG levels between 200 and 399 mg/dl. The result obtained by the present study, is in

agreement with the above findings. Further, the results of the present study show that LDL calculation is accurate only in individuals with TG levels in normal range i.e. <150mg/dl. However, there is one study [14] which reports that the LDL value estimated by the FF was precise for any value of TG <400mg/dl, which do not align with our results. Some studies have shown that FF can also display discrepancies in low TG values. When TG value was <70mg/dl, the estimated LDL-c using the FF showed slightly lower values than that using the direct method [15]. Results of the present study is no different from the above finding. Contradictory results have been demonstrated in other studies, in which serum LDL using the FF was higher than the homogeneous assay for TG <100 or 200mg/dl [16,17].

The present study showed a statistically significant positive correlation between calculated and estimated LDL in group I and II with 'r' values of 0.963 and 0.953 respectively. However, in group III though there was a positive correlation between the two methods it was not as significant as the other groups. The fact emphasizes on the accuracy of direct LDL estimation over calculated LDL in patients with hypertriglyceridemia.

5. Conclusion

On the whole, it can be concluded that, LDL assay by FF method may be replaced by direct method for screening patients with high cardiac risk, due to high accuracy and precision of the latter, thus improving the patient care.

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