

Validation of the Friedewald Formula in Type II diabetes mellitus: An Indian perspective study

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Abstract

Introduction: Diabetes mellitus is characterized by hypertriglyceridemia and abnormal lipoprotein composition. Since LDL is a modifiable risk factor for cardiovascular complications in diabetes, its measurement is recommended in routine clinical practice.

Objective of the study is to assess the accuracy of serum LDL concentration estimation by Friedewald formula and by homogenous method in normal and diabetic patients.

Materials and Methods: Fasting blood samples were collected from 300 subjects (109 normal persons 101 prediabetics, 90 diabetics). Glucose, total cholesterol, LDL and HDL were estimated spectrophotometrically and LDL was calculated by Friedewald formula. LDL calculated by Friedewald formula was compared with that obtained by direct assay.

Results: LDL determined by direct assay correlated highly with calculated LDL in all subjects irrespective of fasting glucose value. Estimated LDL was higher than calculated LDL in normal and diabetic group, indicating the fact that calculated LDL underestimates the true LDL levels irrespective of fasting glucose levels. Further, the difference in the means were significantly high in all the 3 groups with significance being highest in the diabetic group ($p < 0.001$)

Conclusion: it can be concluded that estimation of LDL is always a better investigation technique than following calculations for LDL estimation, considering the importance of patient care in treatment of lipid disorders in diabetes mellitus.

Keywords: Friedewald formula, Direct LDL estimation, diabetes mellitus

1.Introduction

India is racing to be the diabetic capital of the world with more than seventy million diabetics. Type II diabetes mellitus is characterized by a low serum HDL cholesterol, abnormal VLDL and high triglyceride level, with total cholesterol and LDL cholesterol frequently being normal[1]. This is quite unexpected in light of their high coronary heart disease risk. Derranged glucose metabolism is often associated with central obesity and hypertriglyceridemia[2]. Clinical studies have demonstrated a strong positive correlation between LDL and coronary heart diseases[3].

LDL cholesterol is accurately measured by ultracentrifugation or by homogenous enzymatic assays using kits. Since its estimation by Friedewald equation[4] is cost effective most of the clinical labs still follow this indirect method of LDL estimation. The equation depends on three independent factors HDL, total cholesterol and triglyceride (TG) [LDL (mg/dL) = TC (mg/dL) – HDL (mg/dL) - TG (mg/dL)/5]. Since VLDL cholesterol is very small relative to LDL cholesterol, inaccuracy of VLDL component (TG/5) of Friedewald equation can be well tolerated. But, at a very high triglyceride concentration, calculated LDL becomes unreliable.

When triglyceridemia has become a greater problem in insulin resistance and diabetes mellitus[5], the issue needs to be reevaluated in the present era for treatment of these diseases. Hence the aim of the study is to compare LDL by direct assay using commercially available kit that has a synthetic polymer/detergent and that obtained by Friedwald equation in subjects with normal and altered fasting glucose. Thereby reexamine the accuracy of the equation in measuring LDL in diabetics and investigate the accuracy of direct assay in LDL determination in diabetic patients.

2. Methodology

2.1. Study design: Case control study

2.2. Sample size

Fasting blood sample was collected in plain vacutainers from 300 subjects aged between 45-55 years of both the sexes. The study population was divided into three groups based on the fasting glucose (FBG) values. First group included subjects with $\text{FBG} \leq 100\text{mg/dL}$, second group consisted of patients with FBG between 100-125 mg/dL and who were not on any medications and the third included all diabetics with $\text{FBG} \geq 126\text{mg/dL}$ and who were not on lipid lowering drugs. 109 subjects served as controls with normal FBG and normal lipid profile, 101 patients were prediabetics and 90 were diabetics.

2.3. Exclusion Criteria

Patients who were obese, smokers, alcoholics and hypertensives were excluded from the study. Informed consent was collected from all the patients.

2.4. Methodology

Serum samples were used to estimate FBG by GOD-POD method, HDL by direct assay⁵ total cholesterol by cholesterol oxidase –peroxidase method[6], triglyceride by glycerol phosphate oxidase peroxidase method[7], LDL by homogenous direct assay[8] and by calculation using Friedewalds equation. Data were analyzed by SPSS statistics package with difference between group means tested by paired students t test. Correlation coefficients were calculated by Pearson's correlation test. p value less than 0.05 was considered significant.

3. Results

Table 1 depicts baseline lipid profile data of three groups studied, indicating hypertriglyceridemia in diabetic group. Mean values of LDL, both estimated and calculated showed significant difference in all the three groups. The values were highly significant in the diabetic group ($p < 0.001$). Estimated LDL was higher than calculated LDL in all the three groups clearly indicating the fact that calculated LDL underestimates the true LDL concentration. The difference in means were highly significant in group I and group III $p < 0.001$ (**Table 2**) and less significant in group II ($p < 0.05$). Comparison of calculated LDL and direct LDL in all the three groups yielded correlation coefficients from 0.905 to 0.982 depending on the FBS values (**Table 3**). A greater absolute difference in Friedewald estimated LDL versus directly measured LDL occurred at higher FBS group. Percentage error of LDL got by the two methods also increased with higher blood glucose (**Table 4**).

Table 1: Baseline data depicting serum total cholesterol and triglycerides in 3 groups (Mean \pm SD)

FBS	Total cholesterol	Triglyceride
< 100mg/dL	184 \pm 50	142 \pm 85
101-125mg/dL	196 \pm 43	139 \pm 64
$\geq 126\text{mg/dL}$	201 \pm 45	157 \pm 110

Table 2: Comparison of direct LDL and Calculated LDL based on Fasting blood glucose values

FBS	n	Direct LDL	Calculated LDL	Difference	P value
< 100mg/dL	109	89.5 \pm 39.71	86.53 \pm 41.59	2.968	0.001
101-125mg/dL	101	86.82 \pm 35.36	84.21 \pm 39	2.602	0.021
$\geq 126\text{mg/dL}$	90	115 \pm 45.21	108.63 \pm 44.62	7.275	0.001

LDL values expressed as mean \pm SD

Table 3: Correlation coefficients of calculated and estimated LDL in 3 groups

FBS	r value	P value
< 100mg/dL	0.982	0.000
101-125mg/dL	0.905	0.000
$\geq 126\text{mg/dL}$	0.943	0.000

Table 4: Percentage error of the means of LDL estimated by two methods

FBS	n	Mean LDL by Friedewald's Formula	Mean LDL by Direct assay	% error
< 100mg/dL	109	89.5	86.53	2.3
101-125mg/dL	101	86.82	84.21	2.05
$\geq 126\text{mg/dL}$	90	115.9	108.63	6.27

4. Discussion

As the relationship between serum LDL and disorders like metabolic syndrome, coronary artery diseases is increasing, reliable methods of measuring this lipid is needed to treat these patients[9]. Derranged glucose metabolism in diabetes mellitus is often associated with hypertriglyceridemia and abnormal VLDL or LDL composition[2].

The accuracy of calculated LDL becomes progressively less reliable as triglyceride level gets elevated¹⁰.

Despite the classical indication for direct measurement of LDL as TG > 400 mg/dL, some studies have shown that even for triglycerides less than 100mg/dl the LDL got from Freidewald formula is not reliable[11]. Hirany *et al* opined that calculated LDL significantly underestimates actual values in diabetics[12], which is not different from the findings of the present study. There was a significant difference in the estimated LDL and the calculated LDL irrespective of the fasting sugar values, and it is noteworthy to mention that the increase was more significant in diabetic group. The percentage error between direct LDL and calculated LDL rose in diabetics, demonstrating the limited efficacy of Freidewald formula in calculation of LDL in diabetics. Major limitation of Friedewald formula lies in the fact that chylomicron contains proportionately less cholesterol relative to triglycerides than VLDL, their presence leads to the overestimation of VLDL cholesterol and underestimation of LDL cholesterol. Branchi *et al*[13] observed a bias of >10% between calculated and estimated LDL in diabetes mellitus patients than non diabetes mellitus patients. Direct assays have adequate specificity that makes them useful in subjects with established hypercholesterolemia and type I diabetes children[14]. One of the earlier studies indicated that Freidewald formula underestimated LDL in diabetics with or without insulin treatment, even when TG was below 200mg/dl.[12] Hence direct LDL estimation may be recommended in diabetics to prevent the underestimation of risk involved in cardiovascular complications.

The present study showed a statistically significant positive correlation between calculated and direct LDL in all the groups including diabetics, supported by others who made a similar observation in type II diabetes and metabolic syndrome patients, but emphasized on the accuracy of direct LDL estimation over calculated LDL[15][16]. On the contrary, Jose *et al*[17] opined that calculated LDL was reliable in metabolic syndrome patients.

However, Rubies *et al*[18] argued that Freidewald equation should not be used in management of lipid abnormalities in patients with diabetes mellitus.

On the whole, it can be concluded that estimation of LDL is always a better investigation technique than following calculations for LDL estimation, considering the importance of patient care in treatment of lipid disorders in diabetes mellitus. The result of the present study also shows a clear benefit of performing direct LDL estimation as a part of lipid profile even when fasting blood glucose is relatively normal.

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