COMPARATIVE STUDY OF THE ESTIMATION OF LDL CHOLESTEROL BY THE DIRECT METHOD AND FRIEDEWALD EQUATION IN SECONDARY HYPERLIPIDEMIA

T. Ilanchezhian *, R Vanaja and Balaji Rajagopalan

Department of Biochemistry, Shri Sathya Sai Medical College and Research Institute, Ammapettai, Kancheepuram district, Taminadu, India.

ABSTRACT: The objective of the study is to measure the difference between the levels of LDL by Direct method versus Friedewald equation. The study includes 30 patients and 30 controls in the age of 25 – 75 years of both sexes. Fasting blood samples were collected and estimated Total Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein (LDL-C) cholesterol and High density lipoprotein (HDL) cholesterol, LDL cholesterol by direct method and by Friedewald’s formula (FW). There is a significant difference between LDL Direct and LDL-FW at triglyceride range of 1 – 100, (p= 0.01), 201 – 300 (p= 0.01) and no significant difference (p= 0.9) at 101 – 200, (p= 0.3) at 301 – 400 and (p= 0.2) at >400. There is a significant difference between LDL Direct and LDL-FW at total cholesterol range of 200 – 249 and no significant difference (p= 1.0) at 100 – 149, (p= 1.0) at 150 – 199 and (p= 0.9) at >250. There is significant difference between LDL values by direct and FW method (p=0.0490). LDL by Direct method is lower due to non interference of cholesterol and triglyceride, and LDL by FW is higher due to interference by cholesterol and triglyceride. The study has concluded that LDL-C by Direct method is reliable than Friedewald equation.

INTRODUCTION: The LDL is a heterogeneous spherical particle, with hydrophobic oily cores consisting of cholesteryl ester and TG. On an average, LDL carries two thirds of TC in serum. Each LDL particle contains one molecule of Apolipoprotein B-100 (apo B- 100), which is the main protein component of LDL, and the other minor apolipoproteins are apo E and apo C II.

Epidemiological and clinical studies have demonstrated a strong positive correlation between low-density lipoprotein cholesterol (LDL-C) concentrations in serum and the incidence of coronary heart disease (CHD). A reduction of LDL-C decreases the risk and ameliorates the symptoms of CHD by causing a regression in the lesions.

Secondary hyperlipoproteinemia refers to elevated lipid levels in some other diseases like Diabetes mellitus, renal disease, liver disease etc., the symptoms of which resemble that of primary hyperlipoproteinemia. In recent years, several studies investigated the application of the Friedewald formula in patients with secondary hyperlipoproteinemia.
hyperlipidemias. These conditions are characterized predominantly by increased triglycerides, which are well known to make the Friedewald calculation less accurate. It is critical that secondary causes of hyperlipidemias are considered prior to initiation of lipid-Lowering therapy to avoid coronary heart disease (CHD). So, accurate measurement of cholesterol, triglyceride, and HDL, are required to calculate the LDL by Friedewald’s equation (indirectly). Direct method is also used to estimate LDL accurately.

The aim of present study is to estimate and to compare the levels of LDL-C by two methods namely, the Direct method versus Friedewald equation in secondary hyperlipidemia.

MATERIALS AND METHODS:
The study was conducted on 30 secondary hyperlipidemic patients of age group 25 – 75 years of both sexes and compared with age and sex matched 30 controls. All these patients were attending the medical OP of SRM Medical College and Research Centre.

Study protocol:
The oral consent was obtained from patients with secondary hyperlipidemia as well as from controls.

Fasting blood samples were collected and used for estimation of the Total Cholesterol (TC) by enzymatic endpoint CHOD- PAP method. Triglycerides (TG) by Enzymatic Glycerol Phosphate Oxidase/ Peroxidase method. HDL-Cholesterol (HDL-C) by Homogenous enzymatic Direct Assay. LDL-Cholesterol (LDL-C) by Homogenous enzymatic Direct Assay. LDL-Cholesterol (LDL-C) obtained by Friedewald Calculation.

Statistical analysis:
Data are presented as mean ± standard deviation. Student unpaired t-test was used to assess the significance of difference between the groups. The results were analyzed by software spss 15. ‘P’ value less than 0.05 (p<0.05) was considered to be statistically significant.

RESULT: From the present study the following results are reported. Table 1 indicates that there is a statistically significant difference among the levels of lipid profile in controls and the different patients. It is also noted that the LDL-C values are significantly (p>0.05) different when compared with the direct and Friedewald formula.

Table 2 shows the values of LDL-C estimated by direct assay and by Friedewald’s method. They are grouped according to their TG levels. There was significant difference between the two methods at TG levels 1-100, 101-200 mg/dl (p <0.01, < 0.02 respectively). There was no significant difference at TG levels > 201 mg/dl. Table 3 shows the levels of LDL-C at different categories of Total cholesterol. They are grouped according to their TC levels. There was a statistically significant difference in the mean of LDL-C levels obtained by the two methods at TC levels 200-249 mg/dl and >
250 mg/dl (p<0.01, < 0.04) respectively. There was no significant difference at TC levels < 200 mg/dl.

The LDL-C estimated by direct homogenous method and by Friedewald calculation showed a mean of 20.00 to -46.25 (Table 4). At lower TG levels that is, from 1 - 200 mg/dl Friedewald estimation was higher than direct method and the difference was highly significant (p <0.01). At TG levels >200 mg/dl, the direct method showed a higher value than Friedewald's estimation but the difference was not significant (p>0.67).

**TABLE 2: MEAN AND STANDARD DEVIATION (IN mg/dl) OF DIRECT LDL-C AND FRIEDEWALD LDL-C AT DIFFERENT LEVELS OF TRIGLYCERIDE (TG).**

<table>
<thead>
<tr>
<th>TG Range</th>
<th>N</th>
<th>Mean ± SD LDL –C</th>
<th>Direct</th>
<th>Mean ± SD Friedewald LDL-C</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 100</td>
<td>16</td>
<td>58.25 ± 21.29</td>
<td>78.25 ± 21.29</td>
<td>&lt;0.01 S</td>
<td></td>
</tr>
<tr>
<td>101 - 200</td>
<td>20</td>
<td>95.10 ± 25.60</td>
<td>135.55 ± 71.35</td>
<td>&lt;0.02 S</td>
<td></td>
</tr>
<tr>
<td>201 - 300</td>
<td>13</td>
<td>181.69 ± 47.23</td>
<td>174.15 ± 42.82</td>
<td>&gt;0.67 NS</td>
<td></td>
</tr>
<tr>
<td>301 - 400</td>
<td>7</td>
<td>165.00 ± 31.94</td>
<td>140.14 ± 50.16</td>
<td>&gt;0.29 NS</td>
<td></td>
</tr>
<tr>
<td>&gt;400</td>
<td>4</td>
<td>148.00 ± 31.87</td>
<td>101.75 ± 65.82</td>
<td>&gt;0.25 NS</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION:** The diagnosis and management of adults with hypercholesterolemia are largely based on LDL-C concentration. The serum LDL-C concentrations used to classify adults for high risk of heart disease are: Desirable <130 mg/dl, Borderline high-risk 130 – 159 mg/dl, and High risk >160 mg/dl. The goal for secondary hyperlipidemia is to achieve LDL-C of 100 mg/dl. Therefore accurate and precise measurements of patients LDL-C concentrations are necessary to appropriately identify individuals with hypercholesterolemia and to monitor the response to diet and drug treatments.
In the present study, the secondary hyperlipidemia was assessed with LDL-D values and LDL-FW for Diabetes mellitus, renal disease, liver disease, and post menopausal women. It was found that the LDL values by both the methods fell into high risk group in all the above mentioned groups as evidenced in Table 1. Patients with secondary hyperlipidemia have cardiovascular risk factor such as lipid abnormalities. The low level of LDL-FW is due to high triglyceride levels. There is a statistically significant difference (p<0.0490) among LDL-D and LDL-FW in secondary hyperlipidemia.

The previous studies of the Friedewald calculation have determined that at TG concentrations < 200 mg/dl, the Friedewald formula can provide a reliable estimate of LDL-C concentration. Legault et al.\textsuperscript{13} showed that the TG values 200 – 400 mg/dl correlates well with the LDL levels. However, it is shown as in Table 2 these values do not correlate. It was reported that in diabetes mellitus, there is an elevation of LDL due to insulin resistance and in renal disorder the abnormality may be due to increased hepatic production\textsuperscript{14}.

In both the cases, statistically significant difference was observed as per the present study (Table 2). Hyperlipoproteinemia was seen in liver diseases and the LDL values showed a marked difference with both the methods.

The LDL-C estimated by Direct and by Friedewald’s formula showed a significant difference (p < 0.02 and < 0.01) at lower TG ranges of 1 – 100 and 101 – 200 mg/dl respectively. There is no significant difference at TG levels <200mg/dl. It may be due to the interference of high TG levels. When the level of LDL-D was compared to LDL-FW, there was no significant difference at lower cholesterol range of 100 – 149 mg/dl (p> 0.9). The difference in means and SDs were highly significant (p < 0.001) at cholesterol levels of 150 – 199, 200 – 249 and >250 mg/dl.

As shown in Table 3, there was significant difference between the two methods at TG levels 1 – 100 (p< 0.01), 101 – 200 (p< 0.02) and there was no significant difference at TG level 201 – 300 (p>0.67), 301 – 400 (p>0.29) and > 400 (p>0.25) respectively, among the post menopausal women. These results corroborate with the results of Sudha\textit{et al.}\textsuperscript{7} However, as per the present study, the LDL values showed significant difference by both the methods when the TC levels were <200 mg/dl, whereas it is not significant with TC level more than 200 mg/dl.

CONCLUSION: From the present study, it may be concluded that LDL-C (Direct) method is most reliable as it is not affected by different levels of TC and TG. Whereas in LDL-FW, the increased levels of TG above > 200 mg/dl and decreased level of TC below < 200 mg/dl seem to interfere with the estimation. Therefore LDL-C by direct method is most reliable and sensitive in secondary hyperlipidemia than Friedewald method.

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