ASSESSMENT OF LOW-DENSITY LIPOPROTEIN CHOLESTEROL BY HOMOGENEOUS ASSAY VERSUS FRIEDEWALD’S EQUATION - A STUDY OF 50 CASES.

Lincy Jacob¹, Ravikanth Medikonda², Piyush Taylor³, A.K.Bansal¹, Sowjanya Katragadda⁵, Gopinath Agnihotram⁶
¹,²,⁴,⁵,⁶Department of Biochemistry, Govt. Medical College, Jagadalpur, Chattisgarh-494001, INDIA
³Department of Biochemistry, Govt. Medical College, Surat, Gujarat, INDIA

Abstract: The objective of the present study was to assess the difference in estimation of Low Density Lipoprotein by homogeneous assay and by Friedewald’s equation. The present study group comprised of 50 subjects of Hypertensive patients, Diabetic patients, patients diagnosed with Ischaemic Heart Diseases including Healthy. In each of the four groups, estimation of Low Density Lipoprotein- cholesterol by both Friedewald’s equation and Homogeneous assay was compared. It was found that the difference in two methods was found to be significant in all the four groups that is in healthy (10 subjects) p<0.10, in hypertensive (16 subjects) p<0.02, in Diabetic (12 subjects) p<0.10 and in Ischaemic Heart Disease (12 subjects) p<0.01. The difference in LDL-Cholesterol (Direct assay-friedewald’s equation) mean and Standard deviation was also significant in different levels of Total cholesterol, TC 50-149mg% (p<0.01) and TC 150-250mg% (p<0.01) while in different Triglyceride levels were TG 1-100mg% (p>0.10), TG 101-200mg% (p<0.001), TG 201-300mg% (p>0.10) and TG 301-400mg% (p<0.02). It was puzzling to see that in Healthy patients also the difference in LDL-C (Direct assay-Friedewald’s equation) estimation showed significant difference in mean and Standard deviation (p<0.10). Direct assay must be used routinely in biochemical clinical investigation because it is more specific and accurate than Friedewald’s equation.

Key Words
Low Density Lipoprotein-Cholesterol (LDL-C), Friedewald’s equation, Direct Homogeneous Assay, Total Cholesterol (TC), Hypertension (HT), Diabetes, Ischaemic Heart Diseases (IHD).
INTRODUCTION

Diabetes mellitus, Hypertension, Ischaemic Heart Diseases, etc. constitute important elements of the Metabolic Syndrome X. The proportion of these diseases is increasing worldwide, and has acquired a pandemic and magnanimous form. Dyslipidemia, notably Low Density Lipoprotein (LDL-C) constitutes a very important risk factor in this all important group of atherosclerotic diseases and its routine measurement is recommended in the evaluation and management of these disorders. LDL-cholesterol is actually an operational classification, which includes a family of similar particles with hydrated density between 1.006 and 1.063 kg/L under ultracentrifugation. It represents a group of heterogeneous lipoproteins varying in size and density. This heterogeneous fraction indeed composes of main LDL-cholesterol, intermediate density lipoproteins (IDL), lipoprotein(a) (Lp(a)), and remnant of very low-density lipoproteins (VLDL). All have in common a single molecule of apolipoprotein B100 (ApoB) in each particle. Because of this heterogeneity and the availability of different methods for measuring LDL-C, it is necessary to understand which lipoproteins are actually being measured by each individual method and to what extent the atherogenic lipoproteins other than LDL contribute to the observed LDL-C values. If the guidelines have to be implemented into clinical practice, it is best to have the LDL-C assays standardized. Most of the Clinical laboratories use Friedewald’s equation to assess the study of measurement of LDL-cholesterol.

The present study aims at studying the LDL-cholesterol levels by the Friedewald’s equation (Friedewald, W.T, et.al. 1972) and the third generation direct homogenous assay (Bachorik PS et.al, 1997) in different atherosclerotic groups, with the objective of estimating the differences in the two methods, and trying to study the pattern of these differences in various atherosclerotic disorders; thereby recommending a suitable, reliable, convenient method for the LDL-C such that target goals set by the ATP-NCEP (Adult Treatment Panel-National Cholesterol Education Panel) guidelines can be achieved in order to reduce the disease burden of the various atherosclerotic disorders and hence imply positive change in the health care and economy of the person and the society at large.
MATERIALS AND METHODS

The lipid profile analysis was conducted at Clinical Biochemistry Laboratory of Maharani Hospital, Govt. Medical College, Jagdalpur, Chattisgharh. 5ml of venous blood of the enrolled patients was collected in the fasting condition (12-14 hours of fasting was maintained). The blood sample was allowed to clot at room temperature, serum was separated, centrifuged and the following parameters were estimated:

2. Triglycerides by Enzymatic Glycerol phosphate oxidase/peroxidase method. (Cole TG et.al, 1997)
5. LDL-Cholesterol by Friedewald’s Equation (Friedewald, W.T, 1972)

The measurement of parameters like Total Cholesterol, Triglycerides and HDL-Cholesterol were estimated by Actcetol N-H, Lab-care Diagnostics (India) Pvt. Ltd. While measurement of LDL-Cholesterol was estimated by using Reagent Kit from, Diasys Diagnostic Systems GmbH, Alte Strasse 9 65558 Holzheim, Germany, distributed by Sigma Diagnostics. The homogeneous LDL-C assay distributed by Sigma Diagnostics, contains two ready-to-use reagents. Step 1 comprised of Reagent 1 consisting of Good’s buffer [pH 6.8; N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, sodium salt], cholesterol esterase, cholesterol oxidase, catalase, polyamions, and amphoteric surfactants, which selectively protect LDL-C from enzyme reaction. The non-LDL cholesterol reacts with cholesterol esterase and cholesterol oxidase, producing hydrogen peroxide, which is consumed by catalase. Step 2 comprised of reagent 2 having Good’s buffer (pH 7.0), 4-aminoantipyrene, peroxidase, sodium azide, and deprotecting reagent. The nonionic surfactants remove the protecting agent from LDL, enabling the specific reaction of cholesterol esterase and cholesterol oxidase with LDL-C. The resulting hydrogen peroxide yields color with Trinder’s reagent and 4-aminoantipyrene in the presence of peroxidase. The blue color complex produced has an absorbance peak at 620 nm. In 1972, Friedewald et al. published report describing a formula to estimate LDL-C as an alternative to tedious ultra centrifugation, also as VLDL-C carries most of the circulating TGs, VLDL-C can be estimated reasonably well be measured by TG divided by 5 for mg/dl units. LDL-C is then calculated as Total Cholesterol minus HDL-C minus estimated VLDL-C. Then biostatistical analysis Paired t-test was used to find significant difference in comparing the two methods that is both by direct homogeneous assay and by friedewald’s equation in each atherosclerotic groups including healthy subjects.
RESULTS

The difference in LDL-Cholesterol (direct-friedewald estimation) mean and standard deviation in all the four study groups including healthy subjects showed significance (p<0.10, <0.10, <0.02, <0.01 respectively). Out of four groups the difference in LDL-Cholesterol (direct-friedewald estimation), the Hypertensive (p<0.02) and Ischaemic Heart Disease (p<0.01) group when compared to healthy subject were found to be highly significant Table 1.

Table 1. LDL-Cholesterol difference in (direct-friedewald’s estimation) mean and Standard Deviation in different study groups.

<table>
<thead>
<tr>
<th>S.N</th>
<th>Study groups</th>
<th>Number of subjects</th>
<th>Mean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Healthy (HTY)</td>
<td>10</td>
<td>5.3 ± 7.87</td>
<td>p&lt;0.10</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetes Mellitus (DM)</td>
<td>12</td>
<td>10.25 ± 16.84</td>
<td>p&lt;0.10</td>
</tr>
<tr>
<td>3.</td>
<td>Hypertension (HT)</td>
<td>16</td>
<td>9.18 ± 12.66</td>
<td>p&lt;0.02</td>
</tr>
<tr>
<td>4.</td>
<td>Ischaemic Heart Disease (IHD)</td>
<td>12</td>
<td>12.33 ± 12.78</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

Table 1 shows the difference in mean and standard deviation of LDL-Cholesterol measured by direct Homogeneous assay and Friedewald’s equation in different atherosclerotic groups. The difference in LDL-cholesterol in all groups including healthy was found to be significant (p<0.10, <0.10, <0.02, <0.01) respectively.

The difference in mean and standard deviation of estimated LDL-C (direct-calculated) in different levels of Trglycerides, TG 1-100mg% (p>0.10), TG 101-200mg% (p<0.001), TG 201-300mg% (p>0.10) and TG 301-400mg% (p<0.02) Table-2 were less significant when compared with different levels of Total Cholesterol Table-3 TC 50-149mg% (p<0.001) and TC 150-250mg% (p<0.01). Table 4 shows that patients were classified as having high and low cardiac risk taking LDL-C 130mg% as cut off levels. The patients having LDL-C levels <130mg% by direct assay were 42no. (84%) and by Friedewald’s method were 46no. (92%). The patients having LDL-C levels >130mg% by direct assay were 8no. (16%) and by Friedewald’s equation were 4no. (8%). High Cardiac risk factor (LDL-C >130mg%) was found to be twice more by direct assay when compared with (LDL-C >130mg%) measured by Calculated method.
Table 2: LDL-C difference (direct-friedewald’s equation) mean and SD in different categories of Triglycerides.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Triglyceride range</th>
<th>No. of subjects</th>
<th>Mean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-100mg%</td>
<td>15</td>
<td>6.0±13.90</td>
<td>p&gt;0.10</td>
</tr>
<tr>
<td>2</td>
<td>101-200mg%</td>
<td>27</td>
<td>9.4±10.46</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>201-300mg%</td>
<td>5</td>
<td>10.8±21.79</td>
<td>p&gt;0.10</td>
</tr>
<tr>
<td>4</td>
<td>≥301mg%</td>
<td>3</td>
<td>16.3±9.64</td>
<td>p&lt;0.02</td>
</tr>
</tbody>
</table>

Table 2 shows the difference in mean and SD of LDL-C (direct assay- Friedewald’s equation) at different levels of Triglycerides. There was significant difference in LDL-C at TG 101-200mg% (p<0.001) and TG ≥301mg% (p<0.02) and no significance was found at TG 1-100mg% (p>0.10) and TG 201-300mg% (p>0.10).

Table 3. LDL–C difference (direct-friedewald) in mean and SD in different levels of Total Cholesterol.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>TC levels</th>
<th>No.of subjects</th>
<th>Mean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50-149mg%</td>
<td>27</td>
<td>9.70±11.92</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>150-250mg%</td>
<td>23</td>
<td>9.13±14.39</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

Table 3 shows LDL-C difference in mean and SD at different TC levels there was significant difference in LDL-C estimated at TC 50-149mg% (p<0.001) and TC 150-250mg% (p<0.01) respectively.

Table 4. Patients classified into two groups LDL-C measured by two methods.

<table>
<thead>
<tr>
<th>LDL-C levels</th>
<th>No. of subjects by direct assay (%)</th>
<th>No. of subjects by friedewald’s equation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;130mg%</td>
<td>42 (84%)</td>
<td>46 (92%)</td>
</tr>
<tr>
<td>≥130mg%</td>
<td>8 (16%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Total patients</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 4 shows patients were classified as having high and low cardiac risk taking LDL-C 130mg% as cut off levels. The patients in high cardiac risk comprised of 16% according to direct assay and 8% according to friedewald calculation.

**DISCUSSION**

Increase in the level of LDL-C is associated with increased Ischaemic Heart Disease and lowering of LDL-C has been shown to decrease mortality in patients with known CHD. (Sacks FM et.al, 1996).
Also in Diabetic dyslipidemia includes quantitative and qualitative abnormalities in Lipoprotein (Lp) particles, including VLDL-C and their remnants (Patti L et.al 1987, Kasama T et.al, 1987), the use of Friedewald’s equation in diabetic patients has been questioned (Rubies-Prat J et.al, 1993, Hirany S et.al, 1997, Branchi A et.al, 1998). The purpose of the present study was to assess the two methods that is Friedewald equation and Direct Homogeneous assay for estimation of LDL-C in different atherosclerotic groups (table 1). In our present study in all the study groups the LDL-C difference in Mean and SD was found to be significant (Diabetics p<0.10, Hypertension p<0.02, and IHD p<0.01 respectively). Patients were categorized at different TG levels and LDL-C difference in mean and SD were TG 1-100mg% (p>0.10), TG 101-200mg% (p<0.001), TG 201-300mg% (p>0.10) and TG>300mg% (p<0.02) in contrast to study conducted by (Suchanda et. al. 2005) in a similar study demonstrated that LDL-C estimated at TG>300mg% was not significant. Direct LDL-C assays have been developed recently and have been shown to provide accurate and precise measurements of LDL-C (McNamara JR et.al 1995, Whiting MJ et.al 1997, Hirany S et.al 1997, Rifai N et.al 1998, Nauck M et.al 2000) and they overcome TG and fasting limitations of calculated methods which are readily adapted to routine clinical laboratories.

Table 3 of present study shows mean and SD in different categories of Total cholesterol and the difference in LDL-C estimated were found to be very significant, TC 50-149mg% (p<0.001) and TC 150-250mg% (p<0.01) . Yu et al. in their study demonstrated that direct assay provides adequate specificity that make them useful in following subjects with established hypercholesterolemia, in nonfasting samples obtained from children and Type 1 Diabetes Mellitus (Yu, H.H et.al 2000). According to NCEP the desirable LDL-C limit is <130mg% for no cardiac risk hence in our present study we classified patients as having high and low Cardiac Risk taking 130mg% LDL-C as cut off values. LDL-C estimated By direct homogenous assay showed 84% (42 subjects) of study population were at no Cardiac risk whereas 16% (8 subjects) were at Cardiac risk. LDL-C measured by calculation method showed 92% (46 subjects) were at no cardiac risk while 8% (4 subjects) were at Cardiac risk group. (Nauck et al.2002) in their review on LDL-C direct assays compared with Calculated LDL-C concluded that there is evidence which supports recommending the homogeneous assays for LDL-C to supplement the Friedewald Calculation in those cases where Calculation method is unreliable when TG>400mg%. Also direct LDL-C measurement appear to be preferable to a Calculated LDL-C value include not only patients having TG>400mg% but also patients who are unable to fast (Friedewald WT , 1972, McNamara JR et.al 1990).

(Brain et al. 2000) in their review in Diabetic patients showed that N-geneous direct LDL-C assay showed no significant bias associated with increasing Hemoglobin A1c upto 10.3% as measured by HPLC supporting its usefulness in Diabetic patients. Studies also say that Friedewald method underestimates LDL-C (Warnick, G.R. and Wood, P.D 1995).
Even at Low cholesterol levels underestimation of LDL-C occurs (Scharnagl, H et.al, 2001) which is in co-relation to our present study that most of the LDL-C measurements that were done has high values that the LDL-C measured by calculation method.

CONCLUSION
The present study analysis for measurement of LDL-C by direct homogeneous assay versus Friedewald’s equation demonstrated that the difference in LDL-C measured mean and standard deviation showed significance in all the study groups. Also it was puzzling to see that the healthy subjects also demonstrated significant difference in estimation of LDL-C (direct assay-Friedewald’s equation). This study also demonstrated that the high cardiac risk factor was found more by direct assay than by Friedewald’s equation as the calculated method underestimates the estimated LDL-C. Hence this study strongly recommends of using direct assays for measurement of LDL-C in routine clinical biochemistry laboratories rather than using Friedewald’s equation.

ACKNOWLEDGEMENT
We sincerely acknowledge Dr. S. L. Adile, Director of Medical Education, Chattisgarh for providing us with all the facilities in carrying out this entire study and we are also thankful to Dr. S. Bose and Dr. Harminder Singh for their assistance in this study duration.
REFERENCES


Brustein M. Scholnick H.P. and Morfin, R (1970) Cholesterol in high density lipoprotein using Mg++/ PTA; J. Lipid Res. 19.583


International Journal of Applied Biology and Pharmaceutical Technology Page: 9
Available online at www.ijabpt.com


************