Evaluation of different methods for LDL-Cholesterol measurement in Iraqi adult population.

Dr. Saba K. Chlimeran, MSC, assistant Lecturer, Department of Biochemistry, College of Medicine, University of Mosul, Iraq

Abstract

Objective: To evaluate the association of baseline LDL-C concentration as determined by a direct assay with those calculated using friedwald equation in a group of healthy Iraqi population. Subjects and methods: The study was conducted in Mosul city in northern Iraq, from October 2009-April 2010. Fasting venous blood sample were obtained from all subject involved in the survey, by anticubital venepuncture between 8.00 a.m and 10.00 a.m. Serum lipid profile was measured for all subjects following 12 hours fasting, this profile include total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol (Friedewald formula), and direct LDL cholesterol. All the biochemical analyses were performed in the clinical chemistry laboratory of the department of biochemistry, collage of medicine, university of Mosul, Iraq. Result: There was no significant difference between Friedwald formula LDL-C and direct LDL-C. There was a positive correlation between Friedwald formula and direct immunoinhibition (IIH) methods, and The was a positive correlation between LDL-C and HDL-C. Conclusion: The direct assay in this study correlated highly with Friedewald calculation but was 5-10mg/dl the lower LDL-C concentration by direct method, so we can use Friedewald calculation that its less expensive than direct LDL-cholesterol.

Introduction

Low density lipoprotein is the major carrier of cholesterol (as cholesteryl esters) in human. LDL is removed from the circulation by LDL receptor mediated endocytosis (1). LDL is then bound and internalized as the result of the interaction of apoB100 with LDL...
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(B,E)receptor. The cholesteryl esters in the core of LDL are hydrolyzed lysosomes, producing unesterified cholesterol, which can down regulate the LDL receptor and the rate limiting enzyme in cholesterol biosynthesis, hydroxymethylglutaryl co enzyme A (HMGCOA) reductase gene, the sterol regulatory element. About two- third of LDL is removed by the liver and the rest by peripheral tissues(2).

Epidemiological and clinical studies have demonstrated a strong positive correlation between low density lipoprotein cholesterol (LDL_C) concentration in serum and incidence of coronary heart disease (3,4).

Elevated serum levels of LDL are an important risk factor of coronary heart diseases and highly correlated with atherosclerotic lesions (5). Intervention to decrease serum LDL-C can improve the symptoms of CHD and result in regression of the lesions(6).

The common approach for determining LDL_C concentration in the clinical laboratory is the Friedewald formula, which derives LDL_C from total cholesterol, HDL_C cholesterol, and triglycerides in the fasting state(7).

\[
LDL = \text{total cholesterol} - \left( \frac{\text{HDL} + \text{TG}}{5} \right)
\]

Although this method is routinely used and convenient for clinical practice, it is not recommended for use in non-fasting blood samples or in the presence of hypertriglyceridemia (>4.52mmol/L or 400 mg/dl) or type 3 hyperlipoproteinemia (8).

For these reasons, an expert panel of National Cholesterol Education Program (NCEP) in 1995 recommended the development of direct methods for the measurement of LDL_C (9).

In addition, as the Friedewald formula of LDL_C requires 3 primary measurements (total cholesterol HDL_C cholesterol and triglycerides), it potentially decreases the accuracy and the precision of the derived cholesterol concentration (10).

Although direct assays are currently used in clinical laboratories, these assays were only evaluated in small cross-sectional or retrospective studies, with scarce information regarding the association of LDL_C directly with Friedewald calculation in relation with clinical events(11).
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The aim of this study to evaluate the association of baseline LDL_C concentrations as determined by a direct assay with those calculated using Friedewald equation in a group of healthy Iraqi population.

**Subjects & Methods**

A cross-sectional survey for LDL-cholesterol using serum lipid profiles in an apparently healthy adult population in Mosul city, northern Iraq. The study was carried out during October 2009 - April 2010. The subjects included 157 individuals 20-70 years, (mean SD) 41.2±13.8 year, 40 year median). These subjects were apparently healthy volunteers who were divided into 3 groups. The first was composed of 70 individuals 21-70 years, (38.2±13.9, 40 years). They were from the staff of the Mosul College of Medicine, Mosul, Iraq, and from the author's relatives and friends. The second group was composed of 30 individuals 22-68 years (44.2+13, 43 years). These individuals were accompanying the inpatients in the surgical wards of Al-Zahrawi Hospital, Mosul, Iraq. The third group was composed of 57 individuals, 20-40 years (34.0+14.5 years). They were blood donors who were attending the Blood Bank of Al-Zahrawi Hospital.

All subjects were asked to answer a questionnaire, and assessed for the presence or absence of personal or family history of coronary heart disease, diabetes mellitus, hypertension, renal diseases, and features of hyperlipidemia such as lipemia retinalis, tendon xanthoma, or xanthelasma.

Their weight and height were recorded. The ethical approval was obtained subjects' consent according to the recommendations of the scientific committee of Mosul Medical College and Nineveh Province Health Sector, Ministry of Health, Iraq.

Classification of hyperlipidemia according to the cutoff values of different components of serum lipid profile. The prevalence of dyslipidemia, involving one or more of the components of serum lipid profile was calculated. In the first classification, the individual lipid parameter was considered to be abnormal or indicative of dyslipidemic state, according to the recommendations by the NCEP III, at the following cut-off levels:

- a. TG>150mg/dl(2.0mmol/L).
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b. LDL-C >100 mg/dl (2.6 mmol/L).
c. HDL-C <40 mg/dl (1.04 mmol/L).
d. Non-HDL-C >130 mg/dl (3.37 mmol/L).

A second classification was made, according to the recommendations by the BHA, and lipoprotein ratios or indices according to the recommendations of the Joint British Society in terms of risk assessment at the following cut-off levels (12,13):

a. TG >180 mg/dl (2.4 mmol/L).
b. TC >194 mg/dl (5.0 mmol/L).
c. LDL-C >116 mg/dl (3.0 mmol/L).
d. HDL-C <45 mg/dl (1.15 mmol/L).
e. TC-HDL-C >5.0.
f. LDL-C-HDL-C 22.5.
g. TG-HDL-C >3.0.

A venous blood specimen was collected from every subject from 8-10 in the morning, following an overnight fast for not less than 10-12 hours. Measurements of serum lipid components were made and in addition, a number of indices for certain lipid parameters were calculated or derived from the measured values (13). The overall components include the measured parameters (TG, TC, LDL-C and HDL-C). Serum TG and TC were measured by enzymatic methods using kits from bioMerieux (France) (14,15). Serum HDL-C was measured following the precipitation of the apoprotein-B containing chylomicrons and lipoproteins of very low-density lipoprotein and LDL, by phosphotungstic acid in the presence of magnesium ions (16). The supernatant obtained after centrifugation that contains HDL was determined using the cholesterol enzymatic reagents from bio-Merieux (France). Serum LDL-C is calculated by the Friedewald formula (17) using TC, HDL-C, and TG values as:

\[ \text{LDL-C (mg/dl)} = \text{TC-HDL-C (TGx0.2)} \]

or

\[ \text{LDL-C (mmol/L)} = \text{TC-HDL-C (TGx0.455)} \]

and measured by using kit direct immuno-inhibition method. Serum non HDL-C is calculated by subtracting HDL-C value from TC value as recommended by the NCEP III (18). Certain indicators or ratios of lipid profile parameters are calculated by dividing the corresponding value of lipid components. This includes TC-HDL-C (atherogenic index), LDL-
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HDL-C, and TG: HDL-C. All these biochemical analyses were performed in the Clinical Chemistry Laboratory, Department of Biochemistry, College of Medicine, University of Mosul, Iraq.

The statistical methods used included the standard statistical methods of the mean, medium, SD, and range (minimum-maximum). Unpaired Z test was used for comparisons with a statistically significant difference considered to be present at P 0.001.

**Results**

As shown in Table (1), the mean (SD) age of study participants at baseline was 49 years. Fasting blood samples were available in 157 participants.

Compared with fasting Friedwald LDL-C (Table 2), mean concentrations of direct fasting LDL-C and HDL-C with LDL-C & TCH with LDL-C lastly TCH with HDL.

Mean fasting Friedwald LDL-C 130 ± 35 was higher than fasting calculated LDLD by 5.6 mg/dl, the mean fasting HDL-C was 46.6 mg/dl, the mean fasting TG was 198 mg/dl, the mean fasting TCH was 217 mg/dl.

Difference between fasting concentration of direct and Friedewald LDL 1.95, P< 0.52.

Fasting direct LDL-C correlated highly with fasting LDL-C in a linear manner (Pearson's correlation) coefficient r= 0.568, P < 0.001 (Fig 2).

Fasting direct LDL-C also correlated highly with fasting HDL-C (Fig1)

r= 0.014, P= 0.859.

The difference between fasting concentration of direct and Friedewald LDL-C is plotted against their mean in the Bland-Altman graph.

**Discussion**

The direct method used in this study correlated highly with Friedewald formula ,The lower LDL-C concentration measured by this direct assay may misclassify a substantial proportion of individuals into a lower NCEP risk category . Several direct method for measuring LDL-C are currently available, but there are few data evaluating their predictive performance in relation to clinical events. Potential advantage of direct measurement LDL-C are believed to be better precision of the assay owing
to the single measurement and relative lack of effect of the presence of increased triglyceride concentration.

Some studies have shown direct assays to be general accurate compared to the B-quantification reference method or the Friedewald formula, however other studies have questioned the specificity of direct assays and their ability to meet the NCEP goal for a total error of 12 (19).

In addition clinical trials demonstrating the benefit of LDL-C lowering with statin therapy have used Friedewald formula for determining LDL-C concentration with the exception of the heart protection study, which used a direct assay. The present study finding demonstrate no clear advantage for using a direct assay for LDL-C compared with Friedewald formula. Moreover LDL-C concentration with the direct assay used in this study was approximately (5-10mg/dl) lower than by Friedewald calculation. Although small, this systematic difference in mean LDL-C concentration may be clinically important when NCEP risk categories are used to assess the need for drug intervention in a particular individual (20).

There are several possible limitation of the present study lipid measurement could not be corrected for potential regression dilution bias. Although we assesses only the direct Roche method, this assay commonly used and commercially available in the us. The present study included healthcare professionals who were man & women, mostly white apparently healthy and recruited from a variety of geographic locations across the us, thus it is unclear if our result would be applicable to other ethnic populations or men (21).

Time to last meal was self-reported, and we did not have paired samples of fasting measurement in the same individuals. Finally, this was a primary prevention population and further studies are needed before the data can be extended to secondary prevention population that are frequently treated with lipid lowering medication (22).

Strengths of the present study include the large number of healthy man & women participants from whom
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Simultaneous concentrations of direct Friedewald LDL-C were obtained. Additionally all lipid measurement were performed at a core laboratory facility that is certified for lipid testing by the national heart, lung and blood institute centres for disease control and prevention lipid standardization program. Detailed information on cardiovascular risk factor was available allowing for analysis by the presence or absence of these factor such as fasting status (22).

The present study conclude that:

The direct assay used in this study correlated highly with Friedewald calculation but was 5-10 mg/dl the lower LDL-C concentration by direct assay may misclassify a substantial proportion of individuals into a lower NCEP risk category. Although the association of LDL-C with LDL-D by the 2 methods was nearly identical in fasting samples.

References


6. Brown BJ, Albers JJ, Fisher LD. Regression of coronary artery disease as a result of intensive lipid lowering therapy in men with high


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Fig (1): Correlation between HDL-cholesterol values and direct LDL-cholesterol.

Fig (2): Correlation between LDL-Cholesterol values (Friedewald calculation) and direct immunoinhibition (IIH) methods.
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Table (1) : Show the mean &SD of age .TG,TCH,HDL,LDL1 &LDLD

<table>
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<th>Range</th>
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<td>N=157</td>
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<tr>
<td>Age</td>
<td>49.6 ± 7.80</td>
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<td>(25-70)</td>
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<tr>
<td>TG</td>
<td>198.9 ± 104</td>
<td>163</td>
<td>55-355</td>
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<tr>
<td>TCH</td>
<td>217 ± 34</td>
<td>214</td>
<td>136-300</td>
</tr>
<tr>
<td>HDL</td>
<td>46.4 ± 7.34</td>
<td>45</td>
<td>35-80</td>
</tr>
<tr>
<td>LDL1 Friedewalde</td>
<td>130 ± 35.2</td>
<td>132.7</td>
<td>40-262</td>
</tr>
<tr>
<td>LDLD Calculated</td>
<td>127 ± 32.4</td>
<td>128</td>
<td>40-206</td>
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Table (2) Show serum concentration of LDL1-LDLL, HDL-LDLL, TCH-LDLL & TCH-HDL.

<table>
<thead>
<tr>
<th></th>
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<td>N=157</td>
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<tr>
<td>LDL1- LDLLD</td>
<td>5.4 ± 34.4</td>
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<td>HDL-LDLL</td>
<td>-76.4 ± 33.7</td>
<td>-28.3</td>
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<tr>
<td>TCH-LDLL</td>
<td>89.05 ± 39.7</td>
<td>27.9</td>
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<tr>
<td>TCH-HDL</td>
<td>165.5 ± 40</td>
<td>51.67</td>
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الخلاصه:

الهدف:

تقييم العلاقة بين تركيز الكوليسترول خفيف الكثافة بالطريقة المباشرة مع طريقة قياسه عن طريق حساب المعادلة.

طرق أجراء البحث والمشاركين:

الدراسة أجريت في مدينة الموصل خلال الفترة من شرين الثاني ٢٠٠٩ إلى نيسان ٢٠١٠. تم الحصول على عينات الدم وزيادة من كافه المتطوعين المشتركين في الدراسة بين الساعه ٨:٠٠ صباحاً إلى ١٠:٠٠ صباحاً.

تم قياس واجهه شحوم الدم بعد ١٢ ساعة في حاله الصوم تضمنت الكوليسترول الكلي، الشحم الثلاثي، الكوليسترول فريغ الكثافة، الكوليسترول خفيف الكثافة (بطريقة الحساب بالمعادلة)، الكوليسترول خفيف الكثافة، طريقه التثبيط المناعي، كل التحليلات الراضيه أجريت في مختبر الكيمياء السريريه لقسم الكيمياء الحياتيه في كلية الطب جامعه الموصل.

النتائج:

أظهرت النتائج ارتباط إيجابي بين طريقة قياس الكوليسترول خفيف الكثافة بطريقة الحساب بالمعادلة بالمقارنة مع طريقه قياس الكوليسترول خفيف الكثافة بطريقة التثبيط المناعي. هناك ارتباط موجب بين قياس الكوليسترول خفيف الكثافة والكوليسترول فريغ الكثافة.

الخلاصة:

الكوليسترول خفيف الكثافة بطريقة التثبيط المناعي ربط بقياس الكوليسترول خفيف الكثافة بطريقة الحساب بالمعادلة لكن كانت ٥٠ ملغ/ديسيتر تركز الكوليسترول خفيف الكثافة الاوطا بطريقة التثبيط المناعي لذا من يمكن أن نستعمل طريقه الحساب بالمعادلة نظرا لا يوجد اختلاف كبير بالإضافة الي كونه أقل كلفه من الأول.