Variability in calculating non-HDL atherogenic lipoprotein in reference to atherogenic index in type 2 diabetes

Maysoon Al-Haideri *

Abstract

Background and objective: Diabetes mellitus is a disorder that is often associated with cardiovascular diseases and underlying lipid abnormalities. The aim of this study was to calculate the serum level of LDL indirectly, using different equations in type 2 diabetes patients in an attempt to focus on the variation of estimating level which reflected on the decision to prescribe lipid lowering agents.

Methods: A total of 70 patients with type 2 diabetes using oral hypoglycemic agents alone and/or once- or twice-daily insulin, their non-HDL atherogenic lipoprotein in reference to atherogenic index were conducted in Martyr Layla Qasm Center for Diabetes Mellitus in Erbil, Iraq, during the period from June, 2011 to January, 2013.

Results: Age of type 2 diabetes patients ranged from 29 to 82 years with a mean age of 56.6 years with duration of disease ranged between 1.2-39 years. Results revealed that the mean fasting serum glucose and glycosylated hemoglobin were 181.9 mg/dl and 8.428%, respectively. The mean value of serum triglycerides was 171.5 mg/dl which is higher than the cut-off normal value of 150 mg/dl. Results showed significant correlation between atherogenic index and calculated atherogenic lipoprotein and significant correlation between atherogenic index and waist circumference as an indicator of central obesity.

Conclusion: The mean body mass index value indicated that the patients were obese and the mean value of waist circumference did not reach the cut-off level of central obesity. The mean value of atherogenic index indicated that the patients were at increased risk of cardiovascular events. Estimation of LDL value from the direct measurement of lipid profile in type 2 diabetes with high serum triglyceride level is not a reliable method.

Keywords: type 2 diabetes, atherogenic index.

Introduction

Diabetes and its complications are major health-care burden worldwide. Dyslipidaemia is frequently present in type 2 diabetes (T2D) in which its predominant features include increased flux of free fatty acids (FFA), high triglyceride (TG) and low high density lipoprotein cholesterol (HDL-c) levels, a predominance of small, dense (atherogenic) low density lipoprotein cholesterol (LDL) particles, raised apolipoprotein (apo) B values and postprandial hyperlipidaemia may also be present. Atherogenic lipid triad; high serum TG levels, low serum HDL-c levels and a preponderance of small dense LDL-c particles are well seen in T2D complicated with insulin resistance and/or the metabolic syndrome. In T2D, LDL particles are more likely to be vulnerable to oxidation process, an early process of atherosclerosis, due to the impairment of antioxidant potential of LDL particles. Therefore, direct measurement of LDL particles in T2D may give a spurious result. Also the analytical methods that used in measurement of small dense particles like, ultracentrifugation, nuclear magnetic resonance (NMR) spectroscopy and gradient-gel electrophoresis are laborious and expensive for general clinical use. Some authors believe to determine the non-HDL concentration or...
the apo-lipoprotein B instead of using the real LDL level in initiating the management of dyslipidemia because the precipitation methods that used in determination of HDL and LDL are not accurate. The aim of this study was to calculate the serum level of LDL indirectly, using different equations in T2D patients in an attempt to focus on the variation of estimating level which reflected on the decision to prescribe lipid lowering agents.

Methods

This observational study was conducted in Martyr Layla Qasm Center for Diabetes Mellitus in Erbil, Iraq. The study was conducted according to the guidelines from the Declaration of Helsinki with approval from a local ethical review board. All patients gave written informed consent. The entry criteria included patients with T2D using oral hypoglycemic agents alone and/or once- or twice-daily insulin. The present study excluded the patients with a history of familial hyperlipidemia, recent cardiovascular events or patients on lipid lowering agents. Anthropometric determinants including height (m), weight (kg) and waist circumference (cm) were measured. Body mass index (BMI) and waist/height ratio were calculated. Peripheral venous blood was drawn immediately after admission into tubes, then the samples were centrifuged at 2500 rpm for 10 min, and the sera were separated for determination of fasting serum glucose, HbA\textsubscript{1c} (%) and lipid profile. The determinants of lipid profile included fasting serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). The atherogenic index was calculated using the following formula:

\[
\text{Atherogenic index} = \left( \log \frac{\text{TG}}{\text{HDL}} \right)
\]

The patients were categorized according to the value of atherogenic index into:
- Low risk < 0.11
- Intermediate risk 0.11-0.2
- Increased risk > 0.2

The following formulas used for calculating non-HDL or LDL or atherogenic lipoprotein:

1. Friedewald equation:
   \[
   \text{LDL} = \text{Total cholesterol} - [\text{HDL} + (\frac{\text{TG}}{5})]
   \]

2. Non-HDL = TC - HDL

3. Equation 1:
   \[
   \text{Determined-LDL} = 0.98 \text{ TC} - 0.84 \text{ HDL} - 0.12 \text{ TG} + 0.056 \text{ age} + 0.071 \text{ BMI}
   \]

4. Equation 2:
   \[
   \text{Determined-LDL} = 0.98 (\text{ TC} - \text{ HDL}) - 0.12 \text{ TG} + 0.1 \text{ age} + 2.4 \text{ sex} + 0.2 \text{ BMI} \\
   \text{(for male=1 and for female=2)}
   \]

Statistical analysis

Data are expressed as means ± SD or range. Simple correlation, unpaired and paired Student's t-tests were used to evaluate differences in group and between the two groups. For all tests, a two-tailed P ≤0.05 was considered statistically significant. All calculations were made using Excel 2003 program for Windows.

Results

The mean age for the patients was 56.6 years with duration of disease ranged between 1.2-39 years. The mean fasting serum glucose and glycosylated hemoglobin were 181.9 mg/dl and 8.428%, respectively. There were no significant differences between males and females in these variables. The mean body mass index value indicated that the patients were obese and the mean value of waist circumference did not reach the cut-off level of central obesity (Table 1). The mean waist/height ratio as an indicator of increased cardiovascular events approximated 0.6. Table 2 shows non-significant differences in fasting serum lipid profiles between males and females. The mean value of serum triglycerides was 171.5 mg/dl which is higher than the cut-off normal value of 150 mg/dl. Taking the value of calculated LDL by using Friedewald equation as a reference, the atherogenic LDL values were significantly higher than corresponding values using non-HDL equation, determined LDL equation 1 or 2 in both
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also the difference between LDL value determined by equation 1 and 2 reached to significant level. The mean value of atherogenic index indicated that the patients were at increased risk of cardiovascular events.

Table 1: Characteristics and anthropometric measurements of patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male (n=27)</th>
<th>Female (n=43)</th>
<th>Total (70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>58.4±14.9</td>
<td>55.4±14.2</td>
<td>56.6±14.4</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>181.2±7.7</td>
<td>182.3±7.6</td>
<td>181.9±7.6</td>
</tr>
<tr>
<td>HbA1c%</td>
<td>8.563±0.543</td>
<td>8.344±0.626</td>
<td>8.428±0.601</td>
</tr>
<tr>
<td>Duration of diabetes (year)</td>
<td>1.3-39</td>
<td>1.2-37</td>
<td>1.2-39</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>101.55±25.86</td>
<td>96.0±24.84</td>
<td>98.12±25.2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7±0.025</td>
<td>1.659±0.032</td>
<td>1.674±0.035</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>101.88±17.81</td>
<td>98.25±17.5</td>
<td>99.65±17.59</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>35.06±8.73</td>
<td>34.75±8.52</td>
<td>34.87±8.54</td>
</tr>
<tr>
<td>Waist /height ratio</td>
<td>0.599±0.102</td>
<td>0.591±0.101</td>
<td>0.594±0.101</td>
</tr>
</tbody>
</table>

The results are expressed as mean ±SD and range.

Table 2: Fasting serum Lipid profile (mg/dl).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male (n=27)</th>
<th>Female (n=43)</th>
<th>Total (70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>186.1±7.5</td>
<td>186.0±9.2</td>
<td>186.1±8.5</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>173.7±13.2</td>
<td>170.2±11.4</td>
<td>171.5±12.2</td>
</tr>
<tr>
<td>High density lipoprotein (mg/dl)</td>
<td>48.5±2.6</td>
<td>48.65±3.10</td>
<td>48.6±2.9</td>
</tr>
<tr>
<td>Very low density lipoprotein (mg/dl)</td>
<td>34.7±2.6</td>
<td>34.0±2.3</td>
<td>34.3±2.4</td>
</tr>
<tr>
<td>Low density lipoprotein (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Friedewald equation</td>
<td>102.9±7.0</td>
<td>103.4±9.82</td>
<td>103.2±8.8</td>
</tr>
<tr>
<td>Non-HDL equation</td>
<td>137.6±8.3*</td>
<td>137.4±10.5*</td>
<td>137.5±9.6*</td>
</tr>
<tr>
<td>Determined LDL-c equation 1</td>
<td>126.54±7.9*†</td>
<td>126.6±10.4*†</td>
<td>126.6±9.5*†</td>
</tr>
<tr>
<td>Determined LDL-c equation 2</td>
<td>129.3±9.2*††</td>
<td>131.5±11.8*††</td>
<td>130.6±10.9*††</td>
</tr>
<tr>
<td>Atherogenic index (log&lt;sup&gt;TG/HDL&lt;/sup&gt;)</td>
<td>0.553±0.044</td>
<td>0.543±0.047</td>
<td>0.547±0.046</td>
</tr>
</tbody>
</table>

* P < 0.001 in comparison with Friedewald equation, † P < 0.001 in comparison with Non-HDL equation, ‡ P < 0.001 in comparison with Determined LDL-c equation 1.
Figure 1 shows medium strength and significant correlation between atherogenic index and calculated atherogenic lipoprotein and the higher correlation factor observed with LDL calculated by non-HDL equation \((r = 0.598)\). Figure 2 shows significant correlation between atherogenic index and waist circumference as an indicator of central obesity and the higher correlation factor observed with LDL calculated by determined-LDL equation 2 \((r = 0.673)\).

**Figure 1:** Significant correlation between fasting serum LDL and atherogenic index.

**Figure 2:** Significant correlation between fasting serum LDL and waist circumference.
Discussion

The results of this study showed significant differences in the estimation of LDL particles using three different equations compared with the equation of Friedewald which is most commonly used in clinical practice. Such differences may reflect in the using the guidelines of management of dyslipidemia or to identify the people who are at risk of cardiovascular events. Van Deventer et al. found that the risk cardiovascular score was similar for direct and calculated-LDL method in non-hypertriglyceridemia whereas using the equation of non-HDL method showed compatibility with reference measurement procedures for cardiovascular risk score. In this study the mean level of serum triglycerides was high and it exceeded the cut-off limit of metabolic syndrome criteria. Therefore, the calculated LDL by using non-HDL equation may be suitable to identify the patients at cardiovascular disease risk. On the other hand, Yamashita et al. found that estimation of LDL level using Friedewald equation is close to the direct measurement of LDL using different methods; liquid selective detergent method, selective solubilization method, LDL-c, elimination method: LDL-c (E), and enzyme selective protecting method. Moreover, Evans et al. demonstrated that direct and calculated estimation of LDL-c values did not agree with the reference values of ultracentrifugation method and in hypertriglyceridemia the direct method overestimated the actual LDL value whereas the Friedewald calculation underestimated the actual LDL. The results of this study are in agreement with the above mentions studies. Thus, the actual method of determination of LDL i.e. by ultracentrifugation served as a good and reliable method particularly in T2D patients who had high serum triglycerides level. Despite the results of this study showed the correlation between waist circumferences as an anthropometric marker that associated with the cardiovascular event, the limitation of this study is the scoring of cardiovascular event in respect to the estimated LDL level by different equations.

Conclusion

The estimation of LDL value from the direct measurement of lipid profile in T2D with high serum triglyceride level is not a reliable method.

Conflicts of interest

The author reports no conflicts of interest.

References


