ระดับความเข้มข้นของ Non-High Density Lipoprotein Cholesterol และ Apolipoprotein B ในผู้ป่วยโรคเบาหวานชนิดที่ 2

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บทคัดย่อ

ผู้ป่วยเบาหวานชนิดที่ 2 มักมีภาวะระดับolesterol ระดับความเข้มข้นของ non-high density lipoprotein cholesterol (non-HDLc) และ apolipoprotein B (ApoB) สามารถสะท้อนให้เห็นถึงความเสี่ยงของการเกิดโรคหัวใจขาดเลือด งานวิจัยนี้พบว่าความเข้มข้นของ non-HDLc และ ApoB เพิ่มสูงขึ้นในผู้ป่วยเบาหวานชนิดที่ 2 มากกว่า กลุ่มควบคุมที่ไม่เป็นโรคเบาหวานอย่างมีนัยสำคัญทางสถิติ และพบว่า ระดับความเข้มข้นของ Non-HDLc มีความสัมพันธ์กับความเข้มข้นของ ApoB อย่างมีนัยสำคัญทางสถิติ (r = 0.91, p < 0.001) คณะผู้วิจัยสามารถวิเคราะห์ความเข้มข้นประมาณของ ApoB (eApoB) ได้จาก non-HDLc โดยใช้สมการถดถอยเชิงเส้น และใช้การแสดงพื้นที่ใต้กราฟของ receiver operating characteristic curve ซึ่งพบว่า ApoB อยู่ในพื้นที่ใต้กราฟได้ดีกว่า Non-HDLc แสดงว่า ApoB เป็นตัวบ่งชี้ความเสี่ยงต่อภาวะโรคหัวใจและหลอดเลือดในผู้ป่วยเบาหวานชนิดที่ 2 ได้ดีกว่า Non-HDLc ความเข้มข้นของทั้ง Non-HDLc และ ApoB สามารถใช้ประเมินความเสี่ยงต่อภาวะโรคหัวใจและหลอดเลือดได้เฉพาะในผู้ที่มีความเข้มข้นของไตรกลีเซอไรด์สูง และเกินขั้นสูง ซึ่งมักพบในผู้ป่วยเบาหวานชนิดที่ 2 ความเข้มข้นของ ApoB นี้มีประสิทธิภาพในการประเมินภาวะเสี่ยงต่อโรคหัวใจและหลอดเลือดในผู้ป่วยเบาหวานชนิดที่ 2 ได้ดีกว่าความเข้มข้นของ non-HDLc สมการถดถอยเชิงเส้นที่เสนอโดยคณะผู้วิจัยจะมีประโยชน์ในการประมาณความเข้มข้นของ ApoB จาก non-HDLc ในผู้ป่วยเบาหวานชนิดที่ 2 ได้

คำสำคัญ: Non–HDL–Cholesterol, Apolipoprotein B, ปัจจัยเสี่ยงต่อโรคหัวใจและหลอดเลือด, โรคเบาหวานชนิดที่ 2

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Non-High Density Lipoprotein-Cholesterol and Apolipoprotein B Concentration in Type 2 Diabetes Mellitus Patients

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Abstract

Dyslipidemia is common in type 2 diabetes mellitus (T2D) and associated with vascular complications. Non-HDL-cholesterol (non-HDLc) and apolipoprotein B (ApoB) concentration reflects the number of atherogenic particles, increasing in concentration is associated with increased risk of coronary heart disease. This study demonstrated that non-HDLc and ApoB concentrations were considerably higher in T2D than in non-T2D controls ($p < 0.05$). Spearman’s correlation for non-HDLc concentration was significantly correlated with ApoB concentration ($r = 0.91$, $p < 0.001$). ApoB concentration was also estimated from non-HDLc concentration by using a proposed simple linear regression equation. In receiver operating characteristic (ROC) curve, ApoB showed greater area under the curve (AUC) than non-HDLc, indicating ApoB as the better cardiovascular risk marker for T2D. Non-HDLc and ApoB were suitable for estimating cardiovascular risk in subjects with hypertriglyceridaemia, as frequently observed in T2D patients. However, ApoB was more effective to estimate cardiovascular risk in T2D than non-HDLc. The proposed simple linear regression equation may be useful for estimating ApoB concentration from non-HDLc in T2D patients.

Key words: Non-HDL-Cholesterol, Apolipoprotein B, Cardiovascular Risk Factor, Type 2 Diabetes Mellitus

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Introduction

Type 2 diabetes mellitus (T2D) is associated with risk for cardiovascular diseases,\(^1,^2\) which may be due in part to the abnormality in lipid and lipoprotein metabolism.\(^3,^4\) T2D patients may show combined symptoms of dyslipidemia characterized by elevated triglycerides (TG), decreased high-density lipoprotein cholesterol (HDL-C), small dense LDL-particles (independent of low-density lipoprotein cholesterol (LDL-C)), elevated triglyceride-rich remnant lipoproteins (TGRLs), and/or elevated apolipoprotein B (Apo B) concentrations.\(^5\) Although LDL-C levels are the main therapeutic targets in diabetic and non-diabetic dyslipidemia,\(^6\) their concentrations do not equal the entire mass of lipoprotein particles, which also includes intermediate-density lipoprotein (IDL) and very low density lipoproteins (VLDL), for which atherogenicity has been demonstrated.\(^7\) Thus, the sole measurement of LDL-C may underestimate the risk associated with atherogenic lipoproteins. The assessment of atherosclerotic risk in patients with T2D requires careful lipid screening, along with screening for other risk markers.

The advantage of non-HDL-cholesterol (non-HDLc) as an index of risk associated with dyslipidemia lies in the fact that non-HDLc is simply defined as the difference between total and HDL-C, and represents cholesterol carried on all the potentially pro-atherogenic ApoB-containing particles. The ApoB level reflects the particle number, thus accounting for not only remnant and LDL-particles, but also the density of particles when expressed in relation to particle cholesterol content. The measurement of Apo B has been advocated as an alternative index.\(^8\) High ApoB concentration is associated with increased cardiovascular disease (CVD), independent of LDL-C levels.\(^9,^10\)

The aim of this study was to investigate the serum concentrations of non-HDLc and ApoB as cardiovascular risk markers in T2D patients, as well as their association with other traditional cardiovascular risk markers (glucose (Glu), total cholesterol (TC), triglyceride (TG), HDL-C, LDL-C), and to compare the power of ApoB and non-HDLc as markers in T2D subjects.\(^11\) The association of ApoB and non-HDLc concentrations was also evaluated.

Materials and Methods

Subjects

A total of 55 patients with T2D (15 males, 40 females) with a median age of 68.9 years (interquartile range 62-76 years) were selected at random from the Diabetes Care Unit, Uttaradit Hospital, Uttaradit Province. A control group of 40 subjects (14 males, 26 females) with a median age of 64.5 years (interquartile range 57.8-72.7 years) was established from healthy volunteers,
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as based on their medical histories and a physical examination. Diabetes group was diagnosed by the World Health Organization (WHO) criteria.\(^{(12)}\) Participants had diabetes on the basis of clinical diagnosis, diabetes medication used, or fasting glucose \(\geq 126\) mg/dL or at least 200 mg/dL in two hours after an oral glucose tolerance test.\(^{(12)}\) Written informed consent was obtained from all participants and the study protocol was approved by the Ethics Committee of Naresuan University.

**Blood sampling and biochemical determinations**

Venous blood samples were taken without stasis after a 8-12 hour fast. Fasting plasma Glu, serum TC and TG were measured immediately after collection by enzymatic procedures using a Hitachi 912 auto-analyzer (Roche Diagnostic, Switzerland). HDL-C was measured by a direct method using polyethylene-glycol-pretreated enzymes (Roche Diagnostic, Switzerland). LDL-C concentration was calculated according to Friedewald’s formula\(^{(13)}\) and direct measurement of LDL-C was used if TG > 400 mg/dL. ApoB was measured by an immunoturbidimetric method (Roche Diagnostic, Switzerland). Non-HDLc was calculated by subtracting HDL-C from TC.

**Statistical analysis**

Analysis was performed using SPSS version 13.0 (SPSS, Chicago, IL). The variables were expressed as median and interquartile range (Q1-Q3). Mann-Whitney U tests were used to estimate differences between groups. The Spearman’s rank correlation was used to assess the correlation of non-HDLc with ApoB concentrations. The ApoB concentration was also estimated from non-HDLc in T2D patients by using a proposed simple linear regression equation. Both ApoB and non-HDLc were analyzed in terms of receiver operating characteristic (ROC) curves, via calculating the areas under the curves (AUC). In general, an area under the ROC of 0.5 suggests no discrimination, whereas a maximal ROC of 1 corresponds to outstanding discrimination.\(^{(10)}\) Tests were two tailed, and a \(p\)-value < 0.05 was considered significant.

**Results**

None of the 55 T2D patients included in the study were on insulin treatment. Of the T2D patients, 35 (63.6%) were listed as elevated ApoB (\(\geq 90.0\) mg/dL), 38 (69.1%) elevated non-HDLc (\(\geq 130.0\) mg/dL), 34 (61.8%) elevated both ApoB/non-HDLc, 23 (41.8%) elevated ApoB/hypertriglyceridemia (\(\geq 150.0\) mg/dL or 1.69 mmol/L), 12 (21.8%) elevated ApoB/normal TG (\(< 150.0\) mg/dL), 24 (43.6%) elevated non-HDLc/hypertriglyceridemia (\(\geq 150.0\) mg/dL) and 14 (25.5%) elevated non-HDLc/normal TG (\(< 150.0\) mg/dL). The general characteristics and biochemical variables of
the T2D patients and control subjects are shown in Table 1. Non-HDLc, ApoB and lipid profiles were significantly higher in T2D than in control subjects ($p < 0.05$). ApoB was significantly correlated with non-HDLc ($r = 0.91, p < 0.001$) in T2D patients. A simple linear regression equation to estimate the concentration of ApoB from non-HDLc in T2D patients (Fig. 2) was proposed as follows:

$$\text{ApoB (mg/dL)} = 21.35 + 0.52 \times \text{[non-HDLc (mg/dL)]} \quad (I)$$

<table>
<thead>
<tr>
<th>Variables</th>
<th>T2D patients ($n = 55$)</th>
<th>Healthy Controls ($n = 40$)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference (cm)</td>
<td>89.2*</td>
<td>83.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Q1-Q3</td>
<td>85.0-94.2**</td>
<td>78-89</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>118.5</td>
<td>80.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Q1-Q3</td>
<td>95.8-147.5</td>
<td>76.0-90.0</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>202.3</td>
<td>177.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Q1-Q3</td>
<td>185.7-232.2</td>
<td>165.1-193.4</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>170.8</td>
<td>125.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Q1-Q3</td>
<td>123.0-344.2</td>
<td>100.9-170.8</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>45.6</td>
<td>59.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Q1-Q3</td>
<td>37.8-52.1</td>
<td>52.1-67.9</td>
<td></td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>114.3</td>
<td>92.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Q1-Q3</td>
<td>90.2-134.0</td>
<td>78.6-101.5</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dL)</td>
<td>107.0</td>
<td>72.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Q1-Q3</td>
<td>82.0-123.0</td>
<td>65.0-87.0</td>
<td></td>
</tr>
<tr>
<td>Non-HDLc (mg/dL)</td>
<td>160.3</td>
<td>119.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Q1-Q3</td>
<td>129.7-186.7</td>
<td>105.3-131.3</td>
<td></td>
</tr>
</tbody>
</table>

*medians; ** interquartile ranges (Q1-Q3); HDL-C high density lipoprotein cholesterol; LDL-C low density lipoprotein cholesterol; non-HDLc non high density lipoprotein cholesterol
The regression equation (I) was used to calculate the estimated ApoB (eApoB) concentration in T2D patients. The correlation of both assayed ApoB and eApoB was also tested in another group of both type 2 diabetes and non-diabetic subjects. The results were highly correlated ($r = 0.896$, $p < 0.001$) as shown in Fig. 3.

The ROC analysis showed that the AUC of ApoB was greater than those of non-HDLc and LDL-C; the respective probabilities for being associated with the cardiovascular risk markers in T2D patients were 0.825 (95% CI, 0.739-0.912), 0.799 (95% CI, 0.705-0.894), and 0.720 (95% CI, 0.613-0.827) (Table 2 and Fig. 1). The preliminary cut-off of ApoB and non-HDLc values were also proposed. The ApoB cut-off of 90.0 mg/dL had the highest Youden index for detection of cardiovascular risk, with a sensitivity of 70% and a specificity of 80%. The non-HDLc cut-off of 130.0 mg/dL was used for detection of cardiovascular risk, with sensitivity of 74% and a specificity of 72.5%.

**Discussion**

The present study demonstrated that non-HDLc and ApoB were elevated in T2D patients. Both non-HDLc and ApoB were strongly associated with CVD risk ($r = 0.91$) and were better predictors of CVD risk than LDL-C in type 2 diabetes patients.\(^{14, 15}\) In recent study, both non-HDLc and ApoB were used as predictive markers for risk of coronary heart disease.\(^{16}\) Non-HDLc and ApoB may be superior to LDL-C for diagnostic tests of diabetic patients for several reasons: (i) Diabetes is often concomitant with many lipid and lipoprotein abnormalities characterized mainly by elevated VLDL, IDL, and chylomicrons. (ii) The LDL-C level is usually calculated from Friedewald’s equation that requires a fasting

<table>
<thead>
<tr>
<th>Test result variables</th>
<th>Area under the ROC curve ± S.E. (Asymptotic 95% Confidence Interval)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apolipoprotein B</td>
<td>0.825 ± 0.044 (0.739-0.912)</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>Non-HDLc</td>
<td>0.799 ± 0.048 (0.705-0.894)</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.720 ± 0.055 (0.613-0.827)</td>
<td>$&lt; 0.001$</td>
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</tbody>
</table>

S.E. standard error; non-HDLc non high density lipoprotein cholesterol; LDL-C low density lipoprotein cholesterol
TG level < 400 mg/dL (4.52 mmol/L) to accurately calculate the LDL-C level.\textsuperscript{(17)} ApoB reflects the total particle number in these atherogenic lipoproteins, but non-HDLc provides the total cholesterol content of LDL, IDL, and VLDL.\textsuperscript{(17)} Thus, for diabetic patients with combined dyslipidemia, calculated LDL-C fails to be an adequate index of overall lipid-associated risk. Almost all routine determination of LDL-C is not a direct measurement, but rather a calculation based on a measurement of TC, HDL-C, and TG. In fact, the nature of the calculation is to exclude the cholesterol of TGRLs, which are atherogenic particles. The calculated LDL-C concentration has been shown to be significantly different from a direct LDL-C determination by ultracentrifugation in type 2 diabetic patients.\textsuperscript{(18)} The only use of LDL-C in clinical diagnosis ignores the significant contribution of atherogenic VLDL and IDL-C particles. Both non-HDLc and ApoB in our study were superior to LDL-C; thus ApoB and/or non-HDLc may be the primary lipid markers for patients with T2D. Several epidemiological studies have shown that both non-HDLc and ApoB are better predictors for cardiovascular

![ROC Curve](image)

Fig. 1  ROC curve for apo B, non-HDLc, and LDL-C. The areas under the ROC curves were 0.825, 0.799, 0.720 for CHD risk with ApoB, non-HDLc, and LDL-C in type 2 diabetes. ApoB apolipoproteinB; non-HDLc non high density lipoprotein cholesterol; LDL-C low density lipoprotein cholesterol; ROC receiver-operating characteristic; CHD coronary heart disease
events than LDL-C.\textsuperscript{(19-21)} Moreover, increased ApoB is related well to an increased risk of coronary artery disease.\textsuperscript{(19, 22)} ApoB is a better predictor of coronary heart disease (CHD) than non-HDLc and LDL-C.\textsuperscript{(23, 24)} As in our study, ApoB showed the greatest AUC of the ROC curve compared with non-HDLc and LDL-C. ApoB was highly correlated with non-HDLc, and had the same cut-off value (90.0 mg/dL) as reported by Simon \textit{et al.}\textsuperscript{(24)} Wagner \textit{et al.}\textsuperscript{(25)} concluded that both elevated ApoB/hypertriglyceridemia and elevated non-HDLc/hypertriglyceridemia are phenotypes with a predominance of small dense LDL particles, ApoB and non-HDLc seem to be equivalent in hypertriglyceridemic patients ($r = 0.91$).

In fact, non-HDLc is cheap and easy to be calculated therefore it is used as a first line component to evaluate diabetic dyslipidemia. ApoB could identify patients at risk in mild hypertriglyceridemic groups, but it is associated with additional cost. Thus, estimated ApoB concentrations obtained from our proposed simple linear regression equation from non-HDLc could help physicians to use both non-HDLc and ApoB levels for lipid lowering and markers for CHD risk events.
The obvious limitation of our preliminary study was that it was cross-sectional, conducted on examinees from a single hospital and small sample size. In our further study, the observation may be confirmed in a larger population.

**Conclusion**

Non–HDLc and apoB were suitable for estimating cardiovascular risk in subjects with hypertriglyceridaemia, as frequently observed in T2D patients. ApoB has more potential than non–HDLc to estimate cardiovascular risk in T2D. The proposed simple linear regression equation may be useful for estimating ApoB concentration from non–HDLc in T2D patients.

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