Usefulness of Serum Total Cholesterol/Triglyceride Ratio for Predicting the Presence of Small, Dense LDL

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Objective: We examined the usefulness of the serum total cholesterol (TC)/triglyceride (TG) and LDL-cholesterol (LDL-C)/TG ratios for predicting the presence of small, dense LDL, by comparing them with the established indicators of small, dense LDL, such as the LDL-migration index (LDL-MI) and LDL-C/Apolipoprotein B (ApoB) ratio.

Materials and methods: Fasting serum lipid was analyzed in 99 Japanese hyperlipidemic and normolipidemic subjects (34 males and 65 females, 59.4 ± 11.9 years old). Results: A good negative correlation was observed between LDL-MI and log (TC/TG) (R² = 0.473, p < 0.0001). There was a strong positive correlation between LDL-C/ApoB and log (TC/TG) (R² = 0.665, p < 0.0001). Similar results were obtained using LDL-C instead of TC. Using LDL-MI > 0.4 as an indicator of small, dense LDL, the upper limit of TG was estimated to be 140–142 mg/dl.


Key words: Triglyceride, Cholesterol, Lipoprotein, Small, dense LDL, Hyperlipidemia

Introduction

Lowering the LDL cholesterol (LDL-C) level in order to reduce or prevent coronary artery disease (CAD) progression and cardiac events in hypercholesterolemic subjects is now widely accepted. However, large primary and secondary CAD prevention trials have demonstrated that the reduction of LDL-C levels does not entirely explain the reduction of coronary events associated with lipid-lowering therapy. A meta-analysis showed that triglyceride (TG) concentration is an independent risk factor for cardiovascular disease, and also when adjusted for HDL cholesterol (1). A 1-mmol/l increase of TG was associated with a relative risk of 1.3 for men and 1.8 for women.

Plasma LDL comprises multiple discrete subclasses, differing in size and density. Initial studies conducted by Krauss and Burke (2) demonstrated that two distinct LDL subclass phenotypes can be distinguished on the basis of the LDL particle size distribution separated by gradient gel electrophoresis. Pattern A consists of a major LDL peak of greater than 25.5 nm, which in pattern B is less than 25.5 nm. Epidemiological studies have suggested that a predominance of the small-sized LDL particles, which occurs in pattern B, is highly atherogenic and can become a non-traditional risk factor of CAD. Small, dense LDL has been reported to possess several potentially proatherogenic properties, including reduced receptor-mediated clearance, greater arterial wall retention, and increased susceptibility to oxidation (3, 4).

In a previous study, we classified the lipoprotein profile by polyacrylamide gel electrophoresis into four types (SAND), type S (symmetric), type A (asymmetric), type N (nodular), and type D (disrupted), and we showed that
the SAND classification of the lipoprotein profile may offer a new clinical tool to cover the weak points of the WHO classification, particularly in relation to the presence of a midband (5). We also suggested that the TC/TG ratio may be a simple and useful marker of SAND types. In the present study, we examined the usefulness of serum TC/TG and LDL-C/TG ratios for predicting the presence of small, dense LDL as a simple indicator.

Materials and Methods

Subjects
The blood samples used in the present study were the same as those used in the previous study (5). However, some special cases such as hyper-HDL-cholesterolemia and LCAT deficiency were excluded. A total of 99 Japanese subjects (34 males and 65 females, 59.4 ± 11.9 years old) undergoing medical examinations at the Lipid Clinic in Nakatsugawa Municipal Hospital were enrolled. Normolipidemic subjects (n = 10), and Type IIa (n = 54), Type IIb (n = 29), and Type IV (n = 6) subjects according to the WHO classification of hyperlipidemias were included. The guidelines for the diagnosis and treatment of hyperlipidemias in adults proposed by the Japanese Atherosclerosis Society were used for classifying the subjects, as follows (6): hypercholesterolemia, TC ≥ 220 mg/dl, and hypertriglyceridemia, TG ≥ 150 mg/dl. Blood was drawn from each subject after an overnight fast. All studies were approved by the institutional review board in the Nakatsugawa Municipal Hospital, and written consent was obtained from the participants.

Lipoprotein analysis
TC, TG and HDL-C were determined using the following enzyme assay kits (Kyowa Medex, Tokyo, Japan): Determiner L TCII, Determiner L TGII and Determiner L HDL, respectively. LDL-C was determined by a direct assay kit (Cholestest LDL, Daichi Chemicals, Tokyo, Japan). Apolipoprotein B (ApoB) was measured with a turbidimetric immunoassay kit (Auto N Daichi, Daichi Chemicals). The LipoPhor system (Joko, Tokyo, Japan) was used according to the manufacturer’s instructions. The LDL-migration index (LDL-MI) was calculated as described by Mishima et al. (7) shown that LDL-MI > 0.4 indicates the presence of small LDL particles. When LDL-MI was substituted with 0.40 in the regression formula LDL-MI = 0.422 – 0.117 × log (TC/TG) (Fig. 1b), the corresponding TC/TG value was 1.543. Using LDL-MI > 0.40 as the gold standard, the sensitivity of the TC/TG ratio for predicting the presence of small, dense LDL was calculated as 72.7% and its specificity as 83.6% (Table 2). When TC = 220 mg/dl, TG was 140 mg/dl. Similarly, from the regression formula LDL-MI = 0.399 – 0.101 × log (LDL-C/TG) (Fig. 1c), the corresponding LDL-C/TG ratio was 0.977. When LDL-C = 140 mg/dl, the corresponding TG value was 142 mg/dl.

Results
The lipid profiles of the subjects are summarized in Table 1. A good negative correlation was observed between LDL-C/ApoB and LDL-MI with $R^2 = 0.453$, $p < 0.0001$ (Fig. 1a). There was a good negative correlation between log (TC/TG) and LDL-MI with $R^2 = 0.473$, $p < 0.0001$ (Fig. 1b). The correlation between log (LDL-C/TG) and LDL-MI was also good with $R^2 = 0.456$, $p < 0.0001$ (Fig. 1c). There was a strong positive correlation between log (TC/TG) and LDL-C/ApoB with $R^2 = 0.665$, $p < 0.0001$ (Fig. 1d). The correlation between log (LDL-C/TG) and LDL-C/ApoB was also strong with $R^2 = 0.709$, $p < 0.0001$ (Fig. 1e). A very strong positive correlation was observed between TC/TG and LDL-C/TG with $R^2 = 0.959$, $p < 0.0001$.

Mishima et al. (7) showed that LDL-MI > 0.4 indicates the presence of small LDL particles. When LDL-MI was substituted with 0.40 in the regression formula LDL-MI = 0.422 – 0.117 × log (TC/TG) (Fig. 1b), the corresponding TC/TG value was 1.543. Using LDL-MI > 0.40 as the gold standard, the sensitivity of the TC/TG ratio for predicting the presence of small, dense LDL was calculated as 72.7% and its specificity as 83.6% (Table 2). When TC = 220 mg/dl, TG was 140 mg/dl. Similarly, from the regression formula LDL-MI = 0.399 – 0.101 × log (LDL-C/TG) (Fig. 1c), the corresponding LDL-C/TG ratio was 0.977. When LDL-C = 140 mg/dl, the corresponding TG value was 142 mg/dl.

Table 1. Profile of study subjects.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>34</td>
<td>65</td>
<td>99</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.8 ± 13.4</td>
<td>62.3 ± 9.9</td>
<td>59.4 ± 11.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.73 ± 2.52</td>
<td>23.56 ± 3.1</td>
<td>23.96 ± 2.95</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>234.2 ± 52.4</td>
<td>248.3 ± 42.9</td>
<td>243.4 ± 46.6</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>150.3 ± 42.2</td>
<td>159.7 ± 43.2</td>
<td>156.5 ± 42.9</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>51.4 ± 10.9</td>
<td>63.7 ± 15.5</td>
<td>59.5 ± 15.2</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>216.7 ± 180.9</td>
<td>153.2 ± 95.3</td>
<td>175.0 ± 133.7</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>53.8 ± 13.4</td>
<td>62.3 ± 9.9</td>
<td>59.4 ± 11.9</td>
</tr>
<tr>
<td>LDL-MI</td>
<td>0.41 ± 0.04</td>
<td>0.39 ± 0.04</td>
<td>0.40 ± 0.04</td>
</tr>
</tbody>
</table>

Table 2. TC/TG as a diagnostic test for predicting the presence of small, dense LDL.

<table>
<thead>
<tr>
<th>LDL-MI</th>
<th>&gt; 0.4</th>
<th>≤ 0.4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC/TG</td>
<td>&lt; 1.543</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>≥ 1.543</td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>55</td>
<td>99</td>
</tr>
</tbody>
</table>

Sensitivity: 32/44 = 0.727, Specificity: 46/55 = 0.836
Usefulness of Total Cholesterol/Triglyceride Ratio

a. Correlation between LDL-C/ApoB and LDL-MI.

\[
\text{LDL-MI} = 0.620 - 0.190 \times \log \left( \text{LDL-C/ApoB} \right)
\]
\[R^2 = 0.453, \ p < 0.0001\]

b. Correlation between log(TC/TG) and LDL-MI.

\[
\text{LDL-MI} = 0.422 - 0.117 \times \log \left( \text{TC/TG} \right)
\]
\[R^2 = 0.473, \ p < 0.0001\]

c. Correlation between log(LDL-C/TG) and LDL-MI.

\[
\text{LDL-MI} = 0.399 - 0.101 \times \log \left( \text{LDL-C/TG} \right)
\]
\[R^2 = 0.456, \ p < 0.0001\]

d. Correlation between log(TC/TG) and LDL-C/ApoB.

\[
\text{LDL-C/ApoB} = 1.073 + 0.490 \times \log \left( \text{TC/TG} \right)
\]
\[R^2 = 0.655, \ p < 0.0001\]

e. Correlation between log(LDL-C/TG) and LDL-C/ApoB.

\[
\text{LDL-C/ApoB} = 1.171 + 0.446 \times \log \left( \text{LDL-C/TG} \right)
\]
\[R^2 = 0.709, \ p < 0.0001\]

Fig. 1. Linear regression analysis.
Discussion

In Western countries in which coronary artery disease is the leading cause of death, a reduction in serum cholesterol has been considered to be important in the treatment and prevention of coronary artery disease. In Japan, the Japan Atherosclerosis Society Consensus Conference in 1987 proposed a serum TC level of 220 mg/dl, a serum TG level of 150 mg/dl, and an HDL-C level of 40 mg/dl as the levels for initiating treatment. Then, to establish a guideline for the treatment of hyperlipidemias based on the accumulated evidence in Japan, the working committee of the Japan Atherosclerosis Society proposed the Guidelines for Diagnosis and Treatment of Hyperlipidemias in 1997 (8). New guidelines from the Japan Atherosclerosis Society were announced in 2002. The Japan Atherosclerosis Society determined the criteria of hyperlipidemias, using these results as the basis. However, compared with the detailed criteria for the treatment of hypercholesterolemia, hypertriglyceridemia was defined simply as TG less than 150 mg/dl (6).

Evidence is accumulating that the risk for CHD can be reduced further than it is reduced by LDL-lowering therapy alone, through the modification of other risk factors. One potential secondary target of therapy is the metabolic syndrome, which represents a constellation of lipid and nonlipid risk factors of metabolic origin. The National Cholesterol Education Program Adult Treatment Panel III (NCEP III) Guidelines published in 2001 define the metabolic syndrome as a new secondary target for cardiovascular risk reduction therapy beyond LDL cholesterol-lowering (9). One of the major features of the guidelines is that they identify individuals with multiple metabolic risk factors (metabolic syndrome) as candidates for intensified therapeutic lifestyle changes. The atherogenic lipid profile associated with the metabolic syndrome consists of the following: increased apo B, plasma TG, and LDL levels; reduced HDL-C concentration; and smaller, dense, cholesteryl ester-depleted LDL particles.

It has been shown that LDL-MI is a convenient and useful method for identifying LDL particle size. Mishima et al. (7) evaluated the relationships between LDL-MI and the Rf value determined by the LipoPrint LDL™ system and between LDL-MI and LDL particle size measured by non-denaturing 2 to 6% polyacrylamide gradient gel electrophoresis. LDL-MI was strongly positively correlated with Rf value ($r = 0.93063$, $p < 0.001$), and negatively correlated with LDL particle size ($r = –0.78397$, $p < 0.001$). They also showed that an LDL-MI > 0.4 signifies the presence of small LDL particles.

The LDL-C/apo B ratio has also been shown to be another indicator of LDL particle size (6). In the present study, a good correlation was observed between LDL-MI or LDL-C/ApoB and log(TC/TG) or log(LDL-C/TG), respectively. A very strong correlation was observed between TC/TG and LDL-C/TG. Therefore, these results show that TC/TG and LDL-C/TG can be simple and reliable indicators of small, dense LDL.

Lahdenperä et al. (10) reported that serum TG had no effect on LDL size below the threshold level of 150 mg/dl. Griffin et al. (11) showed that the distribution of the LDL subfraction was affected by serum TG when the concentration of TG exceeded 133 mg/dl. However, it has been reported that the prevalence of LDL subclass pattern B increased and LDL peak particle diameter decreased from a low to high TG tertile, even in young Japanese non-obese men who had a mean TG of 62 mg/dl (12). A high prevalence of small, dense LDL has been reported to be a leading cause of CAD in both non-diabetic and diabetic Japanese men (13). In the present study, the threshold level of TG for the presence of small dense LDL was calculated as 140–142 mg/dl. Further studies are necessary to determine the desirable level of TG in relation to the LDL particle size.

In conclusion, TC/TG and LDL-C/TG may offer a convenient and simple clinical tool for predicting the presence of small, dense LDL. While TC/TG and LDL-C/TG showed a similar correlation coefficient, TC/TG could be an easier-to-use indicator of small, dense LDL, particularly for general practitioners.

Acknowledgement: This work was supported by a grant (Gakuen Kenkyuji Joseikin A) from Sugiyama Jogakuen University.

References

6. Japan Atherosclerosis Society: Japan Atherosclerosis Society (JAS) Guidelines for Diagnosis and


