Small Dense Low-density Lipoprotein Cholesterol is a Useful Marker of Metabolic Syndrome in Patients with Coronary Artery Disease

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Aim: An evaluation of the relation between small dense low-density lipoprotein cholesterol (sd-LDL-C) levels measured by the heparin-magnesium precipitation method and metabolic syndrome (MetS).

Methods: We have prospectively measured sd-LDL-C levels by the heparin-magnesium precipitation method in 112 Japanese patients (male/female = 80/32) with coronary artery disease (CAD) who received percutaneous coronary intervention (PCI). Patients were diagnosed with MetS according to modified Japanese criteria.

Results: A total of 36 patients (32%) met the criteria for MetS. Sd-LDL-C levels were significantly higher in the MetS group than non-MetS group (20.7 ± 1.5 mg/dL vs. 17.1 ± 1.0 mg/dL, p = 0.042), especially among patients without lipid-lowering therapy (26.4 ± 2.6 mg/dL vs. 17.5 ± 1.5 mg/dL, p = 0.0034). Sd-LDL-C levels gradually increased with the number of components used to define MetS (0; 14.5 ± 1.8 mg/dL, 1; 16.5 ± 1.8 mg/dL, 2; 16.7 ± 1.3 mg/dL, 3; 19.3 ± 1.7 mg/dL, 4; 23.1 ± 2.1 mg/dL, 5; 40.0 mg/dL, p = 0.0071). High-sensitivity C-reactive protein (hs-CRP) levels were significantly higher in the patients with MetS (1.09 ± 0.17 mg/L vs. 0.67 ± 0.09 mg/L, p = 0.0204).

Conclusion: The sd-LDL-C level measured by the heparin-magnesium precipitation method is a useful marker of MetS in Japanese patients with CAD.


Key words: Small dense low-density lipoprotein cholesterol, Metabolic syndrome, Coronary artery disease, High-sensitivity C-reactive protein

Introduction

Metabolic syndrome (MetS) is a clinical entity characterized by visceral obesity, hypertension, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C), and glucose intolerance, although there has been some incompatibility of diagnostic criteria among countries. In Japan, a definition of and diagnostic criteria for MetS were established in April 2005¹. The incidence of MetS was 7.8% in the general Japanese population². The presence of MetS is associated with increased coronary artery disease (CAD) events, cardiovascular mortality, or reduced survival³-⁵. MetS is also reported to be predictive of subclinical atherosclerosis⁶,⁷.

Small dense low-density lipoprotein (sd-LDL) has been demonstrated to be a new risk factor for the development of CAD in Westerners⁸,⁹ as well as in Japanese, which have relatively low-density lipoprotein cholesterol (LDL-C) levels¹⁰. Subjects with MetS typically do not have elevated levels of LDL-C. However, a qualitative abnormality in low-density lipopro-
tein (LDL) such as sd-LDL is recognized to be frequently associated with MetS. LDL particle size is usually measured by gradient gel electrophoresis using non-denaturing polyacrylamide. This procedure requires a long assay time and does not provide a quantitative determination of sd-LDL.

Recently, Hirano et al. established a simple and rapid method for the measurement of small dense LDL cholesterol (sd-LDL-C) concentrations using heparin-magnesium precipitation. This method is useful for evaluating qualitative and quantitative abnormalities in LDL, and may be applicable to a routine clinical examination.

In Japan as well as in other countries, the definition of and diagnostic criteria for MetS have not included qualitative abnormalities in LDL. Therefore, in this study, we evaluated the relation between sd-LDL-C levels measured by the heparin-magnesium precipitation method and MetS in Japanese patients with CAD.

Subjects and Methods

Subjects
We have prospectively studied 112 patients (male/female = 80/32) with CAD who received percutaneous coronary intervention (PCI) between November 2004 and June 2005. Patients were diagnosed with MetS according to the Japanese criteria, that is, visceral obesity (waist circumference $\geq 85$ cm in men, 90 cm in women) plus two or more of the following: (1) triglyceride $\geq 150$ mg/dL and/or HDL-C $< 40$ mg/dL; (2) systolic blood pressure $\geq 130$ mmHg and/or diastolic blood pressure $\geq 85$ mmHg, or use of anti-hypertensive medication; and (3) fasting plasma glucose $\geq 110$ mg/dL, or history of diabetes. Since waist circumference was not measured in this study, we used a body mass index (BMI) of $\geq 25$ kg/m$^2$ for visceral obesity. Written informed consent was obtained from all patients.

Small Dense LDL Cholesterol Assay

The details and validation of this method have been described elsewhere. In brief, the precipitation reagent (0.1 mL) containing 150 U/mL of heparin sodium salt and 90 mmol/L MgCl$_2$ was added to a serum sample (0.1 mL), and incubated for 10 minutes at 37°C. After centrifugation at 10,000 rpm (5,000×g) for 1 minute, sd-LDL and HDL were collected by filtering off the more buoyant lipoproteins. Then the penetrate solution containing sd-LDL and HDL was removed for the measurement of LDL-C by a direct and selective homogeneous assay method (LDL-EX, Denka Seiken, Tokyo, Japan). This direct LDL-C assay was performed with an autoanalyzer (Type 7170; Hitachi Ltd., Tokyo).

Laboratory Determination

Blood samples were obtained after an overnight fast. Serum total cholesterol and triglyceride levels were measured by standard enzymatic methods. Serum LDL-C and HDL-C levels were measured by direct homogeneous assay using detergents (LDL-EX, HDL-EX, Denka Seiken).

Statistical Analysis

The statistical analyses were performed using Statview 5.0 software (SAS Institute Inc., Cary, NC). All values are expressed as the mean ± SEM. Differences between the means were compared with the unpaired $t$-test. The significance of any differences in proportions was tested with a Chi-square analysis. A one-way analysis of variance (ANOVA) and Fisher’s Protected Least Significant Difference were used to compare mean values of sd-LDL-C among groups from the number of MetS components. A statistically significant difference was defined as $p < 0.05$.

Results

A total of 36 patients (32%) met the criteria for MetS defined in this study. The baseline characteristics of the patients with or without MetS are listed in Table 1. There were no significant differences in age, sex, or total and LDL cholesterol levels between the two groups. However, BMI, triglyceride levels, and the frequency of diabetes mellitus were significantly higher and HDL-C levels were significantly lower in those with MetS. The levels of fasting plasma glucose, HbA1c, and the frequency of hypertension tended to be higher in those with MetS. The rate of statin use was similar between the two groups (Table 1). The characteristics of CAD with or without MetS are shown in Table 2. There were no significant differences between the two groups.

Sd-LDL-C levels were significantly higher in those with MetS (20.7 ± 1.5 mg/dL vs. 17.1 ± 1.0 mg/dL, $p = 0.042$) (Fig. 1a). In addition, apparent differences in sd-LDL-C levels between the two groups were greater when the patients who received lipid-lowering medications such as statins and fibrates were excluded (26.4 ± 2.6 mg/dL vs. 17.5 ± 1.5 mg/dL, $p = 0.0034$) (Fig. 1b). Sd-LDL-C levels gradually increased with the number of components used to define MetS (0; 14.5 ± 1.8 mg/dL; 1; 16.5 ± 1.8 mg/dL; 2; 16.7 ± 1.3 mg/dL; 3; 19.3 ± 1.7 mg/dL; 4; 23.1 ± 2.1 mg/dL; 5; 40.0 mg/dL, $p =$...
Table 1. Baseline characteristics of patients with or without metabolic syndrome

<table>
<thead>
<tr>
<th></th>
<th>MetS (+)</th>
<th>MetS (-)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67 ± 2</td>
<td>70 ± 1</td>
<td>0.1410</td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>29/7</td>
<td>51/25</td>
<td>0.1411</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.4 ± 1.3</td>
<td>159.8 ± 1.0</td>
<td>0.7036</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>70.0 ± 1.7</td>
<td>58.8 ± 1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1 ± 0.4</td>
<td>22.9 ± 0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>183 ± 7</td>
<td>182 ± 3</td>
<td>0.8794</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>120 ± 6</td>
<td>113 ± 3</td>
<td>0.2309</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>40 ± 1</td>
<td>47 ± 1</td>
<td>0.0016</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>139 ± 9</td>
<td>109 ± 4</td>
<td>0.0005</td>
</tr>
<tr>
<td>DM (%)</td>
<td>28 (78%)</td>
<td>32 (42%)</td>
<td>0.0004</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>119 ± 6</td>
<td>110 ± 4</td>
<td>0.2088</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.9 ± 0.3</td>
<td>6.6 ± 0.2</td>
<td>0.5689</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>29 (81%)</td>
<td>49 (64%)</td>
<td>0.0839</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>20 (56%)</td>
<td>41 (54%)</td>
<td>0.8732</td>
</tr>
<tr>
<td>Fibrates (%)</td>
<td>4 (11%)</td>
<td>1 (1%)</td>
<td>0.0191</td>
</tr>
<tr>
<td>ACE inhibitors (%)</td>
<td>8 (22%)</td>
<td>19 (25%)</td>
<td>0.7470</td>
</tr>
<tr>
<td>ARBs (%)</td>
<td>15 (42%)</td>
<td>17 (22%)</td>
<td>0.0347</td>
</tr>
<tr>
<td>β-blockers (%)</td>
<td>13 (36%)</td>
<td>14 (18%)</td>
<td>0.0409</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SEM or number (%). MetS, metabolic syndrome; BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; DM, diabetes mellitus; FPG, fasting plasma glucose; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker.

0.0071) (Fig. 2).

High-sensitivity C-reactive protein (hs-CRP) levels were significantly higher in the patients with MetS (1.09 ± 0.17 mg/L vs. 0.67 ± 0.09 mg/L, p = 0.0204) (Fig. 3a). When we separated the patients according to the type of CAD, we obtained the same results for both stable angina (0.78 ± 0.15 mg/L vs. 0.40 ± 0.09 mg/L, p = 0.0202) and acute coronary syndrome (ACS) (1.64 ± 0.35 mg/L vs. 1.04 ± 0.17 mg/L, p = 0.0893) (Fig. 3b).

### Discussion

In the present study, sd-LDL-C levels measured by the heparin-magnesium precipitation method were significantly higher in the patients with MetS. Furthermore, they increased significantly with the number of components used to define MetS. A previous study based on gradient gel electrophoresis has demonstrated that subjects with MetS have sd-LDL particles (5). In a recent sex-specific cross-sectional study, the number of sd-LDL particles determined by nuclear magnetic resonance spectroscopy was found to be gre-

![Fig. 1](image-url)

Fig. 1.

(a) Small dense LDL cholesterol (sd-LDL-C) levels with or without metabolic syndrome (MetS). (b) Sd-LDL-C levels with or without lipid-lowering therapy. All data are shown as the mean ± SEM. *p < 0.05, **p < 0.01. Lipid-lowering therapy (−), Lipid-lowering therapy (+).
How might MetS lead to an increase in sd-LDL? There is substantial evidence that excess adiposity, especially accompanying diabetes or insulin resistance, leads to increased free fatty acid production by adipocytes. These free fatty acids are taken up by the liver and used to produce triglyceride. As a consequence of increased hepatic triglyceride synthesis, there is increased production and secretion of very low-density lipoprotein (VLDL) particles by the liver. These VLDL particles lead to an increased number of sd-LDL particles. Through the actions of cholesteryl ester transfer protein (CETP), an appreciable amount of triglyceride in VLDL may be exchanged for cholesterol ester in plasma LDL. These triglyceride-enriched LDL particles are a favored substrate for hepatic lipase and may be transformed into smaller LDL by lipase-mediated triglyceride hydrolysis.

Qualitative abnormalities of LDL are not included in either the World Health Organization (WHO), the Adult Treatment Panel III (ATP III), or the Japanese criteria for MetS. Recently, a new definition of MetS was developed by the International Diabetes Federation (IDF). The IDF Consensus has recommended further research on a comprehensive list of other components that should be considered as additions in future definitions of MetS. The list includes adipose tissue biomarkers (adiponectin and leptin), apolipoprotein B, LDL particle size, a formal measurement of insulin resistance and an oral glucose tolerance test, endothelial dysfunction, urinary albumin, inflammatory markers (CRP, tumor necrosis factor alpha, and interleukin-6), and thrombotic markers (plasminogen activator inhibitor factor-1 and fibrinogen).

Hs-CRP level appears to be a strong predictor of future cardiovascular events. The CRP provides prognostic information at all levels of LDL-C and the Framingham Risk Score. It is also clear that CRP levels add clinically important prognostic information regarding MetS. In this study, hs-CRP levels were significantly higher in the patients with MetS. The IDF Consensus recognizes that visceral obesity is an important determinant of MetS, and that there is a strong association between waist circumference, cardiovascular disease, and other components of MetS. The IDF also emphasizes that BMI is not sufficiently sensitive to detect visceral obesity in different ethnic groups. In this study, we tentatively defined MetS using modified Japanese criteria. Since this study was started before the Japanese criteria for MetS were established, waist circumference was not measured. Therefore, we used a BMI of ≥25 kg/m² according to the criteria for obesity of the Japanese Society for the

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**Fig. 2.** Distribution of small dense LDL cholesterol (sd-LDL-C) levels according to the number of components for the definition of metabolic syndrome (MetS). All data are shown as the mean ± SEM. *$p<0.05$, **$p<0.01$, ***$p=0.0071$ with Fisher’s Protected Least Significant Difference.

**Fig. 3.**
(a) High-sensitivity C-reactive protein (hs-CRP) levels with or without metabolic syndrome (MetS). (b) Hs-CRP levels separated from type of coronary artery disease. All data are shown as the mean ± SEM. *$p<0.05$, **$p<0.01$. $\uparrow$: Acute coronary syndrome, $\downarrow$: Stable angina.
Study of Obesity, as the cut-off point for visceral obesity.

In the present study, sd-LDL-C levels measured by the heparin-magnesium precipitation method increased with the number of components used to define MetS. Furthermore, sd-LDL-C levels were significantly lower with than without lipid-lowering therapy in the patients with MetS. As several statins have been known to decrease sd-LDL levels, our data also indicate the effect of statins on sd-LDL-C in those with MetS.

**Conclusion**

In conclusion, the sd-LDL-C level measured by the heparin-magnesium precipitation method is a useful marker of MetS in patients with CAD.

**Study Limitations**

There are some limitations regarding the interpretation of these results and drawing of conclusions. First, this study included 112 patients with CAD. The sample size allowed for only a limited analysis of the relation between MetS and sd-LDL-C levels. Second, about 60% of the patients had received lipid-lowering therapy. Third, since the study was started before Japanese criteria for MetS were established, waist circumference was not measured. Therefore, we tentatively defined MetS using modified Japanese criteria.

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