High-density lipoprotein particles are large in patients with variant angina

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Abstract

Objective: Dyslipidemia in patients with coronary vasospasm may be characterized by low level of high-density lipoprotein (HDL)-cholesterol as well as apolipoprotein (apo) A-I but not high level of low-density lipoprotein-cholesterol. This study sought to examine the HDL particle size in patients with variant angina. Methods: The HDL particle size was examined by analyzing serum lipid levels in 38 patients with variant angina to compare with those of 40 control subjects and 30 normocholesterolemic patients with stable effort angina. Also, actual HDL size distribution was assessed by electrophoresis. Results: The HDL-cholesterol, apoA-I and apoA-II levels were all lower \( P < 0.01 \) for each in patients with variant angina and patients with stable effort angina as compared with control subjects. The apoA-II level was lower \( P < 0.01 \) in patients with variant angina than in patients with stable effort angina. The apoA-I/apoA-II ratio was lower \( P < 0.01 \) in patients with stable effort angina, but not in patients with variant angina as compared with control subjects. In contrast, the HDL-cholesterol/apoA-I ratio was higher in patients with variant angina than in control subjects \( P < 0.01 \) and also patients with stable effort angina \( P < 0.01 \). The slope of the regression line, comparing HDL-cholesterol and apoA-I levels, was greater in patients with variant angina than in control subjects \( P < 0.05 \) and patients with stable effort angina \( P < 0.05 \). Native polyacrylamide gel electrophoresis revealed that HDL particles in patients with variant angina were skewed towards larger sizes compared with control subjects \( P < 0.01 \) and patients with stable effort angina \( P < 0.01 \). The abnormal serum lipid values were normalized in the patients with variant angina after the medical treatment and inactivation of the coronary spasm. Conclusion: High HDL-cholesterol/apoA-I levels associated with low serum HDL-cholesterol and apoA-I levels were characteristic in patients with variant angina, in whom HDL particles were large, cholesterol-rich and possibly malfunctioning. © 1998 Elsevier Science B.V.

Keywords: Variant angina; Coronary spasm; High density lipoprotein; High density lipoprotein cholesterol; Apolipoprotein A-I

1. Introduction

Although it has been established that dyslipidemia in association with high levels of total cholesterol or low-density lipoprotein (LDL) cholesterol and low levels of high-density lipoprotein (HDL) cholesterol are closely related to coronary artery disease [1,2], the association is unclear in cases with coronary vasospasm. No significant elevation has been noted in total cholesterol, triglyceride or LDL-cholesterol in these patients [3–5]. The reported results of HDL-cholesterol levels have been disparate [3–5]. Recently we reported that HDL-cholesterol levels in patients with vasospasm were significantly different between the active and inactive stages of coronary spasm and that the HDL-cholesterol level significantly modified their prognosis [6]. In contrast to HDL-cholesterol, when the level of the main carrier protein of HDL, apolipoprotein (apo) A-I, was examined, the serum level of apoA-I in patients with vasospastic angina was found to be significantly lower than that in those without vasospasm [7] and also apoA-I was the best discriminator between variant angina patients and the control group [8], even in an analysis using a relatively small number of samples. Consequently, these previous studies suggested that a discrepancy between
HDL-cholesterol and apo-A-I levels might exist in patients with active coronary spasm. Possible alterations of HDL-cholesterol construction may be relevant to the alteration of the antiatherogenic function of HDL-cholesterol.

In the present study the HDL particle size was examined by analyzing the HDL-cholesterol/apoA-I ratio and the slope of the regression line between HDL-cholesterol and apoA-I levels for the cholesterol content in HDL particles in patients with variant angina to compare with control subjects without coronary artery disease and also patients with stable effort angina. Also actual HDL particle size distribution was assessed by native polyacrylamide gel electrophoresis.

2. Methods

2.1. Study patients

Thirty-eight consecutive patients with active variant angina undergoing coronary angiography because of chest pain and electrocardiographic ST segment elevation at rest in whom coronary artery spasm was demonstrated angiographically during spontaneous or induced attacks by intracoronary injection of acetylcholine [9] and at least two attacks had occurred during the preceding week, were selected for the study. Patients with severe (> 90% luminal diameter narrowing) organic coronary obstructive lesion were excluded from the study. These 38 subjects comprised 30 men and 8 women with a mean age of 57 ± 2 years. Clinical and angiographic data are listed in Table 1. A significant (> 75% luminal narrowing) organic coronary artery stenosis was found in 11 (29%) patients of the variant angina group. Two other groups of subjects, 30 patients with stable effort angina and 40 age- and sex-matched control subjects without coronary artery disease, were also studied. Patients with a significant organic coronary stenosis having fixed-threshold exercise-induced angina but no rest angina were included in the stable effort angina group. No ischemic ECG changes at rest were detected during Holter monitoring in this group. Chest pain-free subjects who visited our out-patient clinic for some reason, but without any significant disease, having negative treadmill exercise test results, were enrolled in the control group. The stable effort angina group consisted of 23 men and 7 women with a mean age of 61 ± 2 years, including 18 (60%) patients with multivessel disease. The control group included 30 men and 10 women with a mean age of 58 ± 2 years. Patients with hypercholesterolemia (serum total cholesterol level > 240 mg/dl) were excluded from the study. In patients with variant angina, 28 were again subjected to the study for the inactive stage of variant angina after an at least 6-month angina-free period while receiving medical treatment with calcium entry blockers and an attack-free 3-day observation period without medication. Smoking cessation, a reduction in body weight, and an increase in physical activity were, as a rule, recommended to all the patients. Written informed consent was obtained from the study patients, and the study protocol was approved by the ethical committee of our institution.

2.2. Serum analysis

A venous blood sample was obtained in the fasting state. The total cholesterol was measured directly in the serum, and HDL-cholesterol was measured after precipitation of very low-density lipoprotein cholesterol and LDL-cholesterol with dextran sulfate–magnesium chloride by an enzymatic method [10]. The concentration of triglyceride in serum was determined by measuring glycerol after an enzymatic hydrolysis with lipase-esterase. The LDL-cholesterol concentration was calculated according to the following formula: LDL-cholesterol = (total cholesterol) – (HDL-cholesterol) – (triglyceride/5). Urinary cotinine radioimmunoassay was used to verify the patient’s smoking status. Patients with a urinary cotinine concentration above 50 ng/ml were classified as current smokers [11,12]. The concentrations of apo-A-I, apo-A-II and apoB were determined by immunoturbidimetry [13]. The molar ratio of apoA-I/apoA-II and also that of HDL-cholesterol/apoA-I were calculated based on the molecular weight 28 300 for apoA-I, 17 400 for apoA-II and 387 for cholesterol. The slope of the regression line, comparing HDL-cholesterol and apoA-I levels, was determined in each group as an indication of the size of the particles [14].

2.3. HDL particle size analysis

HDL particle size distribution was assessed by native polyacrylamide (8.5%) gel electrophoresis using the
2.4. Statistical analysis

Values are presented as means ± standard error of the mean. Comparisons of serum lipid levels and age among the three groups were performed with one-way ANOVA followed by Scheffé’s test. The clinical characteristics and electrophoretic profiles of the HDL subfractions of the three groups were compared by a χ^2-test with Yates’ correction if one of the frequencies in the contingency table was < 5. Simple linear regression of HDL-cholesterol and apoA-I levels in each group was determined by the least squares method. Analysis of covariance followed by the two-tailed F-test was used to determine the significance of the difference between the slopes of the regression lines in plots of HDL-cholesterol and apoA-I levels. The unpaired or paired Student’s t-test was used to determine the significance of the changes during the follow-up in the serum lipid levels in the variant angina group. The level of significance was set as P < 0.05.

3. Results

Results are summarized in Tables 1–4 and Figs. 1 and 2. As shown in Table 1, gender distribution and mean age were not significantly different among the groups. However, smoking was found to be significantly more prevalent in the variant angina group than in the control group (P < 0.01) and stable effort angina (P < 0.01) groups. The prevalence of diabetes mellitus, hypertension and obesity did not differ significantly between the variant angina and stable effort angina groups.

Table 2

<table>
<thead>
<tr>
<th>Apolipoprotein levels in study patients</th>
<th>C</th>
<th>VA</th>
<th>SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>40</td>
<td>38</td>
<td>30</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>200 ± 5</td>
<td>186 ± 6</td>
<td>208 ± 5^a</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>119 ± 10</td>
<td>115 ± 9</td>
<td>150 ± 16^a</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>126 ± 5</td>
<td>120 ± 5</td>
<td>139 ± 5^a</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>51 ± 1</td>
<td>43 ± 2^b</td>
<td>39 ± 2^b</td>
</tr>
<tr>
<td>apoA-I (mg/dl)</td>
<td>130 ± 3</td>
<td>99 ± 3^b</td>
<td>105 ± 4^b</td>
</tr>
<tr>
<td>apoA-II (mg/dl)</td>
<td>33 ± 1</td>
<td>27 ± 1^b</td>
<td>31 ± 1^ac</td>
</tr>
<tr>
<td>apoB (mg/dl)</td>
<td>98 ± 4</td>
<td>93 ± 3</td>
<td>108 ± 3^a</td>
</tr>
<tr>
<td>apoA-I/apoA-II (molar ratio)</td>
<td>2.48 ± 0.06</td>
<td>2.34 ± 0.08</td>
<td>2.12 ± 0.07ac</td>
</tr>
<tr>
<td>HDL-cholesterol/apoA-I (molar ratio)</td>
<td>28.5 ± 0.5</td>
<td>31.5 ± 1.0bc</td>
<td>27.0 ± 0.7c</td>
</tr>
</tbody>
</table>

C = control subjects; VA = patients with variant angina; SA = patients with stable effort angina.

^aP < 0.05 vs. C.  
^bP < 0.01 vs. VA.  
^cP < 0.01 vs. C.

3.1. Serum lipid profiles

Serum total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol levels in each group are shown in Table 2. The serum total cholesterol, triglyceride and LDL-cholesterol levels in the variant angina group were all somewhat lower as compared with the control group, although no significant difference was found in any of the levels between the variant angina and the control groups. However, the HDL-cholesterol level was significantly (P < 0.01) lower in the variant angina group than control group. Also a significantly (P < 0.01) lower LDL-cholesterol level was noted in the stable effort angina group as compared with the control group, but no significant difference was noted in the serum total cholesterol, triglyceride or LDL-cholesterol level between the stable effort angina and control groups. The total cholesterol, triglyceride and LDL-cholesterol levels were all significantly (P < 0.05 for each) lower in the variant angina group as compared with the stable effort angina group, although no significant difference was noted in the HDL-cholesterol level between the variant angina and the stable effort angina groups.

3.2. Serum apoA-I, apoA-II and apoB levels

Serum apoprotein levels in each group are listed in Table 2. Both apoA-I and apoA-II levels in the variant angina group (P < 0.01 for each) and effort angina group (P < 0.01 for each) were significantly lower than in the control group. Moreover, the apoA-II level was significantly (P < 0.01) lower in the variant angina group than stable effort angina group while no significant difference was noted in the apoA-I level between the groups. The apoB levels were not significantly different between the variant angina and control groups, but apoB levels in both...
of the groups were significantly \((P < 0.01\) for the variant angina group and \(P < 0.05\) for the control group) lower than in the stable effort angina group.

### 3.3. ApoA-I / apoA-II ratio

The calculated molar ratio of apoA-I to apoA-II (apoA-I/ apoA-II) in the stable effort angina group was significantly lower than in the control group \((P < 0.01)\) and in the variant angina group \((P < 0.05)\) (Table 2). No significant difference was found in apoA-I/ apoA-II ratio between the variant angina and control groups.

### 3.4. HDL-cholesterol / apoA-I ratio

The calculated HDL-cholesterol / apoA-I molar ratio was significantly greater in the variant angina group than in the control group \((P < 0.01)\) and also in the stable effort angina group \((P < 0.01)\), although no significant difference was noted between the values of the latter two (Table 2). Since this ratio reflects the core to surface ratio, or the size of the HDL particles, this finding suggests that HDL particles in patients with variant angina are larger. The HDL-cholesterol/ apoA-I molar ratio was not significantly different between the variant angina patients with and without a fixed stenosis (31.7 ± 2.0 vs. 31.3 ± 1.3) and it was significantly larger in both the subgroups with \((P < 0.05)\) and without \((P < 0.05)\) a fixed stenosis than in the control group.

### 3.5. Correlation of HDL-cholesterol levels with apoA-I levels

The slope of the line, comparing HDL-cholesterol and apoA-I levels, is also an indication of the size of the particles [14]. As shown in Fig. 1, HDL-cholesterol and apoA-I levels correlated significantly \((P < 0.01)\) for each in each group. The slope of the regression line was significantly \((P < 0.05)\) greater in the variant angina group \((0.549 ± 0.054, r = 0.87)\) than in the control \((0.398 ± 0.051, r = 0.79)\) and stable effort angina \((0.412 ± 0.050, r = 0.84)\) groups. The slope was not significantly different between the variant angina patients with and without a fixed stenosis \((0.606 ± 0.182 vs. 0.527 ± 0.055)\).

### 3.6. HDL particle size analysis

HDL particle size distribution was also examined by native polyacrylamide gel electrophoresis. As can be seen in Fig. 2, HDL particles were larger in the patients with variant angina as compared with control subjects and patients with stable effort angina. The most predominant

#### Table 3
Comparison of serum lipid levels and HDL particle size between smokers and non-smokers

<table>
<thead>
<tr>
<th></th>
<th>C + (n = 14)</th>
<th>C − (n = 26)</th>
<th>VA + (n = 28)</th>
<th>VA − (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>48 ± 2</td>
<td>53 ± 2</td>
<td>41 ± 2*</td>
<td>n.s. 47 ± 6</td>
</tr>
<tr>
<td>apoA-I (mg/dl)</td>
<td>121 ± 4</td>
<td>135 ± 3</td>
<td>99 ± 3b</td>
<td>n.s. 109 ± 11*</td>
</tr>
<tr>
<td>HDL-cholesterol /apoA-I (molar ratio)</td>
<td>28.8 ± 0.9</td>
<td>n.s.</td>
<td>31.4 ± 1.2</td>
<td>n.s. 31.8 ± 1.4*</td>
</tr>
<tr>
<td>Slope</td>
<td>0.373 ± 0.10</td>
<td>n.s.</td>
<td>0.390 ± 0.075</td>
<td>n.s. 0.514 ± 0.091</td>
</tr>
<tr>
<td>R_m &lt; 0.2</td>
<td>29%</td>
<td>23%</td>
<td>72%*</td>
<td>n.s. 80%*</td>
</tr>
</tbody>
</table>

C = control subjects; VA = patients with variant angina; + = current smoker; − = current non-smoker; Slope = the slope of the regression line, comparing HDL-cholesterol and apoA-I, see text for details; \(R_m\) = the specific rate of migration relative to the albumin fraction of the most predominant HDL band determined by native polyacrylamide gradient gel electrophoresis, see text for details.

\(^aP < 0.05\) vs. C

\(^bP < 0.01\) vs. C.

#### Table 4
Serum lipid level changes in patients with variant angina after follow-up

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Current smokers</td>
<td>19 (68%)</td>
<td>13 (44%)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>186 ± 6</td>
<td>195 ± 6</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>114 ± 10</td>
<td>115 ± 13</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>121 ± 6</td>
<td>123 ± 6</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>42 ± 2</td>
<td>49 ± 2</td>
</tr>
<tr>
<td>apoA-I (mg/dl)</td>
<td>98 ± 3</td>
<td>126 ± 4</td>
</tr>
<tr>
<td>apoA-II (mg/dl)</td>
<td>27 ± 1</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>apoB (mg/dl)</td>
<td>95 ± 4</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>apoA-I / apoA-II (molar ratio)</td>
<td>2.28 ± 0.07</td>
<td>2.52 ± 0.06</td>
</tr>
<tr>
<td>HDL-cholesterol / apoA-I (molar ratio)</td>
<td>3.4 ± 1.0</td>
<td>28.6 ± 0.6</td>
</tr>
</tbody>
</table>
Comparison of the regression lines of HDL-cholesterol and apo A-I plot

![Graphs showing regression lines for different groups](image)

**Fig. 1.** Correlation of HDL-cholesterol levels with apoA-I levels in study patients. C = control subjects (upper left); VA = patients with variant angina (upper right); SA = patients with stable effort angina (lower left). The slope of the regression line indicates the mass ratio of HDL-cholesterol to apoA-I in the particles. It is significantly \( P < 0.05 \) for each greater in VA than C and SA. The regression lines are put together for the sake of comparison (lower right).

HDL subfraction band of \( R_m \) below 0.2, corresponding to the particle diameter > 9.9 nm, was observed in 29 (76%) of 38 patients with variant angina. This prominent HDL subfraction band of \( R_m \) below 0.2 was significantly less prevalently observed in the control subjects (10 of 40, 25%, \( P < 0.01 \)) and patients with stable effort angina (6 of 30, 20%, \( P < 0.01 \)) compared with the patients with variant angina. The prevalence was not significantly different between the variant angina patients with and without a fixed stenosis (9 of 11, 82% vs. 20 of 27, 74%).

### 3.7. Effects of smoking on HDL particle size

The serum lipid levels were compared between current smokers and non-smokers in the control and variant angina groups (Table 3). In the control group, both HDL-cholesterol and apoA-I levels were significantly \( (P < 0.05 \) for each) lower in the smokers than in the non-smokers. However, the HDL-cholesterol/apoA-I ratio was comparable between the smokers and non-smokers. The slope of the regression line in plots of HDL-cholesterol and apoA-I levels was not significantly different between them. Also the prevalence of the most predominant HDL-subfraction band of \( R_m \) below 0.2 was not significantly different between them. In the variant angina group, no significant difference was found between smokers and non-smokers in any of the HDL-cholesterol/apoA-I ratio, the slope of the regression line and the prevalence of the most predominant HDL-subfraction band of \( R_m \) below 0.2. In the smoking subpopulation, both HDL-cholesterol \( (P < 0.05) \) and apoA-I \( (P < 0.01) \) levels were significantly lower in the variant angina group than in the control group. In the non-smoking subpopulation, the apoA-I level was significantly \( (P < 0.05) \) lower, and both the HDL-cholesterol/apoA-I ratio and the slope were significantly \( (P < 0.05 \) for each) higher in the variant angina group than in the control group. In both the smoking \( (P < 0.05) \) and non-smoking \( (P < 0.01) \) subpopulations, the prevalence of the most predominant HDL-subfraction band of \( R_m \) below 0.2 was significantly higher in the variant angina group than in the control group.
Fig. 2. A typical example of non-denaturing polyacrylamide gel electrophoresis of the serum from study patients is shown. Electrophoresis was performed on an 8.5% native polyacrylamide gel after polymerization with the loading gel solution of Sudan Black B dye. Densitometric gel profiles of HDL from study patients are shown. Control = control subjects; VA = patients with variant angina; SA = patients with stable effort angina. The lipoprotein subfractions are identified by their migration distance relative to the albumin fraction: 1.0. Note a prominent peak (arrows) of below 0.2 in.

3.8. Follow-up data in patients with variant angina

The patients in the variant angina group were followed up at our outpatient clinic at monthly intervals. Blood samples were again obtained for the determination of lipid levels after an at least 6-month angina-free period with medical treatment with calcium entry blockers in the 28 patients with variant angina (22 males and 6 females; mean age, 57 ± 2 years). After the follow-up, smoking was significantly (P < 0.01) less prevalent than before (Table 4). Although the HDL-cholesterol level was significantly elevated (P < 0.05), no significant change in total cholesterol, triglyceride or LDL-cholesterol levels was found after the follow-up. However, both apoA-I (P < 0.01) and apoA-II (P < 0.01) levels were significantly elevated after the follow-up while apoB levels did not change significantly. The apoA-I/apoA-II ratio was significantly elevated (P < 0.05) after the follow-up. The HDL-cholesterol/apoA-I ratio in the patients was significantly (P < 0.01) lower after the follow-up than at baseline and then not significantly different from that in the control group. The decrease in the HDL-cholesterol/apoA-I ratio was also significant (P < 0.05) even in the non-smoking patients with variant angina (31.8 ± 1.4 to 28.8 ± 0.8, n = 9).

4. Discussion

In the present study, significantly lower levels of both serum HDL-cholesterol and apoA-I were confirmed in patients with variant angina as well as normocholesterolemic patients with stable effort angina than in control subjects. As the HDL-cholesterol/apoA-I ratio was significantly raised in the variant angina group as compared with the stable effort angina group, the deficiency of apoA-I was apparently more remarkable than that of HDL-cholesterol. The electrophoretic analysis demonstrated that...
larger HDL particles were significantly more predominant in patients with variant angina. In contrast, neither the total nor LDL-cholesterol level was significantly elevated, but rather decreased in the variant angina group, suggesting a lack of apparent association between serum LDL-cholesterol level and coronary spasm. In patients with stable effort angina, a lower HDL-cholesterol level was observed in association with higher prevalence of diabetes mellitus concomitant with higher triglyceride level.

A positive correlation between HDL-cholesterol and acetylcholine-induced coronary vasoreactivity or vasodilatation in both angiographically smooth and diseased coronary segments has been reported [15]. Zeiher et al. [16] reported that coronary arterial segments from patients with elevated serum HDL-cholesterol levels demonstrated a significantly blunted constrictor response to both stimuli (acetylcholine and cold pressor test) compared with segments from patients with low HDL-cholesterol levels, suggesting that HDL-cholesterol exerts a beneficial effect on abnormal vascular reactivity. HDL-cholesterol, aside from its several potential antiatherogenic actions including reverse cholesterol transport [17], an increase in prostacyclin production [18], reduction in platelet aggregability [19], increase in antithrombotic activities [20], stabilizing of serum prostacyclin [21], and promotion of regenerated endothelial proliferation [22], has an antioxidant effect [23–25] preventing LDL oxidation. Increased oxidative susceptibility concomitant with vitamin E deficiency has been recently demonstrated in LDL from patients with active variant angina whose serum LDL-cholesterol level was not elevated [5,26].

The HDL-cholesterol/apoA-I ratio and the slope of the regression line in plots of HDL-cholesterol and apoA-I levels were found to be significantly greater in patients with variant angina than in control subjects and also patients with stable effort angina, suggesting that HDL particles in patients with coronary spasm were cholesterol-rich. Increased size of HDL particles was also confirmed in these patients by native polyacrylamide gel electrophoresis. Recently similar results were reported by Iwanejko et al. who observed that larger HDL particles predominate in the high insulin response group during oral glucose tolerance test in normolipidemic and normoinsulinemic patients with coronary artery disease [27]. Large and cholesterol-rich particles of HDL are functionally distinct from small and cholesterol-poor particles. Smaller HDL particles, HDL$_3$, are known to be more potent in lecithin–cholesterol acyltransferase activity while larger particles, HDL$_2$, contain 3- to 4-fold more cholesteryl ester and triglyceride molecules than HDL$_3$ and are regarded as carrying lipids by proteins more efficiently as a vehicle for cholesterol [17]. The preferential antioxidant effect is suggested to be generally associated with HDL$_1$ [28]. Although it remains to be elucidated how the subfractions obtained by the system that we employed correlate to the HDL subspecies, HDL$_2$ and HDL$_3$, the HDL subfractions with R$_m$ below 0.2 demonstrated in the present study may probably be included in the HDL$_2$. Recently, small and very high density lipoprotein particles were shown to have a more potent antiatherogenic function than normal HDL$_3$ [29]. Cholesterol-rich HDL particles in patients with active variant angina may be less antiatherogenic. Further examination is required to clarify the possible functional changes associated with the alteration of HDL particle size in these patients.

ApoA-I confers resistance to early atherosclerosis when overexpressed in transgenic mice [30]. In contrast, transgenic mice that overexpress mouse apoA-II had elevated HDL-cholesterol concentration, but, nevertheless, exhibited increased atherosclerotic lesion development as compared to normal mice [31]. Several studies have shown that HDL containing both apoA-I and apoA-II are less effective at promoting cholesterol efflux from tissue culture cells than are HDL containing apoA-I alone [32–35]. The percentage of plasma apoA-I not associated with apoA-II was reported to be highly correlated with the subjects’ plasma apoA-I/apoA-II ratio [30]. The significantly lower apoA-I/apoA-II ratio in patients with stable effort angina than in control subjects demonstrated in the present study may suggest functional differences in the antiatherogenic properties of HDL particles. However, this was not documented in the patients with variant angina. The apoA-I/apoA-II ratio appeared to be unrelated to the presence of coronary artery spasm. In contrast, the apoA-II level was found to be significantly lower in our patients with variant angina than in control subjects or patients with stable effort angina. The apoA-II levels were normalized during the inactivation of coronary spasm in these patients, similar to the changes in apoA-I levels. The determination of apoA-II should be a useful surrogate for measuring lipoproteins containing both apoA-I and apoA-II because most serum apoA-II resides in these lipoproteins, although a minor lipoprotein species may contain only apoA-II [36,37]. The pathogenic significance of a low level of apoA-II in patients with variant angina remains unknown.

The underlying mechanisms of the apparently increased HDL particle size in patients with variant angina and the relation between HDL particle size and coronary artery spasm remain to be elucidated. Lifestyle modifications may have influenced the HDL particle size. It is well known that serum HDL-cholesterol, apoA-I and apoA-II levels are all lower in smokers than non-smokers and smoking cessation raises these levels [6,38–40], although the exact mechanisms whereby cigarette smoke or its components alter both HDL-cholesterol and apoA-I as well as apoA-II levels have yet to be determined. High prevalence of current smoking in our patients with variant angina appeared to be related to the decreases in these levels. Also, smoking cessation probably contributed to the normalization of the abnormal serum lipid levels in them after the treatment and the inactivation of coronary spasm. However, no significant difference in HDL particle size.
was demonstrated between smoking and non-smoking control subjects. Importantly, the HDL particles appeared to be larger in patients with variant angina as compared with control subjects both in the smoking and non-smoking subpopulations. Moreover, the apparent HDL particle size decreased after the treatment even in the non-smoking patients with variant angina. Obviously, factors other than simple smoking status, such as diet, exercise, body weight control and also the sensitivity to tobacco smoke as well as smoking dose, appear to be responsible for the alteration in HDL particle size in patients with coronary spasm and require to be further investigated to clarify the causal relation between HDL particle size and coronary spasm.

In conclusion, the present study demonstrated low levels of HDL-cholesterol or apoA-I-containing lipoproteins without high level of either total or LDL-cholesterol in patients with active variant angina, suggesting that these lipoproteins are important negative risk factors for coronary artery spasm. In addition, the higher levels of HDL-cholesterol/apoA-I ratio or larger HDL particles confirmed in these patients as compared with control subjects and patients with stable effort angina might indicate functional alterations of the particles. Determination of apoA-I level in addition to HDL-cholesterol level in blood may be useful for the prediction of HDL particle size or cholesterol content. Dyslipidemia appeared to be characteristically different between patients with coronary artery spasm and organic atherosclerosis, suggesting different pathogenesis and predisposing factors between them.

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