Relationship between Metabolic Syndrome and Early Stage Coronary Atherosclerosis

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Aim: Recent clinical studies using intra-vascular ultrasound have clarified that coronary artery plaque already exists in subjects with normal coronary artery which is diagnosed by coronary angiography; furthermore, culprit lesion on acute coronary syndrome often occurs in mild to moderate angiographical stenotic lesion. The aim of this study is to clarify relationship between metabolic syndrome and early stage coronary atherosclerosis using a 3-dimensional intra-vascular ultrasound.

Methods: 70 subjects with normal coronary artery diagnosed by coronary angiography were enrolled. Proximal range of left anterior descending coronary artery was observed by intra-vascular ultrasound using autopullback methods.

Results: Subjects with metabolic syndrome had significantly high percent plaque volume (31 ± 8% vs 21 ± 8%, p < 0.0001) and frequently detected abnormal plaque quality such as eccentricity, calcification and lipid pool into plaque than those without metabolic syndrome. Multivariate analysis showed that serum adiponectin concentration was the most strongest variable for percent plaque volume (r value = −3.0, p < 0.01). On the other hand, subjects with hypoadiponectinemia were detected high incidence of mild calcification into plaque.

Conclusion: Metabolic syndrome needs to be detected and treated as early as possible. Furthermore, measurement of serum adiponectin concentration and appropriate treatment would prevent acute coronary syndrome.


Key words; Coronary plaque, Intra-vascular ultrasound, Metabolic syndrome, Adiponectin

Introduction

Metabolic syndrome (MS) is characterized by a group of metabolic disorders, which include dyslipidemia (hypertriglyceridemia and low high-density lipoprotein cholesterol), hyperglycemia, hypertension, and visceral fat obesity. Subjects with MS also have a high incidence of cardiovascular events. Coronary angiography has been used to establish and evaluate coronary atherosclerosis; however, recent clinical studies using intra-vascular ultrasound (IVUS) have clarified that coronary artery plaque already exists in subjects with normal coronary arteries, diagnosed by coronary angiography. Furthermore, it has been reported that almost 70% of culprit lesions in acute myocardial infarction, now called acute coronary syndrome and highly correlated with mortality, occur in less than 50% of angiographical stenosis. Therefore, it is difficult to predict the occurrence of acute coronary syndrome, and it is important that we consider the presence of coronary atherosclerosis, especially the presence of vulnerable plaque, before angiographical stenosis. IVUS studies have clarified the future of plaque in acute coronary syndrome; plaque volume is large and shows abnormal plaque quality, such as eccentricity, lipid pool and mild calcified lesions. Therefore, in this study, we examined how MS influenced early stage coronary atherosclerosis using 3-dimensional IVUS, paying special attention to plaque vulnerability.
Methods

Study Population
The study population consisted of 70 subjects who had coronary angiography performed for suspected ischemic heart disease based on symptoms or less invasive procedures, such as exercise electrocardiogram test, ultrasonocardiography and radioimmunoassay methods; however, those who had less than 25% stenotic lesion were estimated by ACC/AHA classification. Subjects with medical treatment for hyperlipidemia, hypertension and diabetes mellitus were excluded. Before the study, oral and written informed consent was obtained from all participants.

Analysis of Blood Samples
Blood samples were drawn in the morning after an overnight fast. Total cholesterol and triglyceride concentrations were measured enzymatically using a kit, and high density cholesterol concentration was measured by the selective inhibition method. Low density lipoprotein cholesterol concentration was calculated by Friedewald’s method. Plasma glucose levels were measured using the glucose oxidase method and insulin levels were measured using the enzyme immunoassay method.

Definition of MS
MS was diagnosed by Japanese criteria, which were reported in 2005. Waist circumference in Japanese criteria is controversial, therefore, we estimated visceral fat volume by not waist circumference but by the visceral fat area at the umbilicus level by computed tomography, and if the visceral fat area was more than 100 cm², visceral fat accumulation was judged as positive.

Angiographic and IVUS Study
Coronary angiography was performed by the transfemoral approach using a standard technique. Two experienced angiographers reviewed all coronary angiograms and confirmed that there were no stenotic lesions, and consequently performed IVUS study. To avoid spasm, 1 to 2 mg of nitroglycerin was adminis-

tered before insertion of the 0.014-inch coronary guidewire and IVUS catheter through a coronary guiding catheter. After the IVUS catheter was inserted more than 20 mm proximal to the left descending coronary artery (LAD), motorized auto pullback was performed at 1 mm/second velocity and the percent plaque volume was calculated for 20 mm length on the LAD proximal side as plaque quantity. We also evaluated plaque quality, such as eccentricity, calcification and lipid pool in plaque formation. Eccentricity was identified when the minimum dimension of plaque thickness/dimension on the other side of plaque thickness in the maxi plaque area was less than 0.5. Calcification was defined when a high echo area with back reflection was detected and a lipid pool was defined as when an echo lucent zone was detected during the observation period. Evaluation of plaque quality was performed by 2 experienced observers who had no knowledge of the clinical data.

Statistical Analysis
A commercially available statistical software program (Stat View-J 5.0; HULINKS Inc., Tokyo, Japan) was used for all statistical analyses. Data are expressed as the mean ± standard deviation. Between-group comparisons were performed using Student’s t-test or the Mann–Whitney U test and, the correlation coefficient was estimated by Spearman rank correlation. Multivariate analysis was performed using stepwise regression analysis and multiple regression analysis. A p value of <0.05 was considered statistically significant.

Visceral fat accumulation (≥ 100 cm² at umbilicus levels by CT scan)

+ ≥ 2 factors

Hypert triglyceridemia (≥ 150 mg/dL) and/or
Low HDL-C (< 40 mg/dL)

High blood pressure Systole; ≥ 130 mmHg and/or
Diastole; ≥ 85 mmHg

High blood glucose levels ≥ 110 mg/dL

Fig. 1. Diagnostic criteria of metabolic syndrome.

The Japanese criteria of metabolic syndrome reported in 2005. Waist circumference in Japanese criteria is controversial, therefore, we estimated visceral fat volume by not waist circumference but by the visceral fat area at the umbilicus level by computed tomography, and if the visceral fat area was more than 100 cm², visceral fat accumulation was judged as positive.
Table 1. Baseline clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>MS (−) (n = 43)</th>
<th>MS (+) (n = 27)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58 ± 10</td>
<td>60 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>20/23</td>
<td>20/7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Smoking</td>
<td>11 (26)</td>
<td>14 (52)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Body mass index</td>
<td>23 ± 3</td>
<td>26 ± 3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Visceral fat area (cm²)</td>
<td>104 ± 61</td>
<td>173 ± 42</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Subcutaneous fat area (cm²)</td>
<td>136 ± 49</td>
<td>147 ± 59</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure (systole, mmHg)</td>
<td>123 ± 17</td>
<td>138 ± 8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blood pressure (diastole, mmHg)</td>
<td>67 ± 14</td>
<td>81 ± 10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>211 ± 35</td>
<td>219 ± 37</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>132 ± 33</td>
<td>138 ± 32</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>120 ± 72</td>
<td>180 ± 71</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>55 ± 12</td>
<td>45 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>98 ± 11</td>
<td>116 ± 25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IRI (µU/mL)</td>
<td>6.5 ± 3.4</td>
<td>8.3 ± 3.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.6 ± 0.9</td>
<td>2.4 ± 1.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>9.3 ± 4.3</td>
<td>5.7 ± 2.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

MS = metabolic syndrome
LDL = low-density lipoprotein
HDL = high-density lipoprotein
IRI = immunoreactive insulin
HOMA-IR = homeostasis assessment insulin resistance
Continuous values are the mean ± SD. ( ): %

Results

Patient Characteristics

Patient characteristics are shown in Table 1. Twenty-seven subjects were diagnosed with MS, showing mild abnormal clinical data, and HOMA-IR as a marker of insulin resistance was significantly higher. Inversely, serum adiponectin concentration was significantly lower in subjects with MS than in those without MS. Occurrence in men was significantly higher in subjects with MS than in those without MS; therefore, we estimated high density cholesterol concentration and serum adiponectin concentration in men and in women separately. However, those two factors were significantly lower in subjects with MS than in those without MS both in men and in women (data not shown).

Relationship between MS and Coronary Plaque

Comparisons of percent plaque volume in subjects with or without MS are shown in Fig. 2A. Percent plaque volume was significantly higher in subjects with MS than in those without MS (31±8% vs 21±8%, p<0.0001). Fig. 2B shows the relationship between MS and plaque quality. Subjects with MS had a higher incidence of abnormal plaque quality such as eccentricity (78% vs 58%, p = NS), calcification (67% vs 33%, p < 0.05) and lipid pools (26% vs 7%, p < 0.05) in plaque than in those without MS. Table 2 shows the relationship between each factor of MS and percent plaque volume. In all subjects, each parameter of MS was significantly correlated with percent plaque volume; however, in subjects with MS, only serum adiponectin concentration was significantly correlated with percent plaque volume (correlation coefficient = −0.63, p<0.0001). Furthermore, we estimated the correlation between serum adiponectin concentration and percent plaque volume in men and in women separately in MS subjects. Serum adiponectin concentration was significantly correlated with
Table 2. Correlation between metabolic syndrome factors and percent plaque volume

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>MS subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log-Triglyceride</td>
<td>0.25*</td>
<td>-0.10</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-0.32**</td>
<td>-0.06</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>0.44***</td>
<td>0.20</td>
</tr>
<tr>
<td>Blood pressure (systole)</td>
<td>0.31**</td>
<td>0.12</td>
</tr>
<tr>
<td>Blood pressure (diastole)</td>
<td>0.27*</td>
<td>0.06</td>
</tr>
<tr>
<td>Visceral fat area</td>
<td>0.43**</td>
<td>0.01</td>
</tr>
<tr>
<td>Log-HOMA-IR</td>
<td>0.33**</td>
<td>0.15</td>
</tr>
<tr>
<td>Log-Adiponectin</td>
<td>-0.52***</td>
<td>-0.63***</td>
</tr>
<tr>
<td>No of MS factors</td>
<td>0.54***</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Data are expressed as the correlation coefficient for percent plaque volume.

*p<0.01, **p<0.001, ***p<0.0001

Abbreviations as in Table 1.

percent plaque volume both in men (correlation coefficient = -0.62, p<0.01) and women (correlation coefficient = -0.73, p<0.05). On the other hand, low-density lipoprotein cholesterol concentration was not significantly correlated with percent plaque volume in all subjects and in MS subjects (data not shown).

Multivariate Analysis for Percent Plaque Volume

All factors of MS were significantly correlated with percent plaque volume; therefore, to clarify which factors of MS were important for percent plaque volume, we performed multivariate analysis of the percent plaque volume among the related factors. First, we performed stepwise regression analysis and three factors (serum adiponectin concentration, visceral fat area, number of MS factors in an individual) were selected. Then we performed multiple regression analysis using these three factors and the serum adiponectin concentration was selected as the strongest variable for percent plaque volume (t value = -3.0, p<0.01) as a subordinate factor (Table 3).

Serum Adiponectin Concentration and Plaque Quality

Fig. 3 shows the relationship between serum adiponectin concentration and plaque quality. We divided the subjects into two groups, higher or lower, by the mean value of log-adiponectin levels (cut-off level, men: 5.6 μg/mL, women: 9.4 μg/mL), which was similar to the mean value in another Japanese report. Subjects in the lower group had a high incidence of abnormal plaque quality compared to those in the higher group. In particular, calcification occurred significantly more frequently in subjects in the lower group (61% vs 32%, p<0.05) than in the higher group. As shown in Table 4, we performed multiple regression analysis for the presence of calcification in plaque formation as a subordinate factor, and age and hypoadiponectinemia were selected as independent variables for calcification (p<0.01, <0.05, respectively) among the factors related to calcification. Furthermore, we estimated the degree of calcification, which was estimated by the arc of calcification and the number of calcified lesions during the observation area in subjects in the lower group (Fig. 4). Concerning the arc of calcifi-

Table 3. Results of multiple regression analysis for percent plaque volume

<table>
<thead>
<tr>
<th>Explanatory factor</th>
<th>Standard regression coefficient</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log-adiponectin</td>
<td>-0.36</td>
<td>-3.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Visceral fat area</td>
<td>0.25</td>
<td>2.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>No of MS factors</td>
<td>0.12</td>
<td>0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Percent plaque volume</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Explanatory factors
Serum triglyceride concentration, Serum HDL-cholesterol concentration, Systolic blood pressure, Diastolic blood pressure, Body mass index, Visceral fat area, HOMA-IR, Serum adiponectin concentration, Sex, Smoking, Age, Number of MS factors
R²=0.35, F value=11.8, p<0.0001, (n=70)
Abbreviations as in Table 1.
Table 4. Results of multiple regression analysis for presence of calcification as plaque

<table>
<thead>
<tr>
<th>Explanatory factor</th>
<th>Standard regression coefficient</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.43</td>
<td>3.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>hypoadiponectinemia</td>
<td>0.32</td>
<td>2.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fasting blood sugar</td>
<td>0.15</td>
<td>1.2</td>
<td>-</td>
</tr>
<tr>
<td>Number of MS factors</td>
<td>0.14</td>
<td>1.1</td>
<td>-</td>
</tr>
<tr>
<td>Visceral fat area</td>
<td>0.12</td>
<td>0.9</td>
<td>-</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-0.03</td>
<td>-0.2</td>
<td>-</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>-0.01</td>
<td>-0.06</td>
<td>-</td>
</tr>
</tbody>
</table>

Subordinate factor
Calcification (−) = 0  Calcification (+) = 1

Hypoadiponectinemia was identified as subjects whose serum adiponectin concentration was less than the mean value of log-adiponectin levels.

R² = 0.42, F value = 17.2, p < 0.0001, (n = 70)
Abbreviations as in Table 1.

Fig. 4. Calcification and hypoadiponectinemia.
These graphs show the degree of calcification in subjects in the lower group. We estimated the arc of calcification, which is the max value (left graph), and the number of calcified lesions (right graph) in the observation area.

Discussion
Subjects with MS had not only a high percent of plaque volume but also abnormal plaque quality in early stage coronary atherosclerosis, which was not detectable by coronary angiography. Each factor of MS was significantly correlated with percent plaque volume; however, multivariate analysis revealed that serum adiponectin concentration was the most important variable for determining percent plaque volume. Furthermore, subjects with hypoadiponectinemia had a close association with calcification in plaque, and future calcification was predicted by a mild degree of plaque formation and plural calcification.

Significance of MS for Plaque Formation in Early Stage Coronary Atherosclerosis
Various diagnostic criteria of MS have been indicated worldwide[14, 19, 20], and in Japan, visceral fat accumulation is considered crucial in MS. In this study, subjects with MS diagnosed by Japanese criteria had a significantly higher percent of plaque volume than those without MS; furthermore, multivariate analysis revealed that the visceral fat area was an independent variable for percent plaque volume. On the other hand, MS subjects showed a high incidence of abnormal plaque quality, such as plaque eccentricity, calcification and lipid pools. Therefore, even though each component of MS plays an important role in the progression of atherosclerosis[21-24], the Japanese criteria of MS, which stress visceral fat accumulation, is a reasonable definition to detect the population at high risk for acute coronary syndrome, and we should detect and treat MS subjects as early as possible. Some reports have emphasized that insulin resistance is a central factor in the pathogenesis of MS[25, 26], and insulin resistance is also known to play an important role in the progression of coronary atherosclerosis[27-30]. Recently, insulin resistance was reported to affect not only skeletal muscle and the liver but also vessel walls[31-35]. Kashiwagi A et al. [36-39] reported that insulin resistance/hyperinsulinemia causes endothelial dysfunction and smooth muscle cell proliferation through the pathway of oxidative stress or inflammation, and consequently insulin resistance promotes atherosclerosis. In this study, there was a significant relationship between HOMA-IR as a marker of insulin resistance and percent plaque volume; however, multivariate analysis revealed that HOMA-IR did not select independent variables for percent plaque volume. Therefore, even though insulin resistance plays an important role in the progression of coronary atherosclerosis, our data indicate that a background of insulin resistance...
such as visceral fat accumulation, hypoadiponectinemia and clustering of MS factors, plays a more important role in plaque formation in the early stage. Insulin resistance also did not have a significant relationship with abnormal plaque quality; however, it is well-known that oxidative stress and inflammation are closely associated with plaque instability. Thus, insulin resistance might promote plaque instability, which is not detectable by gray scale IVUS findings, considering the relationship between insulin resistance and oxidative stress or inflammation in atherosclerosis.

Adiponectin and Plaque Instability

In multivariate analysis, serum adiponectin concentration was selected as the most important factor for percent plaque volume. Recent basic and clinical research clarified a link between adiponectin and atherosclerosis. Eynatten M et al. reported a relationship between hypoadiponectinemia and the severity of coronary atherosclerosis, diagnosed by coronary angiography. Furthermore, our data indicated that hypoadiponectinemia was an important factor in plaque formation in early stage coronary atherosclerosis. Interestingly, in subjects with MS, only serum adiponectin concentration was significantly correlated with percent plaque volume; therefore, our results suggest that blood adiponectin concentration is the most important therapeutic target marker to suppress early stage plaque formation in MS subjects who are considered a high-risk population for cardiovascular events. Nakamura T et al. reported that the clustering of MS components dramatically increases coronary events; however, our data indicated that hypoadiponectinemia was a more important factor for percent plaque volume than clustering of MS components. Actually, hypoadiponectinemia is considered as the cause of clustering of MS components through insulin resistance and hypoadiponectinemia is known to be a useful marker of the pathogenesis of MS. Furuhashi M et al. reported that decreased adiponectin concentration in coronary blood flow, estimated by simultaneous measurement of the aortic root and coronary sinus, reflected the severity of angiographical coronary atherosclerosis. This suggests that a high concentration of adiponectin exists in coronary blood flow, and adiponectin directly resists the progression of coronary atherosclerosis. Therefore, even though clustering of MS components is an important factor in the occurrence of coronary events, the results of multivariate analysis suggest that the decreased defensive effect of hypoadiponectinemia on coronary plaque formation is a more important factor.

Calcification in plaque formation is controversial; however, clinical studies have shown that coronary calcification, detected by computed tomography, is a powerful predictor of cardiac events. Raggi et al. reported that coronary calcification identified by electron-beam computed tomography was highly prevalent in patients with acute myocardial infarction; thus, coronary calcification might be indicated in the presence of vulnerable plaque. Furthermore, a recent IVUS study performed by Ehara et al. reported that culprit lesions in acute coronary syndrome had a significantly higher incidence of mild calcification than in stable angina pectoris. Therefore, our results can be interpreted as follows: from the viewpoint of calcification, subjects with hypoadiponectinemia already have vulnerable plaque in early stage coronary atherosclerosis. The pathogenesis of coronary calcification is not fully understood; however, Wang TJ et al. and Arad Y et al. reported that C-reactive protein as a marker of inflammation is an independent factor of coronary calcification in the subclinical stage. TNF-α is known to be a major inflammatory cytokine, and adiponectin is associated with lower TNF-α expression and has been shown to suppress TNF-α–induced activation of nuclear transcription factor-κB. Therefore, hypoadiponectinemia might be promoted in inflammation of the coronary vessels, and consequently, calcification. Another pathway of calcification by hypoadiponectinemia might exist and, in fact, Maahs et al. reported that hypoadiponectinemia was an independent predictor of the progression of coronary calcification in patients without coronary events, diagnosed by electron-beam computed tomography; however, the details are not fully understood and the precise mechanism should therefore be studied further.

Hypercholesterolemia is established as one of the most important coronary risk factors; however, serum cholesterol levels in subjects with coronary artery disease often appear within the normal range in the Japanese population. There was no relationship between low density lipoprotein cholesterol levels and percent plaque volume or abnormal plaque quality in this study. Recent clinical studies reported that subjects with acute coronary syndrome showed significantly lower adiponectin concentrations than those with stable angina pectoris or control subjects. In this study, on the other hand, hypoadiponectinemia was closely associated with vulnerable plaque formation; therefore, hypoadiponectinemia is considered an important target factor to prevent acute coronary syndrome, more so than hypercholesterolemia, in the Japanese population, whose cholesterol levels are often in the normal range.
Limitations

In this study, the sample volume was small and the observation area by IVUS was limited on the LAD proximal side, because IVUS is an invasive procedure. Therefore, analysis by IVUS study for many participants was very difficult and we observed a near straight line of coronary anatomy for safety. We used HOMA-IR as a marker of insulin resistance. Glucose clamp methods are considered the gold standard to evaluate insulin resistance; however, they are problematic in daily practice, and HOMA-IR is considered a reliable insulin resistance marker in vivo, especially in subjects whose fasting blood glucose concentrations are not so high. Therefore, we used HOMA-IR as a marker of insulin resistance, considering the burden on patients and medical staff. Plaque vulnerability, especially the lipid area in plaque, was very difficult to evaluate by gray scale IVUS. Further study is needed to confirm the relationship between hypoadiponectinemia and the lipid area in plaque, was very difficult to evaluate by gray scale IVUS. Further study is needed to confirm the relationship between hypoadiponectinemia and the lipid area in plaque formation using another method, such as multi-directional computed tomography, virtual histology, integrated backscatter-IVUS, or coronary angiography.

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