Levels of Soluble E-selectin and ICAM-1 in the Coronary Circulation of Patients with Stable Coronary Artery Disease

Association with the Severity of Coronary Atherosclerosis

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SUMMARY

The recruitment of circulating leukocytes to atherosclerotic sites is mediated by a family of adhesion molecules. The objective of the present study was to evaluate the relationship between circulating adhesion molecule levels in the coronary circulation and the severity of coronary atherosclerosis in patients with stable coronary artery disease.

The subjects were 79 patients undergoing coronary angiography. According to the severity of coronary atherosclerosis as assessed by the Gensini Score (GS) of the left coronary artery, they were classified into three groups: group C (no organic stenosis, score 0, \( n=14 \)), group M (mild organic stenosis, score 1-13, \( n=39 \)) and group S (severe organic stenosis, score \( \geq 14 \), \( n=26 \)). Blood samples were taken from the aorta (Ao) and coronary sinus (CS), and plasma levels of soluble E-selectin (sE-selectin) and soluble intercellular adhesion molecule-1 (sICAM-1) were measured by enzyme-linked immunosorbent assay. These levels were then compared between groups.

There were no significant differences in plasma sICAM-1 levels in the Ao or CS between the three groups. The difference in sICAM-1 levels between the CS and Ao (CS-Ao) also showed no significant difference. Plasma sE-selectin levels in both the Ao and CS were significantly higher in group S than in groups C and M (\( p<0.05 \)), but there were no significant differences in CS-Ao. There was a weak but significant correlation between the plasma levels of these adhesion molecules and the number of coronary risk factors present. Multivariate analysis showed that the number of coronary risk factors was the only positive predictor (\( p=0.0048 \)) of the GS; there was no association between the plasma level of either adhesion molecule and the GS.

In patients with stable coronary artery disease, sICAM-1 plasma levels do not indicate the severity of coronary atherosclerosis, while sE-selectin plasma levels appear to reflect the severity of systemic rather than coronary atherosclerosis. (Jpn Heart J 2002; 43: 93-101)
Key words: Coronary artery disease, Soluble adhesion molecules, Intercellular adhesion molecule-1, E-selectin

The adhesion of leukocytes to the vascular endothelium and their subsequent migration into the subendothelial layers are major events in the development of atherosclerosis. Endothelial adhesion molecules are expressed on the surfaces of activated endothelial cells, and play an important role in the recruitment of inflammatory cells and the control of leukocyte migration.\(^1,2\) E-selectin allows the initial movement of leukocytes across the endothelial surface,\(^3\) while intercellular adhesion molecule-1 (ICAM-1) strengthens the attachment of leukocytes to the endothelium and promotes their migration.\(^4\) Soluble types of these adhesion molecules are thought to be shed from activated cells and can now be measured in peripheral blood.\(^5\) Many studies have reported a relationship between levels of circulating adhesion molecules and coronary artery disease;\(^6-17\) however, most investigated levels in peripheral blood. In the present study, we obtained blood samples simultaneously from both the aortic root (Ao) and the coronary sinus (CS), and investigated whether plasma levels of these adhesion molecules in the coronary circulation reflected the severity of coronary atherosclerosis in patients with stable coronary artery disease.

**Patients and Methods**

**Patient groups:** Seventy-nine patients undergoing cardiac catheterization for proven or clinically suspected coronary artery disease were recruited for the present study (63 men and 16 women; mean age 62\(\pm\)11 years, range 21-79 years). Exclusion criteria were acute myocardial infarction within the previous 2 weeks, unstable angina pectoris, New York Heart Association class III or IV congestive heart failure or a left ventricular ejection fraction <25%, active malignancy, and acute or chronic liver or kidney disease. The study protocol was approved by the Niigata University Hospital Ethics Committee, and written informed consent was obtained from each patient before participation.

**Coronary angiography and blood sampling:** In all patients, coronary angiography was performed using the Judkins technique with a 6F catheter (Johnson & Johnson, Miami Lakes, FL). Before coronary angiography, the Judkins catheter was introduced via the femoral artery and advanced into the ascending aorta, and a 7F Simmons catheter (Hanako-Medical Co., Urawa, Japan) was inserted through the femoral vein and advanced into the coronary sinus. Blood samples were then obtained simultaneously from the aortic root and coronary sinus.
Determination of the severity of coronary atherosclerosis: The severity of coronary atherosclerosis was assessed using the Gensini Score (GS),\textsuperscript{(18)} which grades narrowing of the lumens of the coronary arteries as 1 for 1-25% narrowing, 2 for 26-50% narrowing, 4 for 51-75% narrowing, 8 for 76-90% narrowing, 16 for 91-99% narrowing, and 32 for total occlusion. This score is then multiplied by a factor that takes into account the importance of the lesion's position in the coronary arterial tree, for example, 5 for the left main coronary artery, 2.5 for the proximal left anterior descending artery (LAD) or proximal left circumflex artery (LCx), 1.5 for the mid-region of the LAD, and 1 for the distal LAD or mid-distal region of the LCx. For the purposes of this study, the GS was expressed as the sum of the scores for the left coronary arteries. According to this score, the patients were classified into three groups: group C (patients with no organic stenosis and no inducible coronary vasospasm in right and left coronary arteries, score 0, \( n = 14 \)), group M (patients with mild organic stenosis, score 1-13, \( n = 39 \)) and group S (patients with severe organic stenosis, score \( \geq 14 \), \( n = 26 \)).

Measurement of soluble ICAM-1 and E-selectin levels: Plasma was separated from the blood samples by centrifugation and stored at \(-40^\circ\text{C}\) until required for the assay of adhesion molecules. Levels of soluble ICAM-1 and E-selectin were determined using an enzyme-linked immunosorbent assay kit (Genzyme-Techne, Minneapolis, MN). All assays were performed in duplicate.

Data analysis: Detailed clinical information, including age, gender, coronary risk factors, any history of previous myocardial infarction and prior coronary intervention, was compared between the groups. Plasma levels of sICAM-1 and sE-selectin in the Ao and CS samples were evaluated, and differences in these levels between the CS and Ao samples (CS-Ao) were calculated. In addition, we examined the associations between the plasma levels of these adhesion molecules and the number of major coronary risk factors present. A multivariate analysis including age, gender, sICAM-1 and sE-selectin plasma levels and the number of coronary risk factors was also performed to identify any independent predictors of the severity of coronary atherosclerosis.

Statistical analysis: Values are expressed as the mean±SD. Between-group comparisons of soluble adhesion molecule plasma levels, age, systolic and diastolic blood pressure (SBP and DBP), serum total cholesterol, and serum triglyceride levels were performed using a one-way ANOVA followed by the Bonferroni multiple comparison test. Gender, the number of previous myocardial infarctions, prior coronary interventions, the presence of hypertension (SBP\( \geq 140 \) and/or DBP\( \geq 90 \) mmHg), diabetes mellitus and hypercholesterolemia (serum total cholesterol \( \geq 220 \) mg/dL) were compared using the chi-squared test. Stepwise regression analysis was also performed to identify any independent predictors of the severity of coronary atherosclerosis. The variables tested included age, gender,
sICAM-1 and sE-selectin plasma levels, and the number of coronary risk factors. For all analyses, \( P \) values <0.05 were considered statistically significant.

**RESULTS**

**Patient characteristics:** The Table shows the clinical characteristics of the three groups. There were no significant differences in age, gender, the presence of hypertension, SBP, DBP, smoking history, the presence of diabetes mellitus or hypercholesterolemia, and serum total cholesterol and serum triglyceride levels. There were, however, significant differences in the incidence of previous myocardial infarction (\( P <0.05 \)) and the history of prior coronary intervention (\( P <0.01 \)).

**Plasma sICAM-1 levels in the Ao and CS, and CS-Ao values:** There were no significant differences in plasma sICAM-1 levels in the Ao (group C: 220 ± 83 ng/mL, group M: 249 ± 120 ng/mL, group S: 223 ± 72 ng/mL) or CS (group C: 215 ± 71 ng/mL, group M: 258 ± 114 ng/mL, group S: 218 ± 72 ng/mL) between the three groups. The CS-Ao values for sICAM-1 also showed no significant differences (group C: -4.9 ± 35, group M: 8.6 ± 19, group S: -5 ± 27) (Figure 1).

<table>
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<th>Table. Clinical Characteristics of the Study Subjects</th>
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<td>Control (n=14)</td>
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<td>Age (years)</td>
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Data presented are mean value±SD or number (percentage).
Plasma sE-selectin levels in the Ao and CS, and CS-Ao values: The plasma sE-selectin level in the Ao was significantly higher in group S (46±17 ng/mL) than in the other two groups (group C: 36±9 ng/mL, group M: 38±13 ng/mL, p<0.05). The plasma sE-selectin level in the CS was also significantly higher in group S (group S: 46±16 ng/mL, group C: 36±9 ng/mL, group M: 37±12 ng/mL, p<0.05). However, there were no significant differences in the CS-Ao values (group C: 0.143±4.9, group M: -0.923±3.6, group S: 0.077±4.3) (Figure 2).

Plasma levels of adhesion molecules and coronary risk factors: We examined possible associations between adhesion molecule plasma levels and the number of major coronary risk factors, such as hypertension, hypercholesterolemia, diabetes mellitus, and smoking history. There was a weak but significant relationship between the adhesion molecule plasma levels and the number of coronary risk factors present (Figure 3).

Multivariate analysis of potential influences on the severity of coronary atherosclerosis: Multivariate stepwise regression analysis identified the number of risk factors as the only positive predictor of the severity of coronary atherosclerosis (p=0.0048). Plasma sICAM-1 and sE-selectin levels showed no association with the severity of coronary atherosclerosis as assessed by the GS.
Figure 2. Individual and mean values for plasma sE-selectin in the aortic root (Ao) (left-hand panel) and the coronary sinus (CS) (right-hand panel) in patients with normal coronary arteries (C), mild coronary atherosclerosis (M), and severe coronary atherosclerosis (S).

Figure 3. Correlations between the number of coronary risk factors present and the plasma levels of sICAM-1 in the aortic root (Ao) (A) and coronary sinus (CS) (B), and of sE-Selectin in the Ao (C) and CS (D).
DISCUSSION

The major results of the present study can be summarized as follows: 1) In patients with stable coronary artery disease, there was no significant increase in the levels of either sE-selectin or sICAM-1 in the coronary circulation. 2) Plasma levels of sE-selectin in both the CS and Ao were significantly higher in patients with severe coronary atherosclerosis. 3) Plasma levels of sICAM-1 in the CS and Ao were not associated with the severity of coronary atherosclerosis. 4) There was a significant relationship between the adhesion molecule plasma levels and the number of coronary risk factors present. 5) Multivariate analysis showed that only the number of risk factors present influenced the severity of coronary atherosclerosis.

ICAM-1 belongs to the most abundant family of cell surface molecules, the immunoglobulin super-family, and acts to ensure the firm attachment of leukocytes to activated endothelial cells. ICAM-1 levels in the peripheral blood have been shown to increase within 24 hours of the onset of acute myocardial infarction, and to be significantly higher in patients with unstable angina pectoris than in those with stable angina pectoris or control subjects. In the latter study, there was no significant difference in sICAM-1 plasma levels between the patients with stable angina pectoris and the control subjects. A further study also found no significant association between sICAM-1 plasma levels and the severity of atherosclerosis. These studies were all performed using peripheral blood. However, Ogawa, et al reported that mean plasma sICAM-1 levels in both the coronary sinus and the aortic root were significantly higher in patients with unstable angina than in those with stable exertional angina or control subjects; furthermore, the mean level in the coronary sinus was higher than that in the aortic root. In the present study, no association between sICAM-1 levels and the severity of coronary atherosclerosis was detected, and no significant increase in levels in the coronary circulation was observed among patients with stable coronary artery disease. With regard to the associations between plasma sICAM-1 levels and the number of major coronary risk factors, a previous study revealed that sICAM-1 levels were associated with increasing prevalence of cardiovascular risk factors, such as age, diabetes mellitus, smoking, hypertension, low level of high density lipoprotein, and hypertriglyceridemia. Also in our study, there was a significant relationship between the plasma sICAM-1 level and the number of coronary risk factors in patients with stable coronary artery disease. Our results are therefore similar to those of previous studies.

E-selectin belongs to the selectin family and mediates the initial movement of leukocytes across the endothelial surface. With regard to plasma E-selectin levels in coronary artery disease, several studies have reported significant eleva-
tions in patients with acute coronary syndromes compared with control subjects.\textsuperscript{21,22} In contrast to sICAM-1, a number of studies have revealed that plasma sE-selectin levels were significantly higher in patients with stable coronary artery disease than in control subjects. However, full agreement has not been reached regarding the association between sE-selectin levels and the severity of coronary atherosclerosis. One study reported that plasma sE-selectin levels were significantly higher in patients with mild coronary atherosclerosis than in those with severe coronary atherosclerosis or control subjects,\textsuperscript{23} while another found a trend towards a decrease in sE-selectin concentrations as the angiographically assessed extent and severity of coronary artery disease increased.\textsuperscript{12} However, these studies were both performed using peripheral blood, and there are no published studies which have examined changes in sE-selectin levels within the coronary circulation in patients with stable coronary artery disease. In the present study, there was a significant relationship between soluble E-selectin plasma levels in both the coronary sinus and the aortic root and the severity of coronary atherosclerosis, although no increase in levels was observed within the coronary circulation. Moreover, there was a significant relationship between the plasma sE-selectin levels and the number of coronary risk factors. With regard to the associations between plasma sE-selectin level and major coronary risk factors, some previous reports revealed that plasma sE-selectin levels were higher in patients with essential hypertension,\textsuperscript{24} hypercholesterolemia,\textsuperscript{25} and diabetes mellitus.\textsuperscript{26} However, there is no report about the association between the plasma sE-selectin levels and the numbers of the major coronary risk factors. Our results imply that the plasma sE-selectin measured during the study was not derived from the coronary circulation, although levels were higher in the patients with severe coronary atherosclerosis. Indeed, our multivariate analysis indicated that the GS was only influenced by the number of coronary risk factors present, and that the sE-selectin level is not an independent predictor of the severity of coronary atherosclerosis.

In conclusion, the present study showed that plasma sICAM-1 levels do not indicate the severity of coronary atherosclerosis in stable coronary artery disease, and that although sE-selectin levels show some relation to the severity of atherosclerosis, they do not influence the GS. Indeed, the severity of coronary atherosclerosis correlated only with the number of coronary risk factors present. Therefore, plasma sE-selectin levels appear to reflect systemic rather than specific coronary atherosclerosis.

\textbf{REFERENCES}