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Vascular Cell Adhesion Molecule-1 and Intercellular Adhesion Molecule-1 Serum Level in Patients with Chest Pain and Normal Coronary Arteries (Syndrome X)

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Summary

Background: Plasma levels of soluble vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) mediators of leukocyte adhesion to vascular endothelium may implicate in the pathogenesis of the syndrome of chest pain with normal coronary arteries.

Hypothesis: We attempted to determine whether markers of endothelial activation are raised in patients with chest pain and normal coronary arteries.

Methods: We measured plasma VCAM-1, ICAM-1 (ng/ml) in 36 patients (34 men, 2 women, aged 62 ± 9 years) with stable angina, coronary artery disease (CAD), and a positive response to exercise test; in 21 patients (6 men, 15 women, aged 56 ± 9 years) with chest pain and normal coronary arteriograms (syndrome X); and in 11 healthy control subjects (8 men, 3 women, aged 49 ± 14 years).

Results: Plasma ICAM-1 levels were significantly higher both in patients with CAD (mean ± standard error of the mean) (328 ± 26, $p < 0.05$), and in syndrome X (362 ± 22, $p < 0.01$) than in controls (225 ± 29). VCAM-1 levels were also higher in syndrome X (656 ± 42 ng/ml) and in patients with CAD (626 ± 42 ng/ml) than in controls (551 ± 60, $p = 0.09$).

Conclusions: ICAM-1 and VCAM-1 levels are increased both in patients with CAD and with syndrome X compared with control individuals. These findings may suggest the presence of chronic inflammation with involvement of the endothelium in patients with anginal chest pain and normal coronary angiograms.

Key words: endothelium, coronary artery disease, adhesion molecules, syndrome X

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Introduction

Syndrome X describes a group of patients with exertional chest pain associated with ST-segment depression and angiographically normal coronary arteries.[1–4] Studies have shown that these patients may have impaired coronary flow reserve[5–8] and metabolic abnormalities consistent with myocardial ischemia during rapid atrial pacing.[9] It has been reported that coronary microvascular endothelial dysfunction plays an important pathogenetic role in these patients.[10–14] The mechanisms responsible for this endothelial dysfunction in syndrome X are unclear;[15] however, chronic inflammation may lead to vascular dysfunction. This process may involve leukocyte activation and adhesion to vascular endothelium. Different families of cellular adhesion molecules are expressed on the surface of vascular endothelial cells following activation by diverse stimuli and may play a role in microvascular angina.[16] Among adhesion molecules, the expression and biological properties of vascular cell adhesion molecule-1 (VCAM-1) and intracellular cell adhesion molecule-1 (ICAM-1) are well characterized.[17, 18] Circulating forms of VCAM-1 and ICAM-1 have been detected in plasma, and their levels are elevated during inflammatory conditions.[17, 19, 20] A recent study in patients with coronary artery disease (CAD) showed that ICAM-1 and E-selectin are molecular markers for both atherosclerosis and the development of CAD.[21] The present study tested the hypothesis that chronic inflammation and endothelial activation may be present in patients with chest pain and normal coronary arteriograms and may contribute to the pathogenesis of this syndrome.

We compared plasma levels of circulating VCAM-1 and ICAM-1 in patients with CAD, with syndrome X, and in healthy controls, and assessed whether levels of circulating adhesion molecules relate to ST-segment change and clinical presentation.

Methods

Patients

Three groups were studied: (1) 36 patients (34 men, 2 women) aged 44–74 years (62 ± 9 years) with chronic stable angina, CAD, and a positive treadmill exercise test result; (2) 21 patients (6 men, 15 women), aged 39–71 years (56 ± 9 years), with syndrome X; (3) 11 healthy volunteers (8 men, 3 women, 49 ± 14 years). None of the control patients had heart disease,

diseases of other major organs, or chronic inflammatory conditions. Information about medical history and risk factors for CAD was obtained from standardized and validated interviewer-administered questionnaires. A positive exercise test was defined as ≥ 0.1 mV ST-segment depression at between 5 to 7 METS using the modified Bruce protocol. Hypertension was defined as a systolic blood pressure ≥ 150 mmHg, a diastolic blood pressure ≥ 95 mmHg, or current use of antihypertensive medications. Diabetes mellitus was defined as fasting glucose ≥ 140 mg/dl, nonfasting glucose ≥ 200 mg/dl, or a history of treatment of diabetes.

Syndrome X was defined as exertional angina, a positive exercise test with angina, and angiographically normal epicardial coronary arteries with no evidence of coronary spasm.[1,2] None of the patients had a history suggestive of variant angina, and epicardial coronary artery spasm was excluded in all patients on the basis of a negative ergonovine test. Resting electrocardiograms and resting echocardiograms were normal. Left ventricular hypertrophy was assessed by two-dimensional guided M-mode echocardiograms according to the American Society of Echocardiography recommendations.[22] Left ventricular hypertrophy was excluded by using gender-specific normal limits from the Framingham study.[23]

Protocol

Antianginal medication was stopped 24 h prior to the study. The patients were allowed to use sublingual nitroglycerin, as necessary, but no study was performed within 3 h of its administration. The protocol was approved by the Research Ethics Committee, and each patient gave written informed consent.

Measurements

After a 12-h fast, a blood sample was obtained by standard venipuncture into tubes containing ethylene diamine tetraacetic acid (EDTA) (1 mg/dl) and centrifuged at 3000 g at 4°C for 10 min to obtain plasma. Samples were frozen at −20°C until assayed. Levels of serum lipids and hemostatic factors were measured in centralized laboratories by standard and validated methods. Circulating VCAM-1 and ICAM-1 levels were determined by the commercially available ELISA and standards methods.[21]

Statistical Analysis

Data are expressed as mean ± standard deviation of the mean (SD). Those who had extreme values of VCAM-1 and ICAM-1 (± 3 SD from mean) were excluded from statistical analysis. Characteristics of the patients and control subjects were evaluated with Student's *t*-test for continuous variables and with the chi-square test for categorical variables. The skewed distributions of VCAM-1 and ICAM-1 levels were compared between patients and control subjects by the Wilcoxon rank-sum test. A regression analysis was used to evaluate the relationship between adhesion molecule level, lipid level, and exercise test parameters. P values of < 0.05 (two tailed) were considered to indicate statistical significance.

Results

Table I summarizes the clinical characteristics and risk factor distribution of the three groups. A similar proportion of patients with CAD and with syndrome X had a history of hyperlipidemia and their lipid levels were similar (Table I). The plasma level of ICAM-1 (mean ± SEM) was significantly greater in the CAD (328 ± 26, $p < 0.05$), and syndrome X (362 ± 22, $p < 0.01$) groups than in the control group (225 ± 29). The VCAM-1 level was also higher in patients with CAD (626 ± 42) and syndrome X (656 ± 42) than in controls (551 ± 60), but this difference did not reach statistical significance ($p = 0.1$ and $p = 0.09$, respectively) (Fig. 1). In the syndrome X group, there was no significant correlation between VCAM-1 and ICAM-1 levels and maximum ST-segment depression ($r = 0.13$ and $r = 0.19$, respectively).

Discussion

The results of this study show that both in patients with CAD and syndrome X plasma levels of ICAM-1 and VCAM-1 were increased compared with healthy individuals. This finding suggests that inflammatory activity involving the endothelium is present in patients with microvascular angina.

Macrophage adherence to endothelium occurs through binding of highly regulated cell adhesion molecules expressed on the surface of macrophages and endothelial cells.[24] Local accumulation of leukocytes in the vascular wall occurs as a result of initial marginalization and rolling of leukocytes along

TABLE I Clinical and angiographic characteristics of patients

	Stable angina (n = 36)	Syndrome X (n = 21)	*Normal* controls (n = 11)
Age (years)	62 ± 9	56 ± 9	49 ± 14
Gender			
Male	34	6	8
Female	2	15	3
Risk factors			
Hypertension	3	4	0
Hyperlipidemia	13 (36%)	8 (38%)	0
Smoking	6	0	0
Diabetes	4	0	0
Myocardial Infarction	8	0	0
Cholesterol level (mg/dl)	203 ± 6	211 ± 6	187 ± 9
Triglyceride (mg/dl)	162 ± 12	167 ± 22	119 ± 5
HDL (mg/dl)	41 ± 1	47 ± 2	46 ± 3
LDL (mg/dl)	121 ± 5	129 ± 5	103 ± 4
Coronary artery disease extent			
Single-vessel disease	5	—	—
Double-vessel disease	11	—	—
Triple-vessel disease	20	—	—

Abbreviations: HDL = high-density lipoprotein, LDL = low-density lipoprotein.

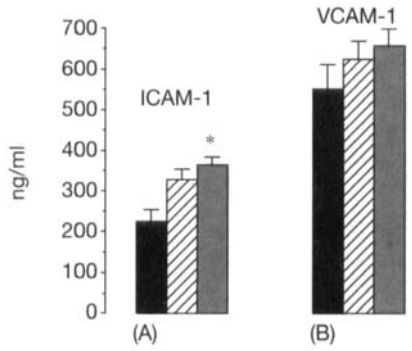

FIG. 1 Bar graph showing mean plasma level of ICAM-1 (A) and VCAM-1 (B) in normals, in patients with coronary artery disease (CAD), and in patients with syndrome X (* = $p < 0.01$ vs. controls). ■ = Control, ▨ = CAD, ■ = syndrome X.

the endothelium.[25] A process mediated by selectins leads to their attachment to endothelial cells and transmigration into the intimal spaces, a process mediated by the adhesive molecules expressed by activated endothelium.[16] Evidence identifying the specific adhesion molecules involved in macrophage adherence comes largely from immunohistochemical and in vitro studies and implicates VCAM-1 and ICAM-1.[26–28] The expression of VCAM-1[28] and ICAM-1[29] has been shown to be upregulated in regions overlying atheromatous lesions. These adhesion molecules are inducible on the endothelial cell surface and can support the adhesion of various leukocytes, including monocytes.[30] Immunohistochemical study has shown expression of ICAM-1 in human atherosclerotic plaques, and it may therefore play an important role in mediating the localization of monocytes in the intima of arteries.[29] A recent study[31] showed that a4 integrin and ICAM-1 play major roles in the recruitment of macrophages to atherosclerotic plaques. In addition, endothelial cells also interact with cells in the bloodstream where their chemotactic action can induce leukocytes and platelets to migrate to the endothelial wall.[29]

Previous studies have shown that the thickness of the intimal lesions of arteries and the intensity of VCAM-1 expression correlated positively.[21] The upregulation of VCAM-1 coincided with the elevated expression of tumor necrosis factor-α in vascular medial and intimal layers as well as in interstitial mononuclear infiltrates.[32] Other studies indicated that soluble VCAM-1 is a good biohumoral correlate of overt atherosclerosis, independent of underlying hypertension, and may be an in vivo marker of endothelial activation.[33]

Recent evidence suggests that lipoprotein(a) contributes to the development of cardiovascular diseases by selectively enhancing the expression of ICAM-1 in endothelial cells.[34] A significant association was found between increasing soluble ICAM-1 and risk of future myocardial infarction, especially among participants with baseline ICAM-1 concentrations in the highest quartile.[35] Moreover, in the ARIC study[21] it has been shown that plasma levels of ICAM-1, E-selectin, and not VCAM-1 may serve as molecular markers for atherosclerosis and the development of CAD. In atherosclerotic plaques, the expression of VCAM-1, ICAM-1, and E-selectin was more prevalent on intimal neovasculature than on arterial luminal endothelium.[36] Furthermore, the presence in neovasculature and neoendothelial cells of VCAM-1 and ICAM-1 was strongly associated with increased intimal leukocyte accumulation.[36]

An association between ICAM-1 and C-reactive protein, a sensitive marker of systemic inflammation, has also been demonstrated.[35] However, unlike C-reactive protein, which is produced by the liver and has an uncertain biological function, ICAM-1 and VCAM-1 have a direct and potentially critical role in the early immune response. Our findings that soluble ICAM-1 level is raised among patients with advanced atherosclerosis as well as in patients with syndrome X extend previous observations about the role of inflammation in coronary atherosclerosis and provide a new insight into the pathogenetic mechanism of syndrome X.

Chronic Inflammation and Endothelial Function in Syndrome X

Evidence suggests that the function of microvascular endothelium is impaired in some patients with chest pain and normal coronary arteriograms.[10, 37] It is known that damaged or activated endothelial cells can secrete vasoconstrictor factors such as endothelin-1.[38] Previous studies have shown that endothelin-1 level is elevated in this group of patients.[39] Moreover, endothelin-1 plasma levels are associated with abnormal coronary blood flow reserve in patients with syndrome X.[40] Our study showed that ICAM-1 levels are increased in syndrome X patients; this may indicate the presence of chronic inflammation in patients with anginal chest pain even in the absence of detectable coronary atherosclerosis.

Conclusions

The present study shows for the first time that patients with syndrome X have raised levels of adhesion molecules similar to those observed in patients with advanced atherosclerosis. Our findings in the CAD group are consistent with other results in the literature and suggest a role of VCAM-1 and ICAM-1 in CAD. Our findings in patients with syndrome X indicate the presence of inflammation with endothelial involvement. Whether raised levels of adhesion molecules are just a marker of inflammation and endothelial dysfunction or a pathogenetic mechanism in syndrome X deserves further investigation.

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