Levels of Soluble Cell Adhesion Molecules in Patients With Angiographically Defined Coronary Atherosclerosis

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Adhesion molecules on the endothelial cell membrane play an important role in the pathogenesis of atherosclerosis. Levels of soluble forms of cell adhesion molecules are reportedly elevated in patients with peripheral artery vessel disease and in patients with an atherosclerotic aorta. The present study investigated the association of serum levels of soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular adhesion molecule 1 (sICAM-1), and soluble P-selectin (sP-selectin) with coronary heart disease (CHD) and the extent of coronary atherosclerosis, and examined the influence of serum levels of lipids, lipoproteins and apolipoproteins (apo) in subjects with (n=52, M/F: 43/9) and without (controls, n=40, M/F:25/15) angiographically proven coronary atherosclerosis. After controlling for age and gender, levels of sVCAM-1 (least squares mean $\pm$ std error: 565 $\pm$ 36 ng/ml vs 540 $\pm$ 41 ng/ml, ns), sICAM-1 (261 $\pm$ 17 ng/ml vs 247 $\pm$ 19 ng/ml, ns), and sP-selectin (142 $\pm$ 8 ng/ml vs 149 $\pm$ 10 ng/ml, ns) in patients with coronary atherosclerosis were not different from those in controls, as assessed by an analysis of covariance. After also adjusting for body mass index, hypertension, diabetes mellitus, and smoking by a multiple logistic function analysis, the association of sVCAM-1, sICAM-1, and sP-selectin with CHD was still not significant. Levels of sVCAM-1, sICAM-1, and sP-selectin were also not related to the extent of coronary atherosclerosis as judged by the number of stenosed vessels. However, inverse ($p < 0.05$) relationships were observed between sVCAMs and serum levels of HDL₃-cholesterol, apo A-II, and lipoprotein containing apo A-I and A-II, between sICAMs and levels of apo A-II and Lp A-I/A-II (Lp A-I/A-II), and between sP-selectin and lipoprotein containing only apo A-I. In conclusion, serum levels of soluble VCAM-1, ICAM-1, and P-selectin were not related to CHD or the extent of coronary atherosclerosis, but were inversely related to serum levels of high-density lipoprotein-related lipoproteins. (*Jpn Circ J* 1999; **63**: 19–24)

Key Words: Atherosclerosis; Coronary angiography; High-density lipoprotein; Soluble cell adhesion molecules

The adhesion of leukocytes to endothelial cells and their transendothelial migration are critical early events in the pathogenesis of atherosclerosis¹ and are mediated by adhesion molecules on the endothelial cell membrane that mainly belong to 2 protein families: the selectins and adhesion molecules of the immunoglobulin superfamily.² The expression of P-selectin (a member of the first group), and of both vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) (members of the second group), has been demonstrated in various cell types forming the atherosclerotic plaque; that is, endothelial cells, vascular smooth muscle cells, and macrophages.^{3–8} Soluble forms of adhesion molecules are probably generated by cleavage at a site close to membrane insertion.⁹ The amount of soluble ICAM-1 has been demonstrated to be directly correlated with the surface expression of ICAM-1 in endothelial cells in culture,¹⁰ and a correlation between soluble VCAM-1 and VCAM-1 mRNA

expression in human atherosclerotic aorta has also been reported.¹¹

Several reports support the notion that the serum levels of soluble adhesion molecules may be useful for providing information on atherosclerotic lesions.^{11–14} Serum levels of soluble P-selectin and ICAM-1 were elevated in patients with peripheral artery vessel disease (pAVD)¹³ and soluble VCAM-1 levels were elevated in patients with an atherosclerotic aorta,¹¹ compared with those in asymptomatic control subjects. Levels of soluble VCAM-1 and ICAM-1 were also elevated in patients with severe hyperlipidemia, in whom advanced atherosclerosis can be expected, and in whom lipid-lowering drugs did not affect the levels of soluble VCAM-1 and ICAM-1.¹⁴ Furthermore, in patients with pAVD, circulating VCAM-1 correlated with the extent of atherosclerosis as defined by the mean internal vessel surface area of the abdominal aorta and pelvic and leg arteries.¹²

In the present study, we studied the serum levels of soluble VCAM-1, ICAM-1, and P-selectin in patients with and without (controls) angiographically defined coronary atherosclerosis. We examined the association of the levels of these soluble adhesion molecules with coronary heart disease (CHD) and the severity of coronary atherosclerosis as judged by the number of stenosed coronary arteries, and the influence of serum levels of lipids and lipoproteins on the levels of these soluble adhesion molecules. To the best of our knowledge, this is the first report of the detailed association between circulating cell adhesion molecules

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Fig 1. Frequency distributions of the levels of (A) soluble vascular cell adhesion molecule 1 (sVCAM-1), (B) soluble intercellular adhesion molecule 1 (sICAM-1), and (C) P-selectin in patients with () and without () coronary heart disease (CHD).

and their relation to lipoproteins and apolipoproteins in a clinical study.

Methods

Patients

The study population included 92 patients who underwent diagnostic coronary angiography for suspected or known CHD or for other reasons (mostly atypical chest pain) at Fukuoka University Hospital from 1994 to 1996. The study was approved by the Ethics Committee of Fukuoka University Hospital and informed consent was obtained from each patient. Controls (without CHD) were defined as those with less than 25% luminal narrowing, and CHD patients were those who had 1, 2 or 3 stenosed (>50% luminal narrowing) epicardial coronary arteries. Patients with vasospastic angina were excluded from the controls and none of the controls had myocardial infarction (MI). As patients with acute MI (within 3 weeks after onset) have been shown to have increased plasma levels of soluble ICAM-1¹⁵ and P-selectin¹⁶, they were excluded from the study. Patients with heart failure (Killip Class ≥ 2 after MI), aortitis treated by prednisolone, hepatic dysfunction (viral and non-viral, transaminases more than 3 times the normal value), or uncontrollable diabetes mellitus (DM) were also excluded from the study. Patients with systolic or diastolic blood pressure >160 mmHg or 95 mmHg, respectively, or who were under anti-hypertensive treatment were considered to have hypertension (HT). Patients under treatment for DM and/or with symptoms of DM and a fasting glucose concentration ≥ 126 mg/dl were considered to have DM. Otherwise, the results of a 75-g glucose tolerance test were used to diagnose DM.

Coronary Angiography

Coronary angiography was performed by the Judkins technique¹⁷ with 5F catheters, and recorded on Kodak 35 mm cinefilm at a rate of 30 frames/s. Coronary arteries were divided into 15 segments, according to the classification of the American Heart Association Grading Committee. Coronary artery segments for reference were carefully selected by 2 expert cardiologists on the basis of smooth luminal borders and the absence of stenotic changes. The presence of stenosis was determined using a computer-assisted coronary angiography analysis system (Mipron 1; Kontron Co) after the direct intracoronary injection of isosorbide dinitrate (ISDN) (2.5 mg/5 ml solution). One minute after the injection of ISDN through the Judkins catheter over 20 s, coronary angiography was performed from several projections over 7 min, as described previously¹⁸. Arterial stenosis that produced more than 50% luminal narrowing was considered significant.

Determination of Serum Lipids, Lipoproteins, Apolipoproteins, and Soluble Cell Adhesion Molecules

Blood was drawn in the morning after an overnight fast. Serum total cholesterol (TC), free cholesterol (FC), and triglyceride (TG) concentrations were determined enzymatically^{19,20}. High-density lipoprotein cholesterol (HDL-C) was determined by the heparin Ca^{2+} precipitation method²¹. HDL subfractions (HDL₂ and HDL₃) were separated by standard sequential preparative ultracentrifugation techniques²². Serum apolipoproteins (apo) A-I, A-II, B, C-II, C-III, and E were determined by the turbidity

immunoassay method²³ Apo A-II levels play a major role in regulating the distribution of apo A-I among the HDL subclasses Lp A-I (lipoprotein containing only apo A-I) and Lp A-I/A-II (lipoprotein containing both apo A-I and apo A-II) in humans²⁴ LpA-I/A-II appears to have a fixed molar ratio of 3:2 for these apolipoproteins.^{25,26} In our preliminary experiments, plasma concentrations of LpA-I isolated by immunoaffinity chromatography²⁷ correlated well with those of LpA-I calculated based on a fixed molar ratio of apoA-I to apoA-II ($n=30$, $r=0.91$, $p<0.001$). Thus, we calculated the plasma concentrations of LpA-I and LpA-I/A-II in the present study.

Levels of sVCAM-1, sICAM-1 and sP-selectin were determined by monoclonal antibody-based enzyme-linked immunosorbent assays (R&D Systems) using frozen serum samples. Briefly, all samples and controls were examined in duplicate, and the concentrations of the samples were determined by analyzing standards with known concentrations of recombinant adhesion molecules coincident with samples and plotting a curve of signal versus concentration. According to the commercial supplier, no cross-reactivity is observed between the aforementioned sCAMs. For all measurements in our laboratory, the interassay and intra-assay coefficients of variation were less than 10% and 5%, respectively, and blinded quality-control specimens were included in each assay.

Statistical Analysis

Statistical analysis was performed using the SAS Software Package (Version 6, Statistical Analysis System, SAS Institute Inc, Cary, NC, USA). Categorical variables (such as gender) and frequency distribution patterns between patients with and without CHD were compared by a Chi-square analysis. The distribution of continuous variables was examined by the Shapiro-Wilk test.²⁶ Differences between patients with and without CHD or among patients with 1-, 2-, and 3-vessel disease were examined by an analysis of variance (ANOVA). Comparisons of 1-vessel and 2- or 3-vessel disease patients were performed with the multiple comparison test of Dunnett.²⁸ Age and gender were adjusted for by an analysis of covariance (ANCOVA).²⁸ The association between CHD and soluble adhesion molecules after controlling for age, gender, and potentially confounding variables was evaluated by a multiple logistic function analysis.²⁹ For all of the adjusted odds ratios, we calculated 95% confidence intervals (CI). For logistic parameter estimates, we showed the standard error. The influence of lipid parameters on levels of soluble adhesion molecules while controlling for age, gender, and body mass index (BMI) was examined by a covariance analysis using the General Linear Model procedure.²⁸ Results of the type II tests are given, where the effects of lipid variables on levels of sCAMs are adjusted for age, gender, and BMI. All p-values are 2-tailed. The significance level was considered to be 5% unless otherwise indicated.

Results

Table 1 shows the characteristics of patients with and without CHD (controls). There were significantly more males, HT, DM and smokers in patients with CHD than in the controls. Age and BMI were not significantly different between the 2 groups. Although all of the women except one were in menopause, none were receiving hormone

Table 1 Clinical Characteristics of the Study Subjects

	Patients without CHD (n=40)	Patients with CHD (n=52)
Age (years)	58±15	64±12
Gender (male/female)	25/15	43/9*
Body mass index (kg/m ²)	23.6±2.9	23.6±2.8
Hypertension (%)	35	60*
Diabetes Mellitus (%)	18	37*
Smokers (%)	45	79*

CHD, coronary heart disease. Values are mean±SD. * $p<0.05$ vs patients without CHD.

Table 2 Age- and Gender-Adjusted Values of Soluble Cell Adhesion Molecules and Lipid Variables

	Patients without CHD (n=40)	Patients with CHD (n=52)
HDL-C (mg/dl)	45.3±2.3	37.6±2.0*
HDL2-C (mg/dl)	32.6±1.9	26.3±1.6*
HDL3-C (mg/dl)	16.6±0.6	16.0±0.5
Apo A-I (mg/dl)	117±4	102±4*
Apo A-II (mg/dl)	28.1±1.0	26.3±0.9
Apo B (mg/dl)	97.0±4.1	105.2±3.5
Apo C-II (mg/dl)	3.1±0.3	3.3±0.2
Apo C-III (mg/dl)	7.9±0.4	7.7±0.4
Apo E (mg/dl)	4.7±0.2	4.8±0.2
Lp A-I (nmol/ml)	17.3±1.1	13.5±0.9*
Lp A-I/A-II (nmol/ml)	24.2±0.9	22.7±0.8
TC (mg/dl)	203±8	194±7
TG (mg/dl)	108±8	125±7
FC (mg/dl)	47.9±1.5	50.5±1.3
LDL-C (mg/dl)	136±8	131±7
sVCAM-1 (ng/ml)	540±41	565±36
P-selectin (ng/ml)	149±10	142±8
sICAM-1 (ng/ml)	247±19	261±17

HDL-C, high density lipoprotein cholesterol; Apo, apolipoprotein; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; FC, free cholesterol; Lp A-I, lipoprotein containing only apo A-I; Lp A-I/A-II, lipoprotein containing both apo A-I and apo A-II; sVCAM-1, soluble vascular adhesion molecule 1; sICAM-1, soluble intercellular adhesion molecule 1.

Data are presented as least-square means±standard error.

* $p<0.05$ vs patients without CHD, as assessed by an analysis of variance.

Table 3 Multiple Logistic Function Analysis of the Association Between Soluble Adhesion Molecules and Coronary Heart Disease, after Considering Age, Body Mass Index, Gender, Hypertension, Diabetes Mellitus and Smoking

Variables included in the model	Parameter estimate ±standard error	Wald Chi-square	Odds ratio (95% confidence interval)
sVCAM-1	0.60±0.51	1.43	1.83 (0.68, 4.93)
sICAM-1	-0.34±0.48	0.48	0.71 (0.28, 1.85)
P-selectin	-0.14±0.49	0.08	0.87 (0.33, 2.28)
Age	0.12±0.52	0.05	1.12 (0.41, 3.10)
Body mass index	-0.13±0.51	0.07	0.88 (0.32, 2.37)
Gender	-0.55±0.66	0.70	0.58 (0.16, 2.09)
Hypertension	0.86±0.51	2.85	2.36 (0.87, 6.39)
Diabetes mellitus	0.59±0.58	1.01	1.80 (0.57, 5.64)
Smoking	1.15±0.58	3.87*	3.16 (1.00, 9.92)

* $p<0.05$, significant association with CHD. see Table 2 for abbreviations.

replacement therapy.

Fig 1 shows the frequency distributions of soluble adhesion molecules in subjects with and without CHD. The distributions of sVCAM-1, sICAM-1, and sP-selectin levels in patients with CHD were not skewed toward higher

levels compared with those in patients without CHD. Indeed, the distribution patterns of sVCAM-1, sICAM-1, and sP-selectin levels between the 2 groups were not different, as assessed by a Chi-square analysis.

Table 2 compares serum levels of lipids, lipoproteins, apolipoproteins, and soluble adhesion molecules in patients with and without CHD after controlling for age and gender. Serum levels of HDL-C, HDL2-C, apo A-I, and Lp A-I were significantly lower in patients with CHD than those without CHD. There were no significant differences in sVCAM-1, sICAM-1, and sP-selectin between patients with CHD and controls after adjusting for age and gender.

Table 3 shows the impact of BMI, HT, DM, and smoking

on the association between levels of soluble adhesion molecules and CHD. A multiple logistic function analysis showed a significant association only between smoking status and CHD. Levels of sVCAM-1, sICAM-1, and sP-selectin were not associated with CHD after controlling for age, gender, BMI, HT, DM, and smoking.

Table 4 compares serum levels of lipids, lipoproteins, apoproteins, and soluble adhesion molecules among patients with 1, 2, and 3 involved vessels, after adjusting for age and gender. Although lower levels of HDL3-C and TC were observed in patients with 3-vessel disease and lower levels of apo B and low density lipoprotein-cholesterol (LDL-C) were observed in those with 2 stenosed vessels, other variables including sVCAM-1, sICAM-1, and sP-selectin were not associated with the extent of coronary atherosclerosis as defined by the number of stenosed vessels.

Table 5 shows the effects of serum levels of lipids, lipoproteins, and apolipoproteins on soluble adhesion molecules after controlling for age, gender, and BMI, using a covariance analysis. As shown in Table 5, levels of sVCAM-1 were inversely ($p<0.05$) related to levels of HDL3-C, apo A-II, apo B, and Lp A-I/A-II; levels of sICAM-1 were inversely related ($p<0.05$) to levels of apo A-II and Lp A-I/A-II; and levels of sP-selectin were related to levels of Lp A-I.

Discussion

Our results showed that the levels of soluble VCAM-1, ICAM-1, and P-selectin were inversely related to serum levels of high-density lipoprotein-related lipoproteins in a group of patients who underwent coronary angiography for chest pain, which may suggest a relation between the extent of atheroma and levels of circulating cell adhesion molecules.

Association Between Circulating Cell Adhesion Molecules and CHD

Elevated levels of soluble adhesion molecules have been reported in patients with pAVD;^{13,30} in patients with an atherosclerotic aorta;¹¹ and in patients with severe hyperlipidemia.¹⁴ However, our results showed that the levels of

Table 4 Age- and Gender-Adjusted Values of Soluble Adhesion Molecules and Lipids in Patients With 1-, 2-, and 3-Vessel Coronary Stenosis

	One vessel (n=23)	Two vessels (n=21)	Three vessels (n=8)
HDL-C (mg/dl)	35.9±2.5	43.6±2.7	30.2±4.4
HDL2-C (mg/dl)	24.9±2.1	30.7±2.2	21.5±3.6
HDL3-C (mg/dl)	16.5±0.6	16.7±0.6	13.2±1.0*
Apo A-I (mg/dl)	101±5	111±5	88±8
Apo A-II (mg/dl)	26.3±1.1	26.4±1.2	25.3±2.0
Apo B (mg/dl)	112.1±4.7	94.8±5.0*	103.1±8.2
Apo C-II (mg/dl)	3.5±0.3	3.0±0.3	2.5±0.6
Apo C-III (mg/dl)	7.8±0.5	7.5±0.6	7.1±0.9
Apo E (mg/dl)	5.0±0.3	4.5±0.3	4.4±0.4
LpA-I (nmol/ml)	13.0±1.2	16.5±1.3	9.4±2.1
LpA-I/A-II (nmol/ml)	22.7±1.0	22.7±1.0	21.8±1.7
TC (mg/dl)	204±6	186±6	180±10*
TG (mg/dl)	135±10	101±10	130±17
FC (mg/dl)	52.5±1.6	48.3±1.7	48.0±2.8
LDL-C (mg/dl)	141±5	122±5*	124±9
sVCAM-1 (ng/ml)	576±53	580±56	533±93
P-selectin (ng/ml)	135±11	142±12	159±19
sICAM-1 (ng/ml)	287±29	252±30	220±50

HDL-C, high density lipoprotein cholesterol; Apo, apolipoprotein; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; FC, free cholesterol; Lp A-I, lipoprotein containing only apo A-I; Lp A-I/A-II, lipoprotein containing both apo A-I and apo A-II; sVCAM-1, soluble vascular adhesion molecule 1; sICAM-1, soluble intercellular adhesion molecule 1.

Data are presented as least-squares means±standard error.

* $p<0.05$, vs patients without CHD, assessed by an analysis of variance.

Table 5 Covariance Analysis of the Influence of Lipid Parameters on Soluble Adhesion Molecules after Considering Age, Gender and Body Mass Index

	Soluble VCAM-1			Soluble ICAM-1			P-selectin		
	Parameter estimates (standard error)	Type II sum of squares	F value	Parameter estimates (standard error)	Type II sum of squares	F value	Parameter estimates (standard error)	Type II sum of squares	F value
HDL-C	-0.70 (1.93)	8248	0.13	-0.95 (0.90)	15228	1.10	-0.60 (0.43)	5993	1.89
HDL2-C	0.13 (2.38)	191	0.003	-0.87 (1.12)	8431	0.61	-0.70 (0.53)	5507	1.73
HDL3-C	-21.7 (7.4)	491150	8.56*	-3.85 (3.63)	15468	1.12	-0.64 (1.76)	421	0.13
Apo A-I	-0.29 (0.98)	5641	0.09	-0.52 (0.46)	17856	1.30	-0.27 (0.22)	4948	1.55
Apo A-II	-9.32 (4.13)	303716	5.10*	-4.18 (1.95)	60897	4.59*	0.32 (0.96)	349	0.11
Apo B	-2.29 (1.05)	281693	4.71*	-0.18 (0.51)	1805	0.13	-0.24 (0.24)	3130	0.98
Apo C-II	-33.2 (16.7)	237287	3.93	4.59 (8.03)	4556	0.33	4.28 (3.85)	3963	1.24
Apo C-III	-12.7 (10.3)	93999	1.52	-3.18 (4.87)	5920	0.43	1.11 (2.35)	721	0.22
Apo E	-36.1 (22.9)	153068	2.50	3.60 (10.9)	1518	0.11	-1.53 (5.26)	274	0.08
LpA-I	5.75 (4.08)	122268	1.98	0.12 (1.94)	58	0.004	-1.98 (0.91)	14509	4.71*
LpA-I/A-II	-10.8 (4.8)	303707	5.10*	-4.84 (2.26)	60899	4.59*	0.37 (1.12)	349	0.11
TC	-0.96 (0.54)	192434	3.16	-0.18 (0.26)	6969	0.50	0.03 (0.12)	158	0.05
TG	-0.35 (0.54)	26081	0.42	0.14 (0.26)	4113	0.30	0.17 (0.12)	6373	2.01
FC	-2.67 (2.95)	51103	0.81	0.69 (1.40)	3433	0.24	-0.45 (0.67)	1469	0.45
LDL-C	-0.92 (0.57)	161288	2.64	-0.15 (0.27)	4197	0.30	0.04 (0.13)	381	0.12

sVCAM-1, soluble vascular cell adhesion molecule 1; sICAM-1, soluble intercellular adhesion molecule 1.

* $p<0.05$, probability of a larger F value for the Type II test, where the effect of the lipid variable is adjusted for those of age, gender and body mass index.

sVCAM-1, sICAM-1, and sP-selectin in CHD patients and controls were similar after adjusting for age and gender (Table 2). The distribution patterns of soluble cell adhesion molecules were compared between patients with and without CHD to exclude the possible influence of distribution, because Hwang et al³¹ reported a skewed distribution for circulating ICAM-1 and VCAM-1, and our sample size was too small to determine the accurate distribution of the levels of soluble adhesion molecules. However, in our study, similar frequency distribution patterns were observed, as shown in Fig 1. After additionally controlling for BMI, HT, DM, and smoking in a multiple logistic function analysis, levels of soluble adhesion molecules were still not significantly associated with CHD (Table 3). These results agree with those of Frijns et al³² who found no difference in sCAM or sVAM in carotid atherosclerosis. Hwang et al reported that plasma levels of ICAM-1 and E-selectin may serve as molecular markers for atherosclerosis and CHD³¹ while Ridker et al³³ showed that the risk of MI associated with raised concentrations of sICAM-1 seemed to increase with the duration of follow-up. However, it is still unclear whether the similar levels of soluble VCAM-1, ICAM-1, and P-selectin in CHD patients and controls are due to differences in the populations studied.

Association Between Circulating Cell Adhesion Molecules and the Extent of CHD

Peter et al reported that soluble VCAM-1 levels, in contrast with soluble ICAM-1 and P-selectin levels, correlated with the extent of human atherosclerosis, as defined by the mean internal vessel surface area of the abdominal aorta and pelvic and leg arteries.¹² Our findings of no association between soluble ICAM-1 or P-selectin levels and the extent of coronary atherosclerosis, as judged by the number of stenosed coronary arteries, are partly similar to those of Peter et al.¹² However, we did not observe a significant association between soluble VCAM-1 and the extent of coronary atherosclerosis.

Influence of Serum Lipids, Lipoproteins and Apolipoproteins on Circulating Cell Adhesion Molecules

As the levels of soluble adhesion molecules were not different between subjects with and without CHD, we studied the influence of serum levels of lipids, lipoproteins, and apolipoproteins on levels of soluble adhesion molecules in all of the subjects. We found that, after controlling for age, gender and BMI, levels of soluble VCAM-1 were inversely related to serum levels of HDL₃-C, apo A-II, and Lp A-I/A-II, soluble ICAM-1 levels were inversely related to serum levels of apo A-II and Lp A-I/A-II, and sP-selectin levels were inversely related to serum Lp A-I levels. Our findings partly support those of Hwang et al³¹ and Ridker et al³³ in that ICAM-1 was negatively associated with HDL-C, and may also be supported by reports that HDL inhibits both the adhesion of monocytes to endothelial cells³⁴ and the expression of adhesion molecules on the surface of endothelial cells.³⁵

Study Limitations

In this angiographic case-control study, patients with CHD were not matched with controls with regard to gender (Table 1). We tried to avoid possible bias by adjusting for age and gender when comparing soluble adhesion molecules and lipid variables between patients with and without

CHD, as VCAM-1 was significantly correlated with age ($r=0.29$, $p<0.01$) in all of the patients.

We selected angiographically defined normal subjects as controls. They had undergone diagnostic coronary angiography because of suspected or known coronary atherosclerosis or other reasons (mostly atypical chest pain). Patients with spastic angina pectoris were excluded from the controls and none of the controls had MI. However, a selection bias is known to exist: angiographically defined normal subjects generally have more risk factors for coronary disease than patients with clinical symptoms but who have not been selected for angiography, because a person with both a chest pain and a known risk factor, such as smoking, may be more likely to be referred for angiography than a person with just a clinical symptom.³⁶ It is possible that the lack of differences in the levels of soluble adhesion molecules between subjects with and without CHD (Table 2) and the lack of an association between the levels of soluble adhesion molecules and the severity of coronary disease as judged by the number of involved vessels (Table 4) in the present study may be related to this bias. Previous angiographic studies, such as the Coronary Artery Surgery Study, have also failed to show associations between classic lipid risk factors and the severity of disease, presumably due to the biases involved in selecting patients for angiography. Thus, biases produced in selecting the controls may have limited the power of this study. Our results do not exclude the possibility that serum levels of soluble cell adhesion molecules may have some clinical value in discriminating CHD patients. Further studies are needed with normal healthy subjects who have no risk factors.

Conclusions

The serum levels of soluble VCAM-1, ICAM-1, and P-selectin were not related to CHD or the extent of coronary atherosclerosis. However, they were inversely related to serum levels of high-density lipoprotein-related lipoproteins.

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