Circulating Vascular Cell Adhesion Molecule-1 (VCAM-1) in Atherosclerotic NIDDM Patients

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Vascular cell adhesion molecule-1 (VCAM-1) has been shown to be highly expressed in atherosclerotic lesions. Although the soluble form of VCAM-1 (sVCAM-1) is detected in human sera, the relation between the degree of atherosclerosis and serum sVCAM-1 level has not been defined. In the present study, sVCAM-1 concentrations were measured in sera from 101 Japanese NIDDM patients. The mean ± SD serum sVCAM-1 concentration in 26 patients with symptomatic atherosclerotic vascular diseases (789 ± 187 ng/ml) was higher than that in 75 patients without the disease (664 ± 175 ng/ml). Among the 101 NIDDM patients, 56 had atherosclerotic change of the carotid arteries, based on the evaluation by high-resolution B-mode ultrasonography. Their sVCAM-1 level was 759 ± 201 ng/ml, higher than that in 45 patients without any detectable atherosclerosis of the carotid arteries (619 ± 130 ng/ml). In addition, there was a positive correlation between sVCAM-1 concentration and thickness of the intimal plus medial complex (IMT) of the carotid arteries in the NIDDM patients (r = 0.41, P < 0.0001). Multivariate regression analysis revealed significant predictors of mean IMT value to be sVCAM-1 concentration (F = 62.88, P = 0.0001) and age (F = 9.59, P = 0.0026). By contrast, sVCAM-1 concentration was not increased in nondiabetic patients with atherosclerotic change of the carotid arteries (668 ± 191 ng/ml; n = 36) compared with those without the atherosclerotic change (632 ± 177 ng/ml; n = 28), and there was no correlation between sVCAM-1 level and IMT of the carotid arteries in the nondiabetic subjects. These results indicate that circulating sVCAM-1 may be a marker of atherosclerotic lesions in NIDDM patients with symptomatic and asymptomatic atherosclerosis. *Diabetes* 46:2096–2101, 1997

Atherosclerosis is characterized by endothelial cell injury, which in turn leads to the adhesion of mononuclear leukocytes to the endothelium, the intimal migration and proliferation of smooth muscle cells, and extracellular matrix deposition (1,2).

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Received for publication 22 August 1996 and accepted in revised form 3 September 1997.

IMT, intimal plus medial complex; sVCAM-1, soluble VCAM-1; VCAM-1, vascular cell adhesion molecule-1.

Much evidence has been accumulated indicating that various adhesion molecules are involved in mononuclear leukocyte adhesion to the vascular endothelium (3–5). The adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) (6,7), intercellular adhesion molecule-1 (ICAM-1) (8,9), E-selectin (10,11), and platelet endothelial cell adhesion molecule (PECAM) (CD31) (11) have been demonstrated to be expressed in atherosclerotic lesions. Thus initial insults may act on vascular cells (e.g., endothelial cells, smooth muscle cells, resident macrophages) to upregulate the expression of these adhesion molecules. In fact, the expression of some of the adhesion molecules has been shown to be enhanced by lysophosphatidylcholine (a component of atherogenic lipoproteins) (12), an atherogenic diet (13), balloon injury (14), shear stress (15), and experimental diabetes (16).

Recent investigations have documented that soluble forms of adhesion molecules are present in endothelial cell culture supernatants and human sera (17–19). Increased levels of the circulating adhesion molecules have been shown in patients suffering from inflammatory diseases, autoimmune disorders, malignancies, and neonatal sepsis (17–23). Although their biological significance is not clear, circulating levels of some adhesion molecules correlated with the disease activity could be useful clinical markers (22,23).

We tested whether circulating levels of adhesion molecules are increased in patients with atherosclerosis. The study protocol was designed to measure serum soluble VCAM-1 (sVCAM-1) concentrations in NIDDM patients with and without atherosclerosis, since diabetes has been shown to predispose to atherosclerosis (24,25). To diagnose atherosclerosis early, we used ultrasound high-resolution B-mode imaging of the carotid arteries (26–29).

RESEARCH DESIGN AND METHODS

Study subjects. We studied 101 Japanese patients who had an established diagnosis of NIDDM as defined by World Health Organization criteria (30). All patients had attended Osaka University Hospital or Aizenbashi Hospital from August 1995 to October 1996. We also studied 64 patients who had essential hypertension and/or hypercholesterolemia but not diabetes as nondiabetic subjects. All the nondiabetic patients had normal levels of fasting plasma glucose (≤6.1 mmol/l) and HbA_{1c} (≤5.8%). Informed consent was obtained from all patients. Patients had complete physical and laboratory examinations before the study. Patients with chronic or acute inflammatory diseases, elevated serum creatinine levels (≥110 μmol/l), abnormal hepatic function tests, malignancies, or autoimmune disorders were excluded from the study.

The diagnosis of atherosclerotic vascular disease (coronary artery disease, cerebrovascular disease, or arteriosclerosis obliterans) was based on laboratory evaluation sufficient to prove the disease status (e.g., angiography, electrocardiography, magnetic resonance imaging of brain, ankle blood pressure measurement, ultrasonic velocity detector).

Diabetic retinopathy was graded as nonproliferative diabetic retinopathy or proliferative diabetic retinopathy based on fundus examination by ophthalmolo-

gists. Diabetic nephropathy was classified as normoalbuminuria, microalbuminuria, or overt albuminuria on the basis of the criteria by Krolewski et al. (31). Diabetic neuropathy was diagnosed when the patients had peripheral sensorimotor neuropathy plus either abnormal nerve conduction in at least two peripheral nerves or abnormal autonomic nerve tests (32).

Blood pressure was measured with a mercury sphygmomanometer, after a supine rest of 5 min. Cigarette-years of smoking were defined as the product of years of smoking and the mean pack-number of cigarettes smoked daily. Patients with hypertension were given ACE inhibitors, calcium-channel blockers, or α- or β-adrenergic antagonists; those with hypercholesterolemia were given pravastatin or simvastatin.

Ultrasonic evaluation of early atherosclerosis. To evaluate early atherosclerosis of the carotid arteries, high-resolution B-mode imaging (26–29) was performed using an echotomographic system (SSA-380A; Toshiba Medical, Tokyo, Japan) with a 7.5-MHz transducer. The axial resolution of this system was at least 0.3 mm. Scanning of the common carotid, the internal carotid, and the external carotid arteries was performed bilaterally in three different longitudinal projections and the transverse projection, as described by Handa et al. (28). The thickness of the intimal plus medial complex (IMT), as defined by Pignoli (26) and Poli et al. (27), was measured as the distance between the lumen-intima interface and the media-adventitia interface. At each longitudinal projection, IMT was determined on three differential sites: the greatest thickness and two other points, 1 cm upstream and 1 cm downstream from the site of the greatest thickness, as reported by Yamasaki et al. (29). These three determinations were averaged, and the greatest value among the six averaged IMT values was defined as the mean IMT. In this study, intimal-medial thickening of the carotid arteries was defined as the mean IMT of ≥1.1 mm, as reported by Yamasaki et al. (33). The plaque lesion was defined when a distinct area with ≥50% greater IMT as compared with neighboring sites was identified, as reported by Salonen et al. (34).

Test procedures. Fasting plasma glucose, HbA_{1c}, serum total cholesterol, serum HDL cholesterol, and serum triglycerides levels were determined by standard laboratory assays. Serum C-peptide and urinary albumin were determined by their specific radioimmunoassay. Serum LDL cholesterol levels were calculated by the equation of Friedewald et al. (35).

To determine sVCAM-1 levels, serum samples were stored at –20°C until assay. Serum concentrations of sVCAM-1 were determined using a commercially available enzyme-linked immunosorbent assay kit (R & D Systems, Minneapolis, MN). Dilution curves of serum samples were parallel to those of standards. Intra- and interassay coefficients of variation were 3.1 and 5.9%, respectively, as determined in representative human serum sample. No cross-reactivity of human IgG, recombinant human soluble ICAM-1, or recombinant human soluble E-selectin was observed, according to the manufacturer's protocol. Control ranges of serum sVCAM-1 were 597 ± 161 ng/ml in our laboratory, and were obtained from healthy subjects (average age, 43 ± 13 year; n = 19).

Statistics. All data are shown as means ± SD. The statistical analysis was performed with the use of unpaired Student's t test or unpaired Welch's t test, as appropriate. Treatment of diabetes and the prevalence of diabetic retinopathy, neuropathy, and nephropathy were compared by χ^2 test or Fisher's exact test, as appropriate. To analyze the effects of different variables on the presence of symptomatic atherosclerotic vascular diseases, we performed logistic regression analysis with Statistical Analysis System computer program (SAS Institute Japan, Tokyo, Japan). To analyze the effects of variables on mean IMT value of the carotid arteries, we performed stepwise multivariate regression analysis as well as univariate regression analysis. In the stepwise multivariate regression analysis, alpha value for inclusion of the variables was set at 0.20. In the logistic regression analysis and the multivariate regression analysis, the explanatory variables were age, BMI, known diabetes duration, type of treatment at study, HbA_{1c}, fasting serum C-peptide, serum LDL cholesterol, serum HDL cholesterol, mean blood pressure, cigarette-years of smoking, retinopathy, neuropathy, nephropathy, and serum sVCAM-1. $P < 0.05$ was considered to be statistically significant.

RESULTS

Serum sVCAM-1 levels in NIDDM patients. Of 101 NIDDM patients in the study, 57 were male and 44 were female; the average age was 61 ± 10 years (range, 31–85). We found that 26 of the NIDDM patients suffered from some of the symptomatic atherosclerotic vascular diseases: 17, coronary artery disease; 10, cerebrovascular disease; and 9, arteriosclerosis obliterans. Among these 26 patients, 7 had multiple atherosclerotic vascular diseases. The clinical characteristics of these 26 patients were compared with those of the other 75 patients without symptomatic atherosclerotic vascular disease (Table 1). The former were older in age, and more had diabetic retinopathy, neuropathy, and nephropathy. However, known diabetes duration, diabetes treatment at time of study, fasting plasma glucose, HbA_{1c}, serum LDL cholesterol, serum triglycerides, and BMI were not significantly different between the groups.

Serum concentrations of sVCAM-1 were measured in the NIDDM patients. The sVCAM-1 concentration in the subjects

TABLE 1
Clinical characteristics of NIDDM patients

	Patients with symptomatic atherosclerotic vascular disease	Patients without symptomatic atherosclerotic vascular disease	P value
n	26	75	
Age (years)	66 ± 8	59 ± 10	<0.005
Sex (M/F)	16/10	41/34	0.54
BMI (kg/m^2)	24 ± 3	24 ± 4	1.00
Known diabetes duration (years)	15 ± 11	11 ± 8	0.10
Treatment at study (diet/sulfonylurea/insulin) (%)	19/39/42	21/53/25	0.25
Fasting plasma glucose (mmol/l)	8.3 ± 2.9	9.1 ± 2.8	0.22
HbA_{1c} (%)*	7.2 ± 2.0	7.6 ± 1.7	0.33
Serum LDL cholesterol (mmol/l)	3.00 ± 0.80	3.16 ± 0.85	0.40
Serum HDL cholesterol (mmol/l)	1.36 ± 0.42	1.40 ± 0.49	0.71
Serum triglycerides (mmol/l)	1.60 ± 0.86	2.01 ± 1.30	0.07
Retinopathy (nil/nonproliferative/proliferative) (%)	42/33/25	64/30/6	<0.05
Neuropathy (absent/present) (%)	42/58	75/25	<0.005
Nephropathy (nonnoalbuminuria/microalbuminuria/overt albuminuria) (%)	38/35/27	61/38/1	<0.0001
Serum sVCAM-1 (ng/ml)	789 ± 187	664 ± 175	<0.005

Data are means ± SD, n, or %. Statistical significance was according to unpaired Student's t test, unpaired Welch's t test, χ^2 test, or Fisher's exact test, as appropriate. *Normal ranges: 4.4–5.8%.

with symptomatic atherosclerotic vascular diseases was 789 ± 187 ng/ml, which was significantly higher ($P < 0.005$) than that in subjects without atherosclerotic vascular disease (664 ± 175 ng/ml) (Table 1). Logistic regression analysis revealed that, among the confounding variables, diabetic nephropathy, age, and serum LDL cholesterol were significantly associated with symptomatic atherosclerotic vascular diseases (Table 2). In this analysis, serum sVCAM-1 concentration was not significantly associated with these disorders.

In the next study, to evaluate early atherosclerosis, high-resolution B-mode ultrasonographic imaging of the carotid arteries was performed. When atherosclerotic change of the carotid arteries was defined as the mean IMT value ≥1.1 mm and/or the presence of plaque lesion (33,34), 56 patients (55%) had atherosclerosis of the carotid arteries. Their serum sVCAM-1 concentration was 759 ± 201 ng/ml, significantly higher ($P < 0.0001$) than that in 45 patients without any detectable atherosclerosis (619 ± 130 ng/ml) (Fig. 1). In addition, there was a significant correlation between serum sVCAM-1 level and mean IMT value of the carotid arteries ($r = 0.41$, $P < 0.0001$) (Fig. 2). Out of 75 patients without symptomatic atherosclerotic vascular disease, 34 (45%) had atherosclerotic change of the carotid arteries. Their serum sVCAM-1 concentration was 732 ± 208 ng/ml, which was higher than in the 41 patients without atherosclerotic change (608 ± 119 ng/ml, $P < 0.005$). Furthermore, a positive correlation was observed in the 75 NIDDM patients without symptomatic atherosclerotic vascular disease ($r = 0.48$, $P < 0.0001$).

Univariate regression analysis in 101 NIDDM patients demonstrated that, among the variables, age was also associated with mean IMT value ($r = 0.40$, $P < 0.0001$) (Table 3). The other variables did not reach statistical significance. Multivariate regression analysis in these patients showed that the risk factors for the mean IMT value were serum sVCAM-1 ($F = 62.88$, $P = 0.0001$) and age ($F = 9.59$, $P = 0.0026$). Cigarette-years of smoking did not contribute to the IMT value. BMI, serum LDL and HDL cholesterol, known diabetes duration, type of treatment at study, HbA_{1c}, fasting serum C-peptide, mean blood pressure, retinopathy, neuropathy, and nephropathy did not enter the regression model when the alpha value for inclusion was set at 0.20. Multivariate regression analysis in 75 NIDDM patients without symptomatic atherosclerotic vascular disease also revealed that serum sVCAM-1 ($F = 13.95$, $P = 0.0004$) and age ($F = 5.13$, $P = 0.027$) were significantly associated with the mean IMT value, but the other variables were not (data not shown).

Serum sVCAM-1 levels in nondiabetic patients. Circulating sVCAM-1 levels in 64 nondiabetic patients (24 male and 40 female) were determined. Their average age was 60 ± 11 years (range, 33–81), which was similar to that in the 101 NIDDM patients. Of the 64 nondiabetic patients, 16 patients had a symptomatic vascular disease: 12, coronary artery disease; 5, cerebrovascular disease; and 1, arteriosclerosis obliterans; in addition, 2 had multiple atherosclerotic vascular diseases. Serum sVCAM-1 concentration in the 16 nondiabetic subjects with symptomatic atherosclerotic vascular diseases was 671 ± 195 ng/ml, not significantly different from that in the other 48 nondiabetic patients who did not have symptomatic atherosclerotic vascular disease (646 ± 182 ng/ml) (Table 4). Among the 64 nondiabetic subjects, 36 (56%) had atherosclerotic change of the carotid arteries on the diagnosis by high-resolution B-mode ultrasonography. Their sVCAM-1 concentration (668 ± 191 ng/ml) was not significantly different from that in the 28 nondiabetic subjects without any detectable atherosclerosis of the carotid arteries (632 ± 177 ng/ml) (Fig. 3), but it was significantly lower than that in 56 NIDDM patients with atherosclerotic change of the carotid arteries (759 ± 201 ng/ml, $P < 0.05$). In these nondiabetic subjects, there was no significant correlation between the serum sVCAM-1 concentration and the mean IMT value of the carotid arteries ($r = 0.14$, $P = 0.28$).

TABLE 2
Logistic regression analysis of the effects of variables on the presence of atherosclerotic vascular diseases

Parameter	Risk ratio	95% CI	*P* value
Nephropathy	4.89	1.20–19.88	0.0001
Age (1 year)	1.12	1.02–1.23	0.0041
Serum LDL cholesterol (1 mmol/l)	1.03	0.99–1.06	0.041
Mean blood pressure (1 mmHg)	1.08	1.01–1.15	0.055

0, normoalbuminuria; 1, microalbuminuria; 2, overt albuminuria for nephropathy.

DISCUSSION

VCAM-1 is a transmembrane glycoprotein that is a member of the immunoglobulin gene superfamily (3,4). It has been shown that VCAM-1 is expressed in various kinds of cells, such as vascular endothelial cells, vascular smooth muscle cells, intravascular macrophages, dendritic cells, renal glomerular epithelial and tubular cells, neural cells, myoblasts, and bone marrow–derived fibroblasts. VCAM-1 expression is enhanced by interleukin-1 or tumor necrosis factor in cultured cells. In addition, sVCAM-1 has been shown to

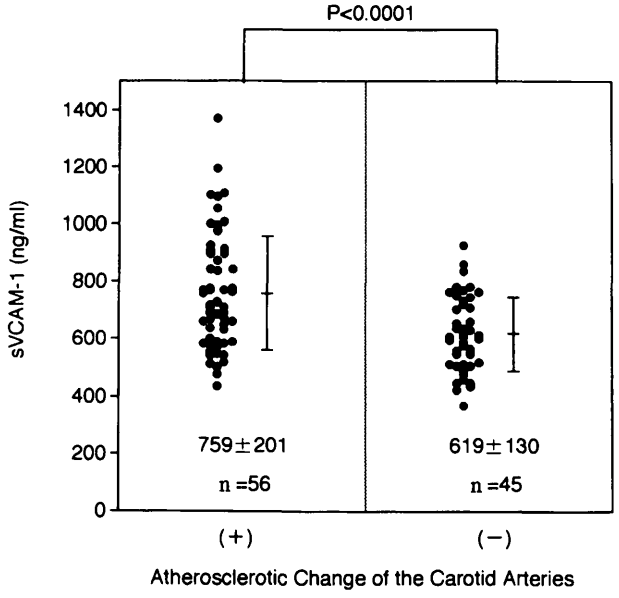

FIG. 1. Serum sVCAM-1 concentrations in 101 NIDDM patients. Patients were divided into two groups: those with atherosclerotic change of the carotid arteries ($n = 56$) and those without any detectable atherosclerosis ($n = 45$). Means ± SD are shown to the right of each column of data points.

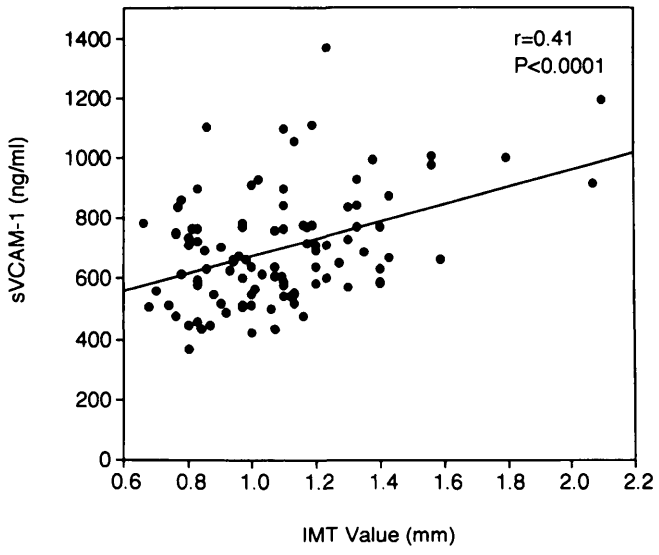

FIG. 2. Correlation between serum sVCAM-1 concentration and mean IMT value of the carotid arteries in 101 NIDDM patients. The correlation was significant (r = 0.41, P < 0.0001).

be released from the cytokine-activated endothelial cells (18). Because the molecular weight of sVCAM-1 in the culture supernatant is smaller than that of VCAM-1 in the cell lysate, sVCAM-1 has been shown to be produced by proteolytic cleavage of the extracellular region of the membrane-bound VCAM-1 at a site near the membrane (18,20). In this regard, there is no evidence that the soluble form is generated by alternative messenger RNA splicing, although an alternate form of the membrane-bound VCAM-1 resulting from alternative messenger RNA splicing has been cloned (36).

In the present studies, we measured serum sVCAM-1 concentrations in Japanese NIDDM patients. We found an increase of sVCAM-1 levels in patients with symptomatic atherosclerotic vascular diseases, compared with the patients who had no symptomatic atherosclerotic vascular disease. In addition, our study revealed that sVCAM-1 concentrations were raised in those with atherosclerotic change of the carotid arteries, based on the diagnosis by high-resolution B-mode ultrasonographic imaging (33,34). sVCAM-1 level was increased as a function of the mean value of IMT. There was no cutoff level of sVCAM-1 with respect to the diagnosis of atherosclerotic change of the carotid arteries. This is plausible because atherosclerosis is not a discrete but a continuous lesion. In the present study, however, only 1 (2%) of 45 patients with no detectable atherosclerosis had sVCAM-1 concentration >900 ng/ml, whereas 12 (21%) of 56 patients with the atherosclerotic change showed sVCAM-1 concentration >900 ng/ml. Thus serum sVCAM-1 level >900 ng/ml suggests the presence of atherosclerotic change of the carotid arteries.

Blann and McCollum (37), in examining circulating sVCAM-1 levels in patients with atherosclerotic vascular disease, failed to demonstrate elevated sVCAM-1 levels in patients with ischemic heart disease or peripheral vascular disease. This is consistent with our results that serum sVCAM-1 concentration was not increased in nondiabetic patients with symptomatic atherosclerotic vascular diseases (Table 4). In nondiabetic subjects, the serum sVCAM-1 level in those with atherosclerotic change of the carotid arteries did not differ from those without any detectable atherosclerosis of the carotid arteries (Fig. 3). In addition, the serum sVCAM-1 level in NIDDM patients with atherosclerotic change of the carotid arteries was higher than that in nondiabetic subjects with the atherosclerotic change. Thus our data indicate that the elevation of circulating sVCAM-1 appears to be specific to the atherosclerosis in diabetic patients. In this regard, circulating sVCAM-1 concentration has been shown to be elevated in diabetic patients, although the relation with atherosclerosis was not examined in that study (38).

TABLE 3
Univariate and multivariate regression analysis on mean IMT value of the carotid arteries

	Univariate regression analysis		Multivariate regression analysis		
Variable	Correlation coefficient	P value	Partial regression coefficient	F	P value
Serum VCAM-1 (ng/ml)	0.41	<0.0001	0.000877	62.88	0.0001
Age (years)	0.40	<0.0001	0.009039	9.59	0.0026
Cigarette-years of smoking	0.07	0.47	0.001339	2.89	0.092
BMI (kg/m^2)	−0.13	0.21		Not entered	
Serum LDL cholesterol (mmol/l)	0.04	0.71		Not entered	
Serum HDL cholesterol (mmol/l)	−0.06	0.55		Not entered	
Known diabetes duration (years)	0.21	0.04		Not entered	
Treatment at study	0.13	0.20		Not entered	
HbA_{1c} (%)	−0.02	0.82		Not entered	
Fasting serum C-peptide (nmol/l)	−0.04	0.74		Not entered	
Mean blood pressure (mmol/l)	−0.05	0.03		Not entered	
Retinopathy	0.13	0.20		Not entered	
Neuropathy	0.17	0.09		Not entered	
Nephropathy	0.10	0.32		Not entered	

Information on multivariate regression analysis for BMI through nephropathy was not entered. Stepwise multivariate regression analysis as well as univariate regression analysis were performed on 101 NIDDM patients. r^2 = 0.47, F = 6.32, and P < 0.0001 in the multivariate regression analysis. Treatment at study was 0, diet; 1, sulfonylurea; 2, insulin; retinopathy was 0, nil; 1, nonproliferative; 2, proliferative; neuropathy was 0, absent; 1, present; nephropathy was 0, normoalbuminuria; 1, microalbuminuria; 2, overt albuminuria.

TABLE 4
Clinical characteristics of nondiabetic patients

	Patients with symptomatic atherosclerotic vascular disease	Patients without symptomatic atherosclerotic vascular disease	*P* value
n	16	48	
Age (years)	64 ± 8	59 ± 12	0.07
Male/female	8/8	16/32	0.23
BMI (kg/m^2)	22 ± 4	24 ± 3	0.04
Serum sVCAM-1 (ng/ml)	671 ± 195	646 ± 182	0.64

Data are means ± SD or *n*. Statistical significance was according to unpaired Student's *t* test, unpaired Welch's *t* test, χ^2 test, or Fisher's exact test, as appropriate.

Atherosclerosis is the outcome of various underlying factors, including lipoprotein abnormalities, hypertension, cigarette smoking, and diabetes. Multivariate regression analysis in all the NIDDM patients (Table 3) revealed that serum sVCAM-1 level was the strongest predictor of mean IMT value of the carotid arteries. Age also contributed to the IMT value, but less significantly. Other variables, including cigarette-years of smoking, BMI, known diabetes duration, type of treatment at study, HbA_{1c}, fasting serum C-peptide, serum LDL cholesterol, serum HDL cholesterol, mean blood pressure, retinopathy, neuropathy, and nephropathy, did not contribute to the IMT value. Therefore, serum sVCAM-1 level may be the most useful marker to predict atherosclerosis of the carotid arteries in NIDDM patients, even when the atherosclerosis is an asymptomatic process. Logistic regression analysis in the NIDDM patients failed to demonstrate significant association of serum sVCAM-1 level with symptomatic atherosclerotic vascular diseases (Table 2). This might be due to the significant contribution of serum sVCAM-1 level to asymptomatic atherosclerosis in patients without symptomatic atherosclerotic vascular disease.

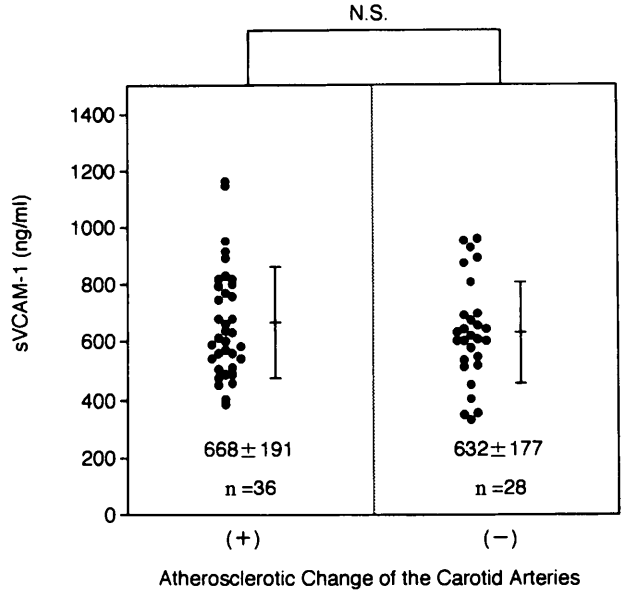

FIG. 3. Serum sVCAM-1 concentration in 64 nondiabetic subjects. Subjects were divided into two groups: those with atherosclerotic change of the carotid arteries (*n* = 36) and those without any detectable atherosclerosis (*n* = 28). Means ± SD are shown to the right of each column of data points.

What links diabetes to increased sVCAM-1 level is unknown. Recently, Schmidt et al. (39) showed that advanced glycation end products (AGEs), which result from nonenzymatic reactions of glucose with proteins, enhanced VCAM-1 expression in cultured endothelial cells via induction of DNA binding activity for NF-κB in the VCAM-1 gene promoter. They also demonstrated that AGEs increased sVCAM-1 release from the endothelial cells. Taken together with our results, accumulation of AGEs on vessel walls of NIDDM patients may trigger increased VCAM-1 expression and release of its soluble form, which is associated with early event of atherosclerosis.

Whether sVCAM-1 has some biological function or not remains unidentified. Recent observation has shown that sVCAM-1 is able to induce angiogenesis in vitro and in vivo (40). Further analysis is necessary to examine whether sVCAM-1 present in human sera plays biological roles in some physiological or pathological conditions.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Ministry of Education, Science, and Culture of Japan and from the Enami Memorial Foundation for Diabetes Research.

We are indebted to Dr. M. Koga for critical reading this manuscript; to Drs. K. Takihara, Y. Miyashita, T. Hirose, T. Motomura, and M. Takagi for expert care of the subjects in the study; to Mr. N. Taki for performing statistical analysis of the data; to Mr. S. Tsubakimori (Osaka Teishin Hospital) for helpful discussion on high-resolution B-mode ultrasonography; and to Keiko Tsujii for her secretarial assistance in preparing the manuscript.

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