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Soluble adhesion molecules and C-reactive protein in the progression of silent cerebral infarction in patients with type 2 diabetes mellitus

Takahiko Kawamura[a,b,*], Toshitaka Umemura[c], Akio Kanai[a], Masahito Nagashima[a], Nobuhisa Nakamura[a], Tomoko Uno[a], Mikihiro Nakayama[a], Takahisa Sano[a], Yoji Hamada[d], Jiro Nakamura[d], Nigishi Hotta[a]

[a]*Department of Metabolism and Endocrine Internal Medicine, Chubu Rosai Hospital, Nagoya 455-8530, Japan*
[b]*Labour Prevention Medical Center, Chubu Rosai Hospital, Nagoya 455-8530, Japan*
[c]*Department of Neurology, Kasugai City Hospital, Nagoya 486-8510, Japan*
[d]*Division of Metabolic Disease, Department of Internal Medicine, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan*

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Abstract

The purpose of this study was to investigate the association between the progression of silent cerebral infarction (SCI) and levels of soluble adhesion molecules and high-sensitivity C-reactive protein (hs-CRP) in type 2 diabetic patients. One hundred twenty middle-aged and elderly diabetic patients without histories of vascular events were followed up for a period of 3 years. We measured levels of soluble intercellular adhesion molecule 1 (sICAM-1), vascular cell adhesion molecule 1, E-selectin, and hs-CRP and assessed brain ischemic lesions by magnetic resonance imaging at baseline and 3 years later. Silent cerebral infarction was observed in 13% of the patients at baseline, and these patients were significantly older and had significantly higher blood pressure than those without SCI. Thirty-two patients had newly diagnosed SCI after 3 years. There were no significant differences in factors such as age, blood pressure, and diabetic control between patients without SCI and those in whom it was newly diagnosed. However, only sICAM-1 levels, but not the other soluble adhesion molecules or hs-CRP, were associated with the progression of SCI, and this relationship remains after adjustment for risk factors. On the other hand, higher levels of sICAM-1 and hs-CRP at baseline were observed in 7 patients who were excluded from the present study because of the onset of symptomatic cerebral infarction during follow-up. Our present study suggests that sICAM-1 levels may be a potential marker for SCI, which may lead to future stroke and vascular dementia, and that this marker could be useful in monitoring disease progression and as a surrogate marker in treatment studies.

1. Introduction

It is widely recognized that the accumulation of multiple risk factors such as dyslipidemia, hypertension, hyperinsulinemia, hypercoagulability, and accelerated fibrinolysis, together with hyperglycemia, leads to the complications of cerebrovascular and cardiovascular diseases in diabetic patients [1,2]. In Japan, the incidence of cerebral infarctions in middle-aged and elderly people is higher than in western countries, especially that of lacunar infarction [3]. In this regard, it has been reported that multiple lacunes are often observed in diabetic patients and are associated with a less favorable functional prognosis. Furthermore, this complication increases mortality in diabetic patients and greatly reduces their quality of life [4].

Recently, the discovery of asymptomatic cerebral infarction has increased with the widespread use of magnetic resonance imaging (MRI) [5-10]. This type of stroke, called silent cerebral infarction (SCI), has been identified as the preclinical stage for symptomatic stroke and vascular dementia [7-9]. The most potent risk factors for SCI have been reported to be hypertension and aging [7-10], and diabetes has been included among them [6-10], although it remains controversial whether diabetes is associated with a higher prevalence of SCI or not.

On the other hand, it has been reported that atherosclerosis is the result of inflammatory change [11], and increased levels of inflammatory markers such as C-reactive protein

* Corresponding author. Labour Prevention Medical Center, Chubu Rosai Hospital, Nagoya 455-8530, Japan. Tel.: +81 52 652 5511; fax: +81 52 651 5567.
E-mail address: kawamura.hsc@chubuh.rofuku.go.jp (T. Kawamura).

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(CRP) may reflect vascular complications and predict future events [12,13]. As for adhesion molecules, it is well known that they are expressed at the initial stage of atherosclerosis and play an important role in its progression [14]. An association between increased levels of soluble adhesion molecules and atherosclerotic disease has been reported, although the pathophysiological role and metabolism of the soluble forms remain unclear [15,16]. Furthermore, recent studies have found high future rates of cardiovascular [17,18] and cerebrovascular disease [19] in healthy subjects with increased levels of soluble adhesion molecules.

Levels of soluble adhesion molecules have been found to be consistently higher in diabetic patients than in nondiabetic subjects [20-23], and elevated levels have been observed in diabetic patients with microvascular and macrovascular complications [24,25]. Thus, diabetic patients with increased levels of adhesion molecules may have a high risk for future vascular events. Recently, the association between cerebrovascular disease and adhesion molecules has been receiving much attention [26-28]. In a previous study, we noted that levels of soluble adhesion molecules were higher in elderly diabetic patients with SCI than those without SCI, suggesting that higher levels may be an independent predictor of future ischemic stroke and vascular dementia [22]. However, there have been few other reports of adhesion molecules being elevated under a chronic condition such as SCI or on the role of adhesion molecules in diabetic patients with cerebrovascular disease [29].

We therefore followed up middle-aged and elderly diabetic patients during 3 years and investigated the association between the progression of SCI and levels of soluble adhesion molecules in comparison with CRP as a potential predictor for these conditions.

2. Patients and methods

2.1. Patients

We recruited consecutively 154 patients with type 2 diabetes mellitus who were older than 45 years and without history or clinical symptoms of cerebrovascular diseases from a group of outpatients at the Chubu Rosai Hospital's Diabetic Center for the present study. Informed consent was obtained from each of the participants, and the study was performed in accordance with the principles of the Declaration of Helsinki. The exclusion criteria were as follows: malignancy, inflammatory disease (such as collagen disease, thyroid disease, and viral hepatitis), severe microvascular complications (such as renal failure), and severe cardiovascular disease (such as myocardial infarction and unstable angina).

During the follow-up period, 21 patients were excluded from the study for malignancy, the progression of diabetic vascular complications, or because they dropped out, and in addition, 7 patients had nonfatal cerebrovascular disease and 5 patients had nonfatal cardiovascular events, and a patient died of cerebral embolism. Finally, 120 patients without vascular events during 3 years were evaluated in the present study (mean, 61.6 ± 8.4 years; range, 46-81 years at baseline; 56 males, 60.7 ± 8.0 years; 64 females, 62.4 ± 8.7 years).

Hypertension was defined by prior diagnosis, current treatment with an antihypertensive agent, or blood pressure greater than 140/90 mm Hg when enrolled in the study. We conducted 3 consecutive measurements of the blood pressure and recorded the average values every month.

2.2. Evaluation of subjects

Fasting blood samples were separated, analyzed, and the serum was stored at −70°C until the assay of adhesion molecules. Total cholesterol, high-density lipoprotein cholesterol, triglycerides, and fasting blood glucose (FBG) were measured by an autoanalyzer using routine enzymatic techniques. Hemoglobin A_{1c} (HbA_{1c}; reference range, 4.3%-5.8%) was measured with a DCA 2000 analyzer (Bayer Ames, Elkhart, IN) every month. Plasma insulin was assayed by radioimmunoassay. For the plasma fibrinogen assay, the Dade thrombin-clotting time methodology was used. High-sensitivity CRP (hs-CRP) was assayed using a monoclonal antibody coated to polystyrene particles and fixed-timed kinetic nephelometric measurements (BN II, Dade Behring, Marburg, Germany) [30].

2.3. Assessment of atherosclerosis

Silent cerebral infarction was diagnosed using MRI (GE Signa Horizon, 1.5 T, Milwaukee, WI), and the criteria used for diagnosis were irregular areas of high signal intensity larger than 3 mm in diameter detected on T2-weighted images, low signal intensity areas on T1-weighted images, and areas of higher intensity than that of the cerebrospinal fluid in proton density images or fluid-attenuated inversion recovery images. Lesions less than 3 mm in diameter or lesions with signal intensity similar to cerebrospinal fluid in proton images and fluid-attenuated inversion recovery images were excluded because of the high possibility that they were enlarged perivascular spaces, even if they demonstrated a high signal on T2-weighted images and a low signal on T1-weighted images. We also excluded leukoaraioses detected only on T2-weighted images from this study.

2.4. Measurement of serum-soluble adhesion molecules

Serum samples for the determination of soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), and soluble E-selectin (sE-selectin) were analyzed in duplicate using the single-step sandwich enzyme-linked immunosorbent assay method (R&D Systems Europe, Abingdon, Oxon, UK) with Model 550 Microplate Reader (Bio-Rad, Hercules, CA) as reported previously [22,23].

2.5. Statistical analysis

Results were expressed as mean ± SD. Statistical analysis was performed by analysis of variance, followed by unpaired

t test for the comparison of 2 means. The χ^2 test for independence, and simple regression, multivariate stepwise regression, and logistic regression analyses were also used where appropriate. Because the distribution of the levels of soluble adhesion molecules and hs-CRP has been reported to be often skewed, differences in concentrations were evaluated through nonparametric statistical procedures by Mann-Whitney U test, and results were expressed as median (interquartile range, 25%-75%). Analysis of covariance was also used for the adjustment of covariables of age, sex, mean blood pressure and HbA_{1c} during the follow-up period, and smoking habit. Statistical analyses were carried out using Statview 5.0 (SAS, Cary, NC) or SPSS 10.1 software (SPSS, Chicago, IL), and $P < .05$ was considered to be statistically significant.

3. Results

3.1. Comparison of clinical and biochemical characteristics between diabetic patients with and without SCI at baseline and after a 3-year follow-up

Silent cerebral infarction was revealed in 15 patients (13%) at baseline and in 47 patients (39%) after 3 years. Significant differences were observed in age, systolic blood pressure, and FBG between diabetic patients with and those without SCI at baseline. In addition, the patients with SCI were much likely to have been treated with hypoglycemic agents or insulin and to have had longer durations of diabetes and microvascular complications at baseline or after the follow-up. However, no significant differences were found in smoking habit, body mass index, and dyslipidemia between the 2 groups (Table 1).

In regard to the levels of soluble adhesion molecules and hs-CRP, sICAM-1 and sE-selectin were found at higher levels in patients with SCI than in those without SCI, whereas there were no differences in the levels of hs-CRP between the two. Soluble VCAM-1 had a tendency to be higher in patients with SCI at baseline.

3.2. Clinical and biochemical characteristics and progression of SCI during a 3-year follow-up

Silent cerebral infarction was newly found in 32 patients after 3 years. No significant differences were observed in age, duration of diabetes, blood pressure, presence of microvascular complications, treatment modality, diabetic control, smoking habit, and dyslipidemia between patients without SCI and those with newly diagnosed SCI (Table 2, Fig. 1).

Multivariate regression analysis of the levels of soluble adhesion molecules and hs-CRP, and the other factors such

Table 1
Clinical and biochemical characteristics between diabetic patients with and without SCI at baseline and after 3 years

	Baseline		After 3 years	
	SCI (−)	SCI (+)	SCI (−)	SCI (+)
No. of patients	105	15	73	47
Male/female	46/59	10/5*	31/42	25/22
No. of infarcts (1/2/>2)	–	4/4/7	–	18/12/17
Age (y)	61.1 ± 7.4	65.3 ± 6.3**	64.2 ± 7.7	65.9 ± 9.2
Duration of diabetes (y)	10.1 ± 8.6	13.4 ± 7.5	12.2 ± 8.8	15.4 ± 8.8*
Treatment (diet/OHA/insulin)	44/51/10	4/7/4	26/39/8	13/20/14**
Diabetic microvascular complications (+/−)	27/78 (25.7%)	7/8* (46.7%)	20/53 (27.4%)	19/28 (40.4%)
Smoking habit (never/ex/current)	63/20/22	6/5/4	46/15/12	23/10/14
Body mass index (kg/m^2)	22.9 ± 3.3	23.6 ± 3.0	22.3 ± 3.5	23.1 ± 2.8
Hypertension (+/−)	43/62 (41.0%)	9/6 (60.0%)	29/44 (39.7%)	26/21* (55.3%)
Systolic BP (mm Hg)	127.6 ± 16.6	137.9 ± 18.8**	131.4 ± 17.0	135.5 ± 16.2
Diastolic BP (mm Hg)	70.9 ± 10.0	76.0 ± 12.0*	68.8 ± 10.3	70.6 ± 9.1
Total cholesterol (mmol/L)	5.38 ± 0.79	5.27 ± 0.89	5.36 ± 0.73	5.24 ± 0.81
High-density lipoprotein cholesterol (mmol/L)	1.50 ± 0.48	1.39 ± 0.34	1.48 ± 0.38	1.36 ± 0.34*
Triglycerides (mmol/L)	1.56 ± 0.89	1.55 ± 0.67	1.33 ± 0.88	1.50 ± 0.74
FBG (mmol/L)	7.78 ± 1.87	8.93 ± 2.88**	7.73 ± 1.80	8.96 ± 2.66***
Fasting IRI (pmol/L)	54.1 ± 29.6	50.0 ± 26.0	57.5 ± 34.6	68.3 ± 35.1
HbA_{1c} (%)	6.78 ± 1.27	7.19 ± 1.04	6.71 ± 0.98	7.09 ± 1.38*
Hematocrit (%)	41.2 ± 4.0	42.9 ± 5.4	40.5 ± 4.0	41.4 ± 4.7
Fibrinogen (μmol/L)	8.30 ± 2.52	8.47 ± 1.93	9.05 ± 2.47	8.98 ± 2.05
hs-CRP (mg/L)	0.67 (0.25-1.45)	0.58 (0.39-1.60)	0.52 (0.26-1.08)	0.51 (0.39-1.31)
sICAM-1 (μg/L)	214 (175-266)	286 (238-334)***	207 (156-235)	241 (207-296)***
sVCAM-1 (μg/L)	440 (377-508)	481 (435-526)*	429 (389-493)	456 (405-534)
sE-selectin (μg/L)	47.6 (35.5-62.2)	63.5 (41.6-78.7)**	42.8 (31.2-52.7)	48.1 (39.6-65.5)**

Data are expressed as mean ± SD or median (interquartile range). The χ^2 test for independence was performed where appropriate. Comparisons of 2 groups are evaluated by unpaired t test or by Mann-Whitney U test. Diabetic microvascular complications (+) indicate presence of clinical retinopathy and/or overt proteinuria and/or neuropathy evaluated by neurologist with symptom, physical examination, and nerve conduction velocity; OHA, oral hypoglycemic agents; BP, blood pressure; IRI, immunoreactive insulin.

* $P < .1$, different from SCI (−).

** $P < .05$, different from SCI (−).

*** $P < .01$, different from SCI (−).

Table 2
Clinical and biochemical characteristics and progression of SCI in diabetic patients after a 3-year follow-up

	N	N-S	*P*
No. of subjects	73	32	–
Male/female	31/42	15/17	.675
No. of infarcts (1/2/>2)	–	17/9/6	–
Age (y)	64.2 ± 7.7	64.7 ± 9.9	.813
Body mass index (kg/m^2)	22.3 ± 3.5	23.2 ± 2.8	.165
Duration of diabetes (y)	12.2 ± 8.8	15.0 ± 9.4	.147
Treatment (Diet/OHA/insulin)	26/39/8	9/15/8	.179
Diabetic complication (+/−)	21/52 (28.8%)	11/21 (34.4%)	.497
Smoking habit (never/ex/current)	46/15/12	17/5/10	.226
Hypertension (+/−)	29/44 (39.7%)	18/14 (56.3%)	.117
Baseline (mm Hg)			
Systolic BP	125.7 ± 115.9	132.0 ± 17.7	.077
Diastolic BP	70.5 ± 9.6	71.8 ± 11.1	.561
3 y later (mm Hg)			
Systolic BP	131.4 ± 17.0	135.1 ± 15.9	.295
Diastolic BP	68.8 ± 10.3	70.9 ± 9.2	.316
During follow-up (mm Hg)			
Mean systolic BP	129.6 ± 12.3	131.3 ± 11.2	.484
Mean diastolic BP	68.5 ± 6.9	70.6 ± 6.6	.160
HbA_{1c} (%)			
Baseline	6.67 ± 1.22	7.01 ± 1.37	.214
3 y later	6.71 ± 0.98	6.93 ± 0.97	.300
Mean during follow-up	6.78 ± 0.90	7.02 ± 0.95	.214
Dyslipidemia (+/−)	37/36 (50.7%)	18/14 (56.3%)	.599
Fibrinogen (μmol/L)	9.05 ± 2.47	8.10 ± 1.99	.483
Hematocrit (%)	40.5 ± 4.0	41.1 ± 5.0	.552

Data are expressed as mean ± SD. Comparisons of 2 groups are evaluated by unpaired *t* test. The χ^2 test for independence is performed where appropriate. Mean systolic BP, diastolic BP, and mean HbA_{1c} are shown by averaging values during 3 years. N indicates patients without SCI during 3 years; N-S, patients with newly diagnosed SCI after 3 years.

as shown in Table 1, revealed that the most potent predictor for the progression of SCI was sICAM-1 (r^2 = 0.144, F = 5.99, P < .005). Only the levels of sICAM-1, but not the levels of sVCAM-1, sE-selectin, or hs-CRP, were significantly lower in patients without SCI than in those with newly diagnosed SCI after the follow-up period as shown in Fig. 1.

We then evaluated the statistical differences in the sICAM-1 levels between patients without SCI and those with newly diagnosed SCI after adjustment by covariables of age, sex, mean of blood pressure and HbA_{1c}, during follow-up, and smoking habit by analysis of covariance. Significantly lower levels of sICAM-1 were observed in patients without SCI at baseline (F = 9.95, P = .002) and 3 years later (F = 6.61, P = .012) independently of these factors. The relative risk for the progression of SCI over 3 years was about 8 times in the highest quartile (>277.2 μg/L) of sICAM-1 levels at baseline as compared with the lowest quartile (<184.1 μg/L) after adjustment for covariables (Table 3). These risks remained significant after adding hs-CRP and sE-selectin to the model (data not shown).

3.3. Levels of soluble adhesion molecules and hs-CRP during the progression of ischemic stroke

Seven patients, of whom 6 patients had SCI at baseline, developed symptomatic infarction during follow-up. We examined soluble levels of adhesion molecules and hs-CRP of those patients including patients with symptomatic infarction in Fig. 1. Significantly higher levels of sICAM-1 and hs-CRP were found in patients with symptomatic infarction as compared with those without SCI at baseline.

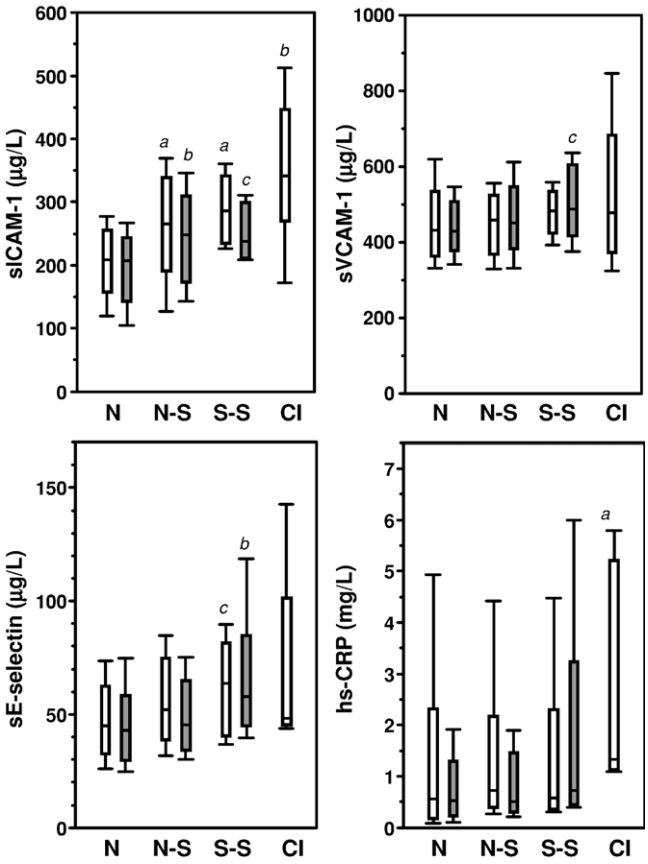

Fig. 1. Levels of sICAM-1 at baseline and 3 years later during the progression of ischemic stroke. Data are expressed as median (interquartile range, 25%-75%) (μg/L). The open and gray columns show the data at baseline and after the follow-up, respectively. Differences among groups are evaluated by Mann-Whitney *U* test. N indicates patients without SCI during the follow-up (n = 73); N-S, patients with newly diagnosed SCI (n = 32); S-S, patients with detected SCI at baseline and after the follow-up (n = 15); CI, patients with symptomatic infarction excluding a cerebral embolism during the follow-up (n = 7). [a]P < .001, [b]P < .01,[c]P < .05, different from N, respectively.

Table 3
The relative risk for the progression of SCI over 3 years according to sICAM-1 levels at baseline

Quartiles of sICAM-1 (μg/L)	Crude[a] OR (CI)	Adjusted[b] OR (CI)
Q1 (<184.1)	1.00	1.00
Q2 (184.1-226.4)	0.65 (0.16-2.63)	0.67 (0.16-2.81)
Q3 (226.5-277.2)	1.80 (0.53-6.15)	1.68 (0.46-6.10)
Q4 (>277.2)	8.22 (2.27-29.7)*	8.61 (2.04-36.3)*

ORs were derived from logistic regression analyses (n = 105). OR indicates odds ratio; CI, % confidence interval.

[a] Adjusted for age and sex.

[b] Additionally adjusted for mean systolic BP, mean diastolic BP, and mean HbA_{1c} during 3 years, and smoking habit.

* P < .005.

The number of infarcts detected with MRI increased after 3 years in 6 of 15 patients with SCI at baseline. Taken together, the progression group (patients with newly diagnosed SCI after 3 years, with increased numbers of infarcts after 3 years in patients with SCI at baseline and with the onset of symptomatic infarction) showed markedly higher levels of sICAM-1 as compared with the other patients (293 μg/L [228-342 μg/L] vs 211 μg/L [180-256 μg/L], $P < .0001$ at baseline; 251 μg/L [206-312 μg/L] vs 211 [161-237 μg/L], $P < .0005$ after follow-up). Higher levels of hs-CRP were observed at baseline in the progression group (0.81 mg/L [0.49-1.68 mg/L] vs 0.57 mg/L [0.22-1.44 mg/L], $P < .05$), whereas no significant differences were found in levels of sVCAM-1 and sE-selectin (data not shown) (Fig. 1).

4. Discussion

Ischemic cerebrovascular disease can be classified into 2 main types—atherosclerotic large-vessel disease and hypertension-related small-vessel disease excluding cerebral embolism. Recently, the widespread use of MRI has drawn medical attention to the frequency of unexpected white matter lesions [5-10]. Silent cerebral infarction, which is now classified as a type III cerebrovascular disorder proposed by the National Institute of Neurological Disorders and Stroke, is recognized not only as powerful predictor of symptomatic stroke, but also as a specific marker of target organ damage in the brain in conditions such as vascular dementia [7-10]. It is thought that most of the lesions of SCI occur in small vessels of the brain and that one of the underlying causes for such small-vessel disease is endothelial cell dysfunction [31], although the mechanism has not been fully resolved. Because dyslipidemia and hypertension occur together with hyperglycemia and hyperinsulinemia in diabetic patients, it is likely they are responsible for endothelial dysfunction that may lead to microvascular disorders in the brain such as SCI [1,2]. This is suggested by our observation that increased levels of sICAM-1 and sE-selectin, which reflect endothelial function, were found in patients with SCI.

Hypertension and aging are well established as risk factors for SCI [7-10], and the findings of our present study are in agreement with those of previous studies in this regard. However, our study showed that classic risk factors such as blood pressure, glycemic control, and age were not associated with occurrence of SCI during the follow-up period.

We have reported that sICAM-1 and sVCAM-1 levels were elevated in elderly diabetic patients with SCI [22]. In the present study we found that levels of sICAM-1 and sE-selectin were elevated in subjects including middle-aged subjects with SCI at baseline and after follow-up. However, sICAM-1 level was the only factor found to be closely associated with the progression of SCI in the present study. The relationship between sICAM-1 levels and SCI was still significant after adjustment for smoking and other risks, although we had observed that smoking increased sICAM-1 levels in a previous study [23]. Tanne et al [19] also reported the association of future stroke in healthy subjects with high levels of sICAM-1. Although it is unclear why only sICAM-1 levels should be associated with the progression of ischemic stroke, our study suggested some possibilities.

It has been reported that brain cells produce cytokines and chemokines and can express adhesion molecules. In this regard, tumor necrosis factor α (TNF-α) may exert a primary effect on the microvascular inflammatory response as reflected by TNF-α–induced neutrophil adhesion to brain capillary endothelium through the up-regulation of endothelial adhesion molecules. Actually, many leukocytes (primarily neutrophils) in vessels in ischemic tissue have been observed to adhere to the endothelium [27]. It has also been reported that ICAM-1 is expressed on the cell surface with exposure to cytokines such as TNF-α and interleukin 1β during ischemia [32] or free radicals induced after reperfusion [33]. Because ICAM-1 is widely expressed on leukocytes including neutrophils, fibroblasts, and epithelial cells in contrast to VCAM-1, it is likely that ICAM-1 expressed on neutrophils is strongly associated with microvascular occlusion in the brain. Although this suggests differences in the action of these adhesion molecules [16,34], further studies will be needed to clarify it.

It has also been established that atherosclerosis is associated with inflammatory changes [11], and CRP has been reported to be a powerful marker for predicting future events [12,13]. Although 7 patients with advanced nonfatal ischemic stroke had high levels of hs-CRP and sICAM-1 at baseline, we did not find a significant relationship between hs-CRP levels and SCI. It is therefore likely that hs-CRP is indicative of atherosclerotic large-vessel disease in the brain, but not small-vessel disease. Rader [35] has mentioned that the levels of soluble adhesion molecules may provide predictive information augmenting that from inflammatory markers such as CRP. Our result that sICAM-1 levels can still be an independent risk factor for ischemic stroke after adjustment for hs-CRP may support this. However, owing to the small number of events in the present study, a further prospective study will be needed to confirm the significance of using adhesion molecules and CRP independently predicting future cerebrovascular events and to evaluate the usefulness of their combined use in screening subjects for cerebrovascular risk.

The present study had some limitations. The sample size was relatively small. Some patients were receiving drugs like statins and angiotensin-converting enzyme inhibitors, which can reduce levels of adhesion molecule and hs-CRP [36,37]. However, data obtained after the period of follow-up were similar to those at baseline, indicating that it did not influence the results, even if the drugs were having a beneficial effect on adhesion molecule expression.

In conclusion, the present study showed that ischemic cerebrovascular disease was more likely in diabetic patients with higher sICAM-1 levels. Therefore, sICAM-1 levels may be a potential marker for SCI, which may lead to future

stroke and vascular dementia, and that this marker could be useful in monitoring disease progression and as a surrogate marker in treatment studies.

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