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Soluble adhesion molecule E-selectin predicts cardiovascular events in Japanese patients with type 2 diabetes mellitus

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Abstract

Soluble adhesion molecule E-selectin (sE-selectin) is a marker of endothelial activation. To investigate whether high serum concentrations of sE-selectin could predict cardiovascular events, we followed 392 Japanese patients with type 2 diabetes mellitus who had no history of cardiovascular disease for a mean period of 6 years. The cardiovascular end points were defined as fatal and nonfatal myocardial infarction, angina pectoris, stroke, and sudden death. During the follow-up period, 51 patients reached end point. Patients who reached end point were significantly older and had longer duration of diabetes, higher systolic blood pressure, higher hemoglobin A1c, higher plasma glucose, higher sE-selectin, and lower high-density lipoprotein cholesterol compared with those free of such events. The mean serum concentration of sE-selectin was higher in patients who reached end point (81.1 ± 32.2 ng/mL) than event-free patients (66.7 ± 33.7 ng/mL, mean ± SD; $P < .01$). Multiple logistic regression analysis identified age, systolic blood pressure, total cholesterol, sE-selectin, and low high-density lipoprotein cholesterol as independent factors related to cardiovascular events. The odds ratio for cardiovascular events for 1-SD increase in sE-selectin concentration was 1.45 (95% confidence interval, 1.22-1.71). Kaplan-Meier analysis demonstrated a significantly higher cardiovascular event rate in the highest tertile of sE-selectin compared with the lowest or middle tertile of sE-selectin ($P < .01$). The results suggest that high serum concentrations of sE-selectin can predict cardiovascular events in Japanese patients with type 2 diabetes mellitus.

1. Introduction

Endothelial activation and/or dysfunction plays important roles in atherosclerogenesis [1,2] and can be evaluated by flow-mediated vasodilatation [3]. However, the technique of flow-mediated vasodilatation is complex; and trained technicians are needed. In contrast, serum markers of endothelial activation, such as von Willebrand factor, thrombomodulin, and C-reactive protein (CRP), are widely used for such evaluation because of their simplicity [4-6]. These serum markers are elevated in patients with atherosclerotic cardiovascular diseases as determined in both cross-sectional and longitudinal studies [4-6]. Soluble adhesion molecule E-selectin (sE-selectin) is used as a marker of endothelial activation [7]. Several studies reported that sE-selectin levels are elevated in patients with type 2 diabetes mellitus [8-10]. We also reported previously that serum levels of sE-selectin correlated with hyperglycemia, insulin resistance, and adiposity in Japanese patients with type 2 diabetes mellitus [11-15]. High levels of sE-selectin have been reported in diabetes, hypertension, dyslipidemia, obesity, and smoking [11]. Thus, atherogenic conditions may activate the aortic endothelium and lead to elevation of serum sE-selectin. These observations suggest that high serum concentrations of sE-selectin may predict future cardiovascular events.

In the present 6-year longitudinal study, we investigated the predictive power of high serum concentrations of sE-selectin for cardiovascular events in Japanese patients with type 2 diabetes mellitus.

2. Materials and methods

A total of 392 Japanese patients with type 2 diabetes mellitus who had no history of cardiovascular disease gave informed consent to participate in this hospital-based observational study. Type 2 diabetes mellitus was diagnosed according to the criteria of the World Health Organization

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[16]. The exclusion of patients with cardiovascular disease was conducted by taking detail medical history from each patient and checking routine electrocardiogram (ECG). The study protocol was approved by the ethics committee of Sasebo Chuo hospital. All patients were recruited from the Outpatient Clinic of Sasebo Chuo Hospital.

Baseline blood samples were drawn at 1 to 3 hours after breakfast. Plasma glucose, hemoglobin A_{1c} (HbA_{1c}), total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were measured. Plasma glucose level was measured by the glucose oxidase method (Kyoto Daiichi Kagaku, Kyoto, Japan). Hemoglobin A_{1c} level was measured by high-performance liquid chromatography method (Tosoh, Tokyo, Japan). Total cholesterol and TG levels were measured by the enzymatic method (Kokusai Shiyaku, Kobe, Japan). High-density lipoprotein cholesterol was determined after isolation by precipitation method. We also measured sE-selectin, soluble intercellular adhesion molecule−1 (sICAM-1), and soluble vascular cell adhesion molecule−1 (sVCAM-1) by commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN). The intra- and interassay coefficients of variations were less than 6% for all 3 proteins.

We followed 392 patients (men, 60.5%; age, 60.1 ± 10.4 years; body mass index [BMI], 23.6 ± 3.4 kg/m^2; mean ± SD) for 1 to 8 years (mean, 6 years). During the follow-up period, 24 patients were lost to follow-up; and thus, data of only 368 patients were available for analysis (follow-up rate, 93.9%). The end points of this study were (1) fatal and nonfatal myocardial infarction, (2) angina pectoris, (3) fatal and nonfatal stroke, and (4) sudden death. Acute myocardial infarction was diagnosed based on typical ECG changes with elevation of cardiac enzymes. Angina pectoris was diagnosed by history of typical chest pain and ECG changes or coronary angiography. Stroke was diagnosed by neurologic deficit(s) and confirmed by computed tomography or magnetic resonance imaging.

Unpaired data were analyzed by Student *t* test or contingency table analysis. Independent factors related to cardiovascular events were analyzed by multiple logistic regression analysis. The time-dependent effects of serum E-selectin on cardiovascular events were analyzed by Kaplan-Meier survival curve with log-rank test. Data are presented as mean ± SD. Differences were considered statistically significant at $P < .05$. Statistical analysis was performed using Statview 5.0 (SAS, Cary, NC) software package.

3. Results

During the follow-up period, 51 patients reached end points (13.9% during 6 years). The details of cardiovascular events were as follows: 6 patients had myocardial infarction, 17 had angina pectoris, 26 had stroke, and 2 had sudden death. The baseline clinical characteristics of patients who reached end points and event-free patients are listed in Table 1. Patients who reached end points were significantly older and had longer duration of diabetes, higher systolic blood pressure, higher HbA_{1c}, higher plasma glucose, and lower HDL-C levels than event-free individuals. Sex, the treatment mode of diabetes, smoking status, BMI, TC, and TG were not significantly different between the 2 groups.

Serum concentrations of sICAM-1 tended to be higher in patients who reached end point (231.0 ± 86.2 ng/mL) compared with event-free patients (212.8 ± 89.5 ng/mL), but these differences did not reach statistical significance ($P = .17$). Similar differences were noted in sVCAM-1 (end point, 902.2 ± 316.9 ng/mL; event-free, 866.8 ± 294.1 ng/mL; $P = .43$). In contrast, serum concentrations of sE-selectin were significantly higher in patients who reached end point (81.1 ± 32.2 ng/mL) than event-free patients (66.7 ± 33.7 ng/mL, $P < .01$, Fig. 1).

To investigate the independent factors related to cardiovascular events in Japanese diabetic patients, we performed multiple logistic regression analysis. The following independent variables were included in the model: age, duration of diabetes, sex, smoking, BMI, systolic blood pressure, diastolic blood pressure, HbA_{1c}, plasma glucose, TC, TG, HDL-C, sICAM-1, sVCAM-1, and sE-selectin. Table 2 shows the independent factors related to cardiovascular events. The odds ratio and 95% confidence interval (CI) for every 1-SD difference are listed. Age, systolic blood pressure, TC, HDL-C, and sE-selectin were independent factors related to cardiovascular event. Duration of diabetes was significantly related to cardiovascular events in unpaired *t* test. However, in the multiple logistic regression model, duration of diabetes ($P = .47$) was not independently related to events. Age was still an independent predictor of event in that model ($P < .01$). Thus, age had stronger factor than duration of diabetes. Soluble E-selectin was a significant and

Table 1
Baseline clinical characteristics of patients with and without cardiovascular events

	With CVD (n = 51)	Event-free (n = 317)
Age (y)	65.8 ± 9.1*	60.2 ± 10.4
Duration of diabetes (y)	9.0 ± 7.7*	6.6 ± 6.0
Sex (male)	36 (70.6)	185 (58.4)
Therapy (D/O/I)	17/22/12 (33.3/43.1/23.5)	140/117/60 (44.2/36.9/18.9)
Smoking (yes)	17 (33.3)	103 (32.5)
BMI (kg/m^2)	24.4 ± 12.2	23.5 ± 11.5
Systolic blood pressure (mm Hg)	140 ± 18*	132 ± 19
Diastolic blood pressure (mm Hg)	75 ± 12	74 ± 11
HbA_{1c} (%)	7.5 ± 1.7*	7.0 ± 1.6
Plasma glucose (mg/dL)	191 ± 64*	167 ± 72
TC (mg/dL)	202 ± 50	193 ± 37
TG (mg/dL)	168 ± 127	141 ± 89
HDL-C (mg/dL)	51.9 ± 13.5*	58.6 ± 16.7

Data are mean ± SD or number (percentage). CVD indicates cardiovascular disease; D, diet therapy; O, oral hypoglycemic agent therapy; I, insulin therapy.

* $P < .05$ vs event-free.

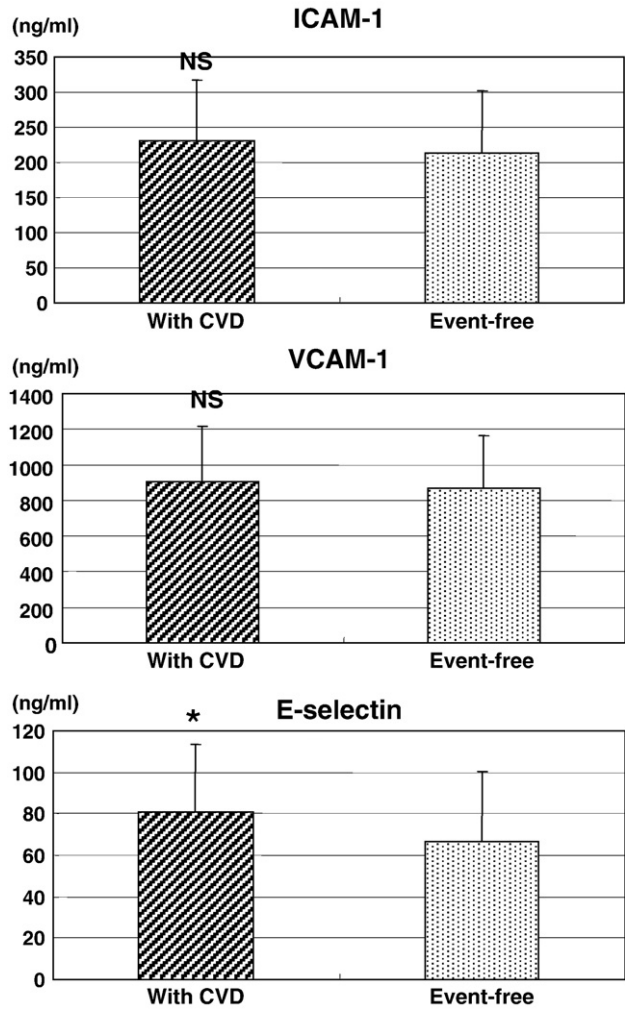

Fig. 1. Serum concentrations of sICAM-1, sVCAM-1, and sE-selectin in patients with and without cardiovascular events. *$P < .01$ vs event-free patients. CVD indicates cardiovascular disease.

independent determinant of cardiovascular events, with odds ratio of 1.45 (95% CI, 1.22-1.71) for 1-SD increase in sE-selectin concentration.

To determine the time-dependent effects of sE-selectin, we performed Kaplan-Meier analysis according to the tertile of sE-selectin. All patients were subdivided into 3 groups: low sE-selectin (7.6-52.3 ng/mL), mid sE-selectin (52.7-75.0 ng/mL), and high sE-selectin (75.2-225.9 ng/mL) levels. Fig. 2 shows the Kaplan-Meier survival curves. Patients with high serum sE-selectin had significantly higher cardiovascular event rate compared with those of low and mid sE-selectin levels ($P < .01$).

4. Discussion

We reported previously that high serum concentrations of sE-selectin, a marker of endothelial activation, related to atherosclerotic conditions such as insulin resistance, obesity, or clustering of multiple risk factors in Japanese patients with type 2 diabetes mellitus [11-15]. In the present study, we have documented in a prospective longitudinal study that a high serum concentration of sE-selectin is a significant and independent predictor of cardiovascular events in Japanese patients with type 2 diabetes mellitus.

Table 2
Independent factors related to cardiovascular events in multiple logistic regression analysis

	Odds ratio	95% CI	*P* value
Age (every 1 SD)	2.08	1.37-3.10	<.01
Systolic blood pressure (every 1 SD)	1.57	1.05-2.39	.03
TC (every 1 SD)	1.54	1.06-2.25	.03
HDL-C (every 1 SD)	0.62	0.08-0.99	.04
sE-selectin (every 1 SD)	1.45	1.22-1.71	.03

We also reported previously that high serum concentrations of CRP and insulin resistance (measured by insulin tolerance test) could predict all-cause death and cardiovascular events in Japanese patients with type 2 diabetes mellitus [17]. C-reactive protein is widely used as a marker of inflammation [6]. Inflammation can activate the vascular endothelium [18], and CRP levels correlate with serum concentrations of sE-selectin [19]. These early findings suggest that high serum concentrations of sE-selectin could reflect the presence of vascular endothelial inflammation. Furthermore, insulin resistance could also induce endothelial activation [18]. We reported that serum concentrations of sE-selectin correlated with insulin resistance measured by insulin tolerance test [13,14]. Leinonen et al [20] reported that insulin resistance measured by homeostasis model correlated with either sE-selectin or CRP. Considering these relationships, it would be interesting to add CRP and insulin resistance marker in the regression analysis to analyze the importance of sE-selectin in comparison with other markers. Unfortunately, we did not evaluate CRP and insulin resistance in this follow-up study.

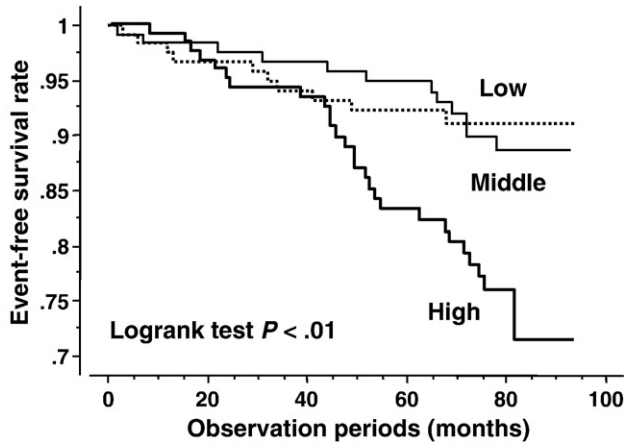

Fig. 2. Kaplan-Meier analysis according to the tertile of sE-selectin. All patients were subdivided into 3 groups: low sE-selectin (7.6-52.3 ng/mL), mid sE-selectin (52.7-75.0 ng/mL), and high sE-selectin (75.2-225.9 ng/mL) levels.

A recent study indicated that albuminuria can be also used as a predictor of cardiovascular event [21]. Although the precise mechanism of the development of atherosclerosis in a state of albuminuria is elusive at present, albuminuria may correlate with vascular dysfunction [21]. In this regard, a number of studies reported the correlation of microalbuminuria with high serum concentrations of sE-selectin [22,23]. Therefore, high serum concentrations of sE-selectin may correlate with albuminuria and cardiovascular event. Unfortunately, we did not measure urinary albumin level in the present longitudinal study.

One of the most important issues is whether sE-selectin is only a risk marker or whether it has a role as a mediator of atherosclerosis. Elevated levels of sE-selectin may reflect the increased expression of E-selectin on the surface of aortic endothelium. Furthermore, Koch et al [24] reported that soluble form of E-selectin per se could induce angiogenesis and accelerate the progression of atherosclerosis. These results suggest that sE-selectin may be not only a risk marker but also a disease mediator for atherosclerosis.

In the present study, we draw the blood samples at postprandial state. Postprandial glucose rather than fasting glucose is known as a predictor of cardiovascular disease [25]. Patients who reached end point showed higher postprandial glucose than event-free patients. However, postprandial glucose was not independently related to cardiovascular events in multiple logistic regression analysis. Marfella et al [26] and Ceriello et al [27] reported that hyperglycemia could increase the serum concentrations of soluble adhesion molecules. However, in the multiple logistic regression analysis, sE-selectin was an independent factor related to cardiovascular event. Thus, elevated levels of sE-selectin were not explained as a result of postprandial hyperglycemia alone.

Soluble VCAM-1 is reported as a marker of the extent of atherosclerosis [28,29]. We also reported that serum concentrations of sVCAM-1 were elevated in the presence of carotid atherosclerosis [30]. Thus, high serum concentrations of sVCAM-1 may be associated with the extent of atherosclerosis at a point in a cross-sectional study. Jager et al [31] reported that high serum concentrations of sVCAM-1 could predict cardiovascular events in white people in a population study. However, in Japanese patients with type 2 diabetes mellitus, Kawamura et al [32] reported no relationship between serum concentrations of sVCAM-1 and the progression of silent cerebral infarction. In our present study, sICAM-1 could not predict cardiovascular events either. In our former cross-sectional study, serum concentrations of sICAM-1 were elevated in diabetic patients with microangiopathy (retinopathy), but were not elevated in patients with carotid atherosclerosis [30]. Taken together, sVCAM-1 and sICAM-1 could not predict cardiovascular events in Japanese patients with type 2 diabetes mellitus.

In conclusion, the combination of old age; high systolic blood pressure, TC, and sE-selectin; and low HDL-C can predict cardiovascular events in Japanese patients with type 2 diabetes mellitus. Thus, sE-selectin, reflecting endothelial activation, could be a marker of future cardiovascular events.

References

[1] Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000;101:1899-906.

[2] Quyyumi AA. Prognostic value of endothelial function. Am J Cardiol 2003;91:19H-24H.

[3] Inoue T, Matsuoka H, Higashi Y, et al. Flow-mediated vasodilation as a diagnostic modality for vascular failure. Hypertens Res 2008;31: 2105-13.

[4] Spiel AO, Gilbert JC, Jilma B. von Willebrand factor in cardiovascular disease: focus on acute coronary syndromes. Circulation 2008;117: 1449-59.

[5] Salomaa V, Matei C, Aleksic N, et al. Soluble thrombomodulin as a predictor of incident coronary heart disease and symptomless carotid artery atherosclerosis in the Atherosclerosis Risk in Communities (ARIC) Study: a case-cohort study. Lancet 1999;353:1729-34.

[6] Ridker PM, Rifai N, Rose L, et al. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002;347:1557-65.

[7] Ferri C, Desideri G, Baldoncini R, et al. Early activation of vascular endothelium in nonobese, nondiabetic essential hypertensive patients with multiple metabolic abnormalities. Diabetes 1998;47:660-7.

[8] Cominacini L, Pasini AF, Garbin U, et al. Elevated levels of soluble E-selectin in patients with IDDM and NIDDM: relation to metabolic control. Diabetologia 1995;38:1122-4.

[9] Albertini JP, Valensi P, Lormeau B, et al. Elevated concentrations of soluble E-selectin and vascular cell adhesion molecule–1 in NIDDM. Diabetes Care 1998;21:1008-13.

[10] Boulbou MS, Koukoulis GN, Vasiou KG, et al. Increased soluble E-selectin levels in type 2 diabetic patients with peripheral arterial disease. Int Angiol 2004;23:18-24.

[11] Matsumoto K, Sera Y, Nakamura H, et al. Serum concentrations of soluble adhesion molecules are related to degree of hyperglycemia and insulin resistance in patients with type 2 diabetes mellitus. Diabetes Res Clin Pract 2002;55:131-8.

[12] Matsumoto K, Nakamura H, Ueki Y, et al. Correction of hyperglycemia reduces insulin resistance and serum soluble E-selectin levels in patients with type 2 diabetes mellitus. Diabet Med 2001;18:224-8.

[13] Matsumoto K, Sera Y, Abe Y, et al. Serum concentrations of soluble vascular cell adhesion molecule–1 and E-selectin are elevated in insulin resistant patients with type 2 diabetes. Diabetes Care 2001;24: 1697-8.

[14] Matsumoto K, Sera Y, Miyake S, et al. Serum levels of adhesion molecules correlate with insulin resistance. Atherosclerosis 2002;161: 243-4.

[15] Matsumoto K, Sera Y, Abe Y, et al. High serum concentrations of soluble E-selectin correlate with obesity but not fat distribution in patients with type 2 diabetes mellitus. Metabolism 2002;51:932-4.

[16] Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97.

[17] Matsumoto K, Sera Y, Abe Y, et al. Inflammation and insulin resistance are independently related to all-cause of death and cardiovascular events in Japanese patients with type 2 diabetes mellitus. Atherosclerosis 2003;169:317-21.

[18] Carter A, Grant P. Vascular homeostasis, adhesion molecules, and macrovascular disease in non–insulin-dependent diabetes mellitus. Diabet Med 1997;14:423-32.

[19] Matsumoto K, Sera Y, Abe Y, et al. Elevated high-sensitivity C-reactive protein correlates with insulin resistance and hyper-E-selectinemia in patients with type 2 diabetes. J Japan Diabet Soc 2003;46:1-5.

[20] Leinonen E, Hurt-Camejo E, Wiklund O, et al. Insulin resistance and adiposity correlate with acute-phase reaction and soluble cell adhesion molecules in type 2 diabetes. Atherosclerosis 2003;166: 387-94.
[21] Hillege HL, Fidler V, Diercks G, et al, for the Prevention of Renal and Vascular End Stage Disease (PREVEND) Study Group. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. Circulation 2002;106: 1777-82.
[22] Tatasciore A, Zimarino M, Renda G, et al. Awake blood pressure variability, inflammatory markers and target organ damage in newly diagnosed hypertension. Hypertens Res 2008;31:2137-46.
[23] Lopes-Virella MF, Carter RE, Gilbert GE, et al, Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications Cohort Study Group. Risk factors related to inflammation and endothelial dysfunction in the DCCT/EDIC cohort and their relatioship with nephropathy and macrovascular complications. Diabetes care 2008;31:2006-12.
[24] Koch AE, Halloran MM, Haskell CJ, et al. Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule–1. Nature 1995;376:517-9.
[25] DECODE Study Group. Glucose tolerance and cardiovascular mortality: comparison of fasting and 2-hour diagnostic criteria. Arch Intern Med 2001;161:397-405.
[26] Marfella R, Esposito K, Giunta R, et al. Circulating adhesion molecules in humans: role of hyperglycemia and hyperinsulinemia. Circulation 2000;101:2247-51.
[27] Ceriello A, Quagliaro L, Piconi L, et al. Effect of postprandial hypertriglyceridemia and hyperglycemia on circulating adhesion molecules and oxidative stress generation and the possible role of simvastatin treatment. Diabetes 2004;53:701-10.
[28] Peter K, Nawroth P, Conradt C, et al. Circulating vascular cell adhesion molecule–1 correlates with extent of human atherosclerosis in contrast to circulating intercellular adhesion molecule–1, E-selectin, P-selectin, and thrombomodulin. Arterioscler Thromb Vasc Biol 1997;17:505-12.
[29] De Caterina R, Basta G, Lazzerini G, et al. Soluble vascular cell adhesion molecule–1 as a biohumoral correlate of atherosclerosis. Arterioscler Thromb Vasc Biol 1997;17:2646-54.
[30] Matsumoto K, Sera Y, Ueki Y, et al. Comparison of serum concentrations of soluble adhesion molecules in diabetic microangiopathy and macroangiopathy. Diabet Med 2002;19:822-6.
[31] Jager A, Van Hinsbergh V, Kostence PJ, et al. Increased levels of soluble vascular cell adhesion molecule 1 are associated with risk of cardiovascular mortality in type 2 diabetes. The Hoorn Study. Diabetes 2000;49:485-91.
[32] Kawamura T, Umemura T, Kanai A, et al. Soluble adhesion molecules and C-reactive protein in the progression of silent cerebral infarction in patients with type 2 diabetes mellitus. Metabolism 2006;55:461-6.