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Circulating Cell Adhesion Molecules (sICAM-1 and sVCAM-1) and Microangiopathy in Diabetes Mellitus

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Abstract

Background/Aim: Diabetic microvascular complications are not uncommon. This study was done to investigate the relationship between biochemical parameters of diabetes mellitus (DM) and diabetic microangiopathy and serum levels of soluble intercellular cell adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) in DM. **Patients and methods:** The study included 35 type-1 and 25 type-2 DM patients along with 20 age- and sex-matched healthy controls. For each studied subject, thorough clinical examination and laboratory evaluation [fasting blood sugar (FBS), glycosylated hemoglobin (HbA1c), and lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides)] were performed. The serum level of C-peptide was estimated by radioimmunoassay (RIA) while levels of sICAM-1 and sVCAM-1 were determined by ELISA. **Results:** A significant elevation of both sICAM-1 and sVCAM-1 was detected in both type-1 and type-2 DM patients ($P < 0.001$), with no significant difference between the two patient groups. Levels of sICAM-1 and sVCAM-1 in patients with microangiopathy were significantly ($p < 0.05$) higher than that of patients lacking this complication. Patients with microangiopathy had older age and longer duration of DM, but there was no difference between patients with and those without microangiopathy regarding FBS, HbA1c or lipid profile. There was no significant effect of the disease duration on sICAM-1 or sVCAM-1 levels. Moreover, there was no correlation between circulating CAMs and FBS, HbA1c, cholesterol, triglyceride or C-peptide levels. **Conclusion:** sICAM-1 and sVCAM-1 serum levels are elevated in DM patients and may have a role in pathogenesis of diabetic microvascular complications. They may be predictors for these complications.

Key words: Adhesion molecules, diabetes mellitus, microangiopathy, nephropathy, neuropathy, retinopathy, sICAM-1, sVCAM-1.

Introduction

Diabetes mellitus (DM) is characterized by fasting hyperglycemia and development of chronic vascular complications. Microvascular disease has been strongly related to glycemic control (1-3). However, immune interaction may contribute to the pathogenesis of diabetic microvascular complications. Adhesion proteins are molecules which regulate the interaction between the endothelium and leucocytes (4). They play a role during leucocyte emigration from blood vessels and may be involved in the regulation of the immune system (5). They are involved in the process of atherosclerosis because an increase in their expression on the endothelial surface causes increased adhesion of leucocytes (1,6).

Cell adhesion molecules (CAMs) are expressed by activated endothelium and are shed into the circulation (7). Serum concentrations of circulating CAMs may reflect endothelial activity and /or damage (2,7) because endothelial cells release soluble forms of CAMs (sCAMs) in correlation to their surface expression (8). Although the cellular expression of CAMs is difficult to assess clinically, sCAMs can be detected in the circulation and may serve as markers for CAMs (9). Raised concentrations of sCAMs were found in a variety of disorders and in different immune-mediated diseases (7,10). They may indicate endothelial activation and increased interaction with leucocytes (7,11).

Intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are two

of the main CAMs which are expressed on the endothelium and associated with leucocyte activation. ICAM-1 interacts with a number of leucocytes (including neutrophils) and mediates their firm adherence and activation. VCAM-1 supports the adherence of lymphocytes, monocytes and eosinophils to the endothelium (12,13). Surface expression of VCAM-1 is rather specific for endothelium. In contrast, the origin of ICAM-1 is widespread and thus does not allow conclusions on endothelial state (14).

In DM, little information is available about the relation of sCAMs with the disease duration, metabolic control of DM and development of late diabetic microvascular complications. Therefore, we measured serum concentrations of sICAM-1 and sVCAM-1 in type 1 and in type 2 DM patients who had variable durations of the disease and presented with variable degrees of metabolic control and diabetic microvascular complications.

Materials and Methods

Study subjects:

The study included 60 DM patients (35 type 1 and 25 type 2), who were selected from the Menoufia University Hospital. Type 1 DM patients included 14 males and 21 females with age range from 11-40 years (31 ± 11) while type 2 DM patients included 10 males and 15 females with age range from 41-63 years (49 ± 10). In addition, 20 healthy subjects of matched age (41 ± 17 , range 16-60 years) and gender (8 males and 12 females) served as controls. The duration of the disease was variable in the studied patients (1-36 years). All subjects were evaluated by a full history and complete physical examination and laboratory investigations. They were free of clinically manifest infections and other DM-unrelated illnesses. None of the control subjects had family history of DM. All the studied subjects were non-smokers. An informed consent was obtained from each subject before enrollment in the study. Personal and clinical characteristics and biochemical data of the studied patients and controls are presented in table (1).

Diagnosis of neurovascular complications

Diabetic retinopathy was diagnosed by ophthalmoscopy after pupil dilatation by a specialized ophthalmologist. Diabetic neuropathy was diagnosed when peroneal conduction velocity (PNCV) was less than 40 m/s. Diabetic nephropathy was diagnosed by detection of frank proteinuria (> 300 mg/day) or microalbuminuria (30-300 mg/day).

Sampling

Venous blood was aseptically obtained after 12 hours fasting in vacutainer tubes without anticoagulant or preservative and allowed to clot at room temperature and serum was immediately separated by centrifugation at 1000 g and divided into 3 aliquots. One aliquot was used for immediate assay for lipid profile and fasting blood sugar (FBS) and the other 2 aliquots were stored at -70°C for subsequent assay of sCAMs and C peptide. In addition, 2 ml of venous blood were obtained on EDTA for estimation of HbA1c. 24 hour urine samples were collected from each patient for the detection of microalbuminuria. Samples which were positive by Combur test strips (Boehringer Mannheim, Diagnostics & Biochemicals Ltd, UK) were excluded.

Analytical methods

Fasting blood glucose was assayed by enzymatic method (glucose oxidase) using Synchron CX-5 autoanalyzer (Beckman Ins. Inc, CA, USA). Serum cholesterol and triglyceride assays were carried out by enzymatic method using Synchron CX-5 autoanalyzer (Beckman Ins. Inc, CA, USA). HDL cholesterol was determined after precipitation by phosphotungstic acid (BioMerieux, France). LDL cholesterol was calculated according to the Friedewald formula (15). Glycated hemoglobin (HbA1c) was quantified by a colorimetric method using a kit from Stanbio (Stanbio Laboratory Inc., San Antonio, Texas, USA). In this method, HbA1c was separated by ion exchange resin. Non-glycated Hb binds to a weakly binding cation exchange resin, leaving HbA1c free to be removed by resin separators in supernate. The percent of HbA1c is determined by measuring absorbance values of HbA1c and total Hb fractions at 415 nm (16). Determination of 24 hour urinary albumin excretion was done by competitive enzyme-linked immunosorbent assay (ELISA) using a kit from Randox (Randox Laboratory Ltd, UK). In this method, albumin from sample and albumin coated to microtiter plate compete for binding sites on antibody conjugate. After incubation, the plate was washed and the substrate for alkaline phosphatase was added. The absorbance was measured at 405 nm (17). Estimation of C-peptide was performed by a competitive radioimmunoassay (18), using a commercial kit (Diagnostic Systems Laboratories Inc, Texas, USA.)

Assessment of sICAM-1 and sVCAM-1 serum levels was done by ELISA kits (R & D Systems Europe Ltd, Abingdon, Oxon, UK). This involved a double antibody ELISA using antibodies directed against different epitopes on the ICAM-1 and VCAM-1 molecules. A second antibody conjugated with horseradish-peroxidase was used [19,20] Internal standards were used to construct reference curves. Instructions supplied by the Manufacturer were followed.

Statistical analysis

Data were collected, tabulated and statistically analyzed by Microstat software program. Student's t and correlation coefficient tests were done at 5% level of significance.

Results

Figure (1) shows that serum levels of both sICAM-1 and sVCAM in type 1 and type 2 DM patients were significantly ($p < 0.001$) higher than that of the controls. However, no significant difference was detected between the 2 types of DM patients.

Table 2 and Figure 2 show serum levels of sICAM-1 and sVCAM-1 in relation to microangiopathy. sICAM was significantly ($p < 0.05$) higher only in patients with neuropathy, however, sVCAM-1 was significantly ($p < 0.05$) higher in patients having retinopathy, nephropathy or neuropathy compared to diabetic patients without these complications (Table 2).

However, when all the studied DM patients with microangiopathy were compared to those lacking this complication, both sICAM-1 and sVCAM-1 were significantly ($p < 0.05$) higher in patients with microangiopathy (Figure 2).

Table 1: Clinical and biochemical data of the study subjects.			
Studied parameters	Type 1 DM (n=35)	Type 2 DM (n=25)	Controls (n=20)
Age (years)	11-40 (31 ± 11)	41-63 (49 ± 10)	16-60 (41 ± 17)
Gender (Males/Females)	14/21	10/15	8/12
Duration of diabetes (years): (<10/>10)	16/19	12/13	-
Microangiopathy	15*	14*	-
Retinopathy	9	8	-
Nephropathy	6	5	-
Neuropathy	7	5	-
FBS (mg /dl)	210 ± 98	168 ± 63	88 ± 23
HbA1c (%)	7.9 ± 1.2	7.3 ± 1.0	5.2 ± 0.2
C-peptide (ng/ml)	1.01 ± 0.18	1.91 ± 0.6	Not done
Triglycerides (mg/dl)	252 ± 61.1	269 ± 91.1	130 ± 25
Total cholesterol (mg/dl)	238 ± 64	242 ± 47	182 ± 24
HDL cholesterol (mg/dl)	47 ± 12	48 ± 9.2	40 ± 6.3
LDL cholesterol (mg/dl)	168 ± 52	172 ± 29.8	139 ± 13.5
*6 type 1 and 4 type 2 DM patients had combined microangiopathy.			

Table 2. Levels of sICAM-1 & sVCAM-1 and microangiopathy status in the studied DM patients.					
<i>Type 1 DM patients (n=35)</i>					
Microangiopathy	Adhesion molecules	Positive	Negative	T value	P Value
Retinopathy		(n=9)	(n=26)		
	sICAM-1 (ng/ml)	304 ± 51	269 ± 60	1.56	> 0.05
	sVCAM-1 (ng/ml)	879 ± 98	768 ± 121	2.48	< 0.05
Nephropathy		(n=6)	(n=29)		
	sICAM-1 (ng/ml)	317 ± 54	270 ± 59	1.80	> 0.05
	sVCAM-1 (ng/ml)	898 ± 107	776 ± 112	2.44	< 0.05
Neuropathy		(n=7)	(n=28)		
	sICAM-1 (ng/ml)	320 ± 66	268 ± 54	2.18	< 0.05
	sVCAM-1 (ng/ml)	887 ± 110	774 ± 119	2.54	< 0.05
<i>Total patients with microangiopathy</i>		(n=15)	(n=20)		
	sICAM-1 (ng/ml)	314 ± 73	251 ± 79	2.41	< 0.05
	sVCAM-1 (ng/ml)	874 ± 187	739 ± 179	2.17	< 0.05
<i>Type 2 DM patients (n=25)</i>					
Microangiopathy	Adhesion molecule	Positive	Negative	t value	P value
Retinopathy		(n=8)	(n=17)		
	sICAM-1 (ng/ml)	334 ± 57	291 ± 72	1.48	> 0.05
	sVCAM-1 (ng/ml)	893 ± 130	767 ± 142	2.12	< 0.05
Nephropathy		(n=5)	(n=20)		
	sICAM-1 (ng/ml)	349 ± 51	294 ± 58	1.75	> 0.05
	sVCAM-1 (ng/ml)	906 ± 101	782 ± 123	2.04	< 0.05
Neuropathy		(n=5)	(n=20)		
	sICAM-1 (ng/ml)	346 ± 55	295 ± 59	2.11	< 0.05
	sVCAM-1 (ng/ml)	898 ± 112	784 ± 104	2.16	< 0.05
<i>Total patients with microangiopathy</i>		(n=14)	(n=11)		
	sICAM-1 (ng/ml)	330 ± 69	273 ± 58	2.20	< 0.05
	sVCAM-1 (ng/ml)	861 ± 117	738 ± 108	2.70	< 0.05
Levels of VCAM-1 were significantly higher among patients with retinopathy, nephropathy and neuropathy while that of sICAM-1 were significantly higher among patients with neuropathy as compared to patients without these complications.					

Table 3. Diabetic microangiopathy in relation to patient's age, disease duration, metabolic control and biochemical parameters of diabetes

Patients	The studied parameters	Patients without microangiopathy	Patients with microangiopathy	t value	P value
Type 1DM patients	Number of patients	(n=20)	(n=15)		
	Age	30 ± 11.5	46 ± 15.3	3.54	<0.001
	Duration of the disease (years)	9.5 ± 5.3	16.3 ± 6.8	3.33	< 0.01
	FBS (mg /dl)	204 ± 54	230 ± 61	1.33	> 0.05
	HbA1c (%)	7.7 ± 0.9	8.1 ± 0.4	1.60	> 0.05
	Total cholesterol (mg/dl)	217 ± 51	249 ± 43	1.96	> 0.05
	HDL cholesterol (mg/dl)	46 ± 9	47 ± 8.5	0.33	> 0.05
	LDL cholesterol (mg/dl)	158 ± 43	175 ± 59	0.99	> 0.05
	Triglycerides (mg/dl)	246 ± 49	268 ± 64	1.15	> 0.05
Type 2DM patients	Number of patients	(n=14)	(n=11)		
	Age	43 ± 10.5	54 ± 12.5	2.41	< 0.05
	Duration of the disease	10.4 ± 5.5	17 ± 9.4	2.2	< 0.05
	FBS (mg /dl)	158 ± 57	171 ± 49	0.6	> 0.05
	HbA1c (%)	7.18 ± 0.5	7.5 ± 0.8	1.23	> 0.05
	Total cholesterol (mg/dl)	241 ± 38	245 ± 43	0.25	> 0.05
	HDL cholesterol (mg/dl)	46 ± 6.8	47 ± 8.2	0.33	> 0.05
	LDL cholesterol (mg/dl)	166 ± 15	178 ± 18	1.82	> 0.05
	Triglycerides (mg/dl)	241 ± 41	273 ± 52	1.72	> 0.05

There was a significant relation between presence of microangiopathy in relation to age of the patients and duration of the disease in among both type 1 and type 2 DM. There was no relation with biochemical parameters of the disease.

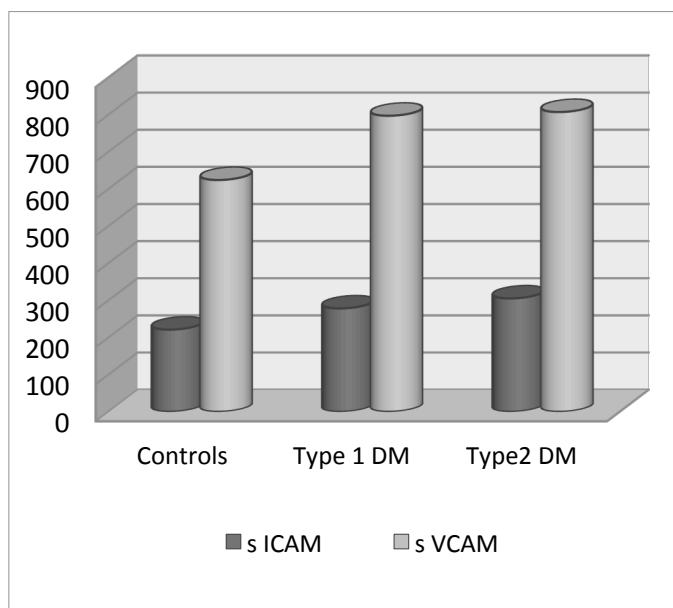


Figure 1. Circulating adhesion molecules in the studied DM patients. Data are expressed as means. Values of patients were compared to that of the controls by Student's t test at 5% level of significance. Both type 1 and type 2 diabetes patients had significantly higher levels of sICAM-1 ($p < 0.01$) and sVCAM-1 ($p < 0.001$) compared to controls. No significant difference was detected between type 1 and type 2 DM.

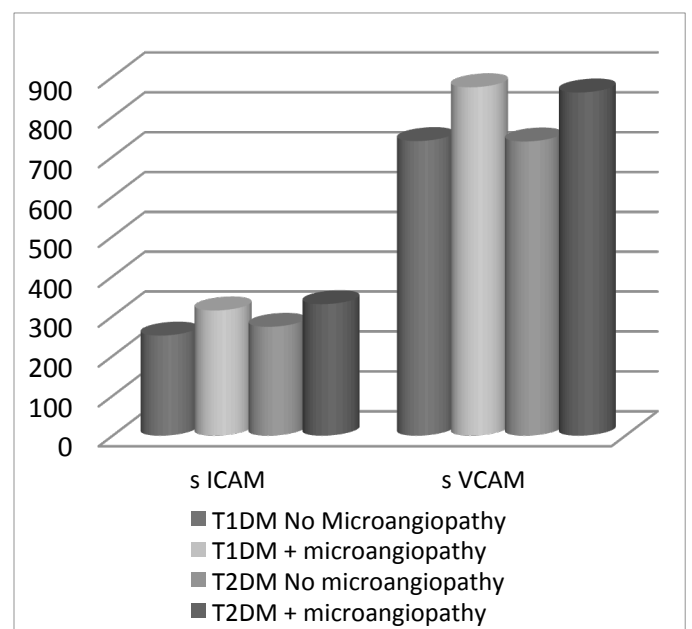


Figure 2. Circulating cell adhesion molecules in relation to microangiopathy in the studied DM patients. Data are means. Levels were compared by the Student's t test at 5% level of significance. Levels of both sICAM-1 and sVCAM-1 were significantly ($p < 0.05$) higher among patients with microangiopathy in type 1 and type 2 DM.

Table 4. Correlation of circulating adhesion molecules with biochemical data and duration of the disease in DM patients

Study parameters	Type 1 DM patients		Type 2 DM patients	
	sICAM-1	sVCAM-1	sICAM-1	sVCAM-1
Disease duration (years)	0.17	0.24	0.19	0.26
FBS (mg /dl)	0.18	0.12	0.08	0.16
HbA1c (%)	0.19	0.25	0.21	0.23
Total cholesterol (mg/dl)	0.24	0.16	0.10	0.18
HDL cholesterol (mg/dl)	0.13	0.07	0.11	0.12
LDL cholesterol (mg/dl)	0.11	0.31	0.18	0.30
Triglycerides (mg/dl)	0.17	0.09	0.16	0.10
C-peptide (ng/ml)	0.12	0.18	0.05	0.08

Comparison between patients with microangiopathy and those without it is illustrated in table 3. Patients with microangiopathy were significantly older and had a longer duration of the disease. However, no significant difference was detected regarding FBS, HbA1c, cholesterol, triglyceride or C-peptide levels.

The relation between sICAM-1 and sVCAM-1 levels in diabetic patients and duration of the disease is demonstrated in Figure 3. There was no significant difference between patients having DM for more than or less than 10 years. Table (4) shows that there was no correlation between circulating serum levels of sICAM-1 or sVCAM-1 and duration of the disease, FBS, HbA1c, cholesterol, triglyceride or C-peptide levels. However, there was a significant positive correlation ($p < 0.01$) between the levels of sICAM-1 and sVCAM-1 in the studied DM patients.

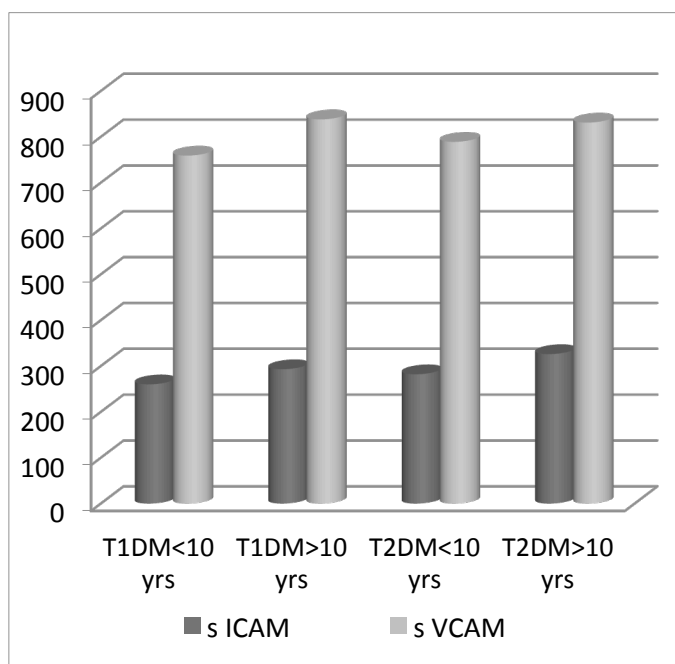


Figure 3. Circulating adhesion molecules in relation to duration of the disease in the studied DM patients. Data were expressed as mean \pm SD. Values between patients less than and more than 10 years duration of DM were compared by the Student's t test at 5% level of significance. Both sICAM-1 and sVCAM-1 levels were not significantly different in relation to duration of the disease among type 1 and type 2 DM patients.

Discussion

In this study, significantly higher levels of sICAM-1 and sVCAM-1 were found in the sera of type 1 and type 2 DM patients, a finding which was reported by some investigators (7, 21-23). Increased concentrations of sICAM-1 and sVCAM-1 were detected in patients with either long or short duration of DM. High levels of circulating CAMs have been observed in DM, even in absence of diabetic complications (24,25). Raised levels of CAMs are believed to result from increased endothelial cell expression and shedding following activation (7). After expression of CAMs on the cells, they are shed into plasma. Thus, plasma concentrations of CAMs may be representative for endothelial activation, damage or turnover (26).

This study showed higher levels of CAMs in DM patients with compared to those without microvascular complications, indicating that these CAMs may be implicated in the development of microangiopathy (27). Elevated concentrations of sICAM-1 and sVCAM-1 in diabetic patients reflects endothelial activation and stimulation of leucocytes and suggests potential involvement of CAMs in diabetes-associated micro- and macrovascular disease (3,28). Endothelial cell activation and expression of CAMs may modify immunologic responses, lead to dysregulation of the coagulation system (29), and mediate leucocyte adhesion to the endothelium (25,30). Increased expression of CAMs enhances stickiness of endothelium to cellular components of the blood stream, most specifically monocytes (30). Monocytes are activated by exposure to the increased levels of cytokines in the plasma of DM patients (31), resulting in increased expression of growth factors, cytokines and free radicals damaging the endothelium (32). This leads to platelet adhesion, thrombus formation and stimulation of vascular smooth muscle proliferation (33). Neutrophils may act as mediators of vascular damage by releasing a variety of toxic agents, including elastase and other proteases, toxic oxygen radicals and leukotrienes on activation (34).

In this study, sVCAM-1 was significantly higher in patients having neuropathy, nephropathy or retinopathy. However, sICAM-1 was significantly elevated only in cases with neuropathy. This may indicate that VCAM-1 may be more important than ICAM-1 in pathogenesis of microvascular complications. The level of sVCAM-1, but not sICAM-1, was reported to be markedly elevated in type 1 DM patients who had neuropathy, retinopathy,

or nephropathy than those without these microvascular complications (2,3,21,22). However, elevated levels of sICAM-1 were found in subjects with vascular disease (35), and also in DM patients with or without vascular disease (25,36). Because sVCAM-1 release is almost completely restricted to endothelial cells (9), its elevated serum concentration seems to reliably reflect endothelial activation and vascular endothelial damage than sICAM-1, which is also related to lymphocyte activation and non-endothelial injury (37). Therefore, elevated serum concentration of sVCAM-1 as an indicator of widespread endothelial damage may serve as a risk marker for both presence and progression of microangiopathy in DM patients (38). The levels of sVCAM-1 and sICAM-1 were correlated with the presence of retinopathy (27,28,39), nephropathy (3,28), and neuropathy (8). Also, a significant elevation of these molecules was found in patients with microalbuminuria (40).

Our study showed that patients with microangiopathy were significantly older and had a longer duration of the disease, indicating increased occurrence of microvascular complications among patients with long-standing disturbed metabolic status. It was reported that advanced glycation endproducts which are present for long times within the cell wall may have a more sustained effect on endothelial expression of VCAM-1 and other stimuli as cytokines (41). However, no significant correlation was detected between duration of the disease and sICAM-1 or sVCAM-1 level, a finding which was also reported by Fasching et al (21). This may indicate that expression of CAMs does not increase with age and the sustained effect of long-standing disturbance may be more important in pathogenesis of diabetic microangiopathy.

This study demonstrated a non significant correlation between the levels of sICAM-1 or sVCAM-1 and the metabolic control status of the disease as indicated by blood glucose, HbA1c, cholesterol or triglyceride levels. Our finding and that of others (21,25,42) indicate that increased ICAM-1 and VCAM-1 expression and shedding may occur irrespective of metabolic control of the disease. Moreover, the parameters of metabolic control of the disease (levels of blood glucose, HbA1c, cholesterol and triglycerides) in DM patients with microangiopathy were not significantly different compared to that of patients without this complication. However, the metabolic disturbance in DM may lead to increased release of CAMs and may play a role in endothelial injury encountered in these patients (6,13). It was demonstrated that patients with hypertriglyceridemia and low HDL had increased levels of sICAM-1 and sVCAM-1 (9), and sICAM-1 was correlated with hyperglycaemia (43,44). Hyperglycaemia could accelerate oxidation processes and cause endothelial damage by LDL (36). Hypercholesterolemia promoted VCAM-1 expression (2), and oxidized LDL particles were found to induce expression and release of CAMs from prestimulated endothelial cells (45). Moreover, a correlation between LDL cholesterol and sVCAM-1 levels was documented only in patients with diabetic microangiopathy, but not in those without this complication (21).

In conclusion, sICAM-1 and sVCAM-1 levels are high in DM patients, reflecting increased expression and shedding of these CAMs into the circulation. This increased expression and shedding may occur irrespective of metabolic control of the disease and reflect ongoing endothelial cell stimulation and leucocyte activation. Patients having microangiopathy display higher concentrations of sICAM-1 and sVCAM-1 and these CAMs (especially sVCAM-1). Therefore, these molecules may be markers of vascular complications in DM and their serial assessment may be helpful in identifying patients at high risk for development of clinically apparent vascular complications.

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Abbreviations: CAMs: cell adhesion molecules, DM: diabetes mellitus, HbA1c: glycosylated hemoglobin, FBS: fasting blood sugar, RIA: radioimmunoassay, sICAM-1: soluble intercellular cell adhesion molecule-1, sVCAM-1: soluble vascular cell adhesion molecule-1