Circulating E-Selectin, Vascular Cell Adhesion Molecule-1, and Intercellular Adhesion Molecule-1 in Men With Coronary Artery Disease Assessed by Angiography and Disturbances of Carbohydrate Metabolism

Irina Kowalska, Marek Strączkowski, Małgorzata Szelachowska, Ida Kinalska, Jolanta Prokop, Hanna Bachórzewska-Gajewska, and Agnieszka Stępien

It is hypothesized that adhesion molecules could be an early predictor of coronary artery disease. Therefore we investigated the relationship between the concentrations of soluble forms of adhesion molecules and disturbances of glucose metabolism in 78 men referred for coronary angiography but with no previous history of diabetes. The group consisted of 78 men (mean age, 47.6 ± 7.0 years; mean body mass index [BMI], 28.4 ± 3.24 with the symptoms of angina pectoris and positive exercise test. All subjects were given a standard oral glucose tolerance test (OGTT) with glucose and insulin estimations. Fasting plasma concentrations of the soluble (s) forms of E-selectin, intercellular adhesion cell molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and HbA_{1c} were also measured. According to the OGTT, 10.2% of the patients (n = 8) fulfilled the criteria for type 2 diabetes mellitus and 44.9% (n = 35) for impaired glucose tolerance (IGT). The highest concentrations of sE-selectin were observed in patients with type 2 diabetes mellitus and were significantly higher in comparison to the group with normal glucose tolerance and IGT. The concentration of sVCAM-1 increased with the progression of disturbances of glucose metabolism and remained the highest in type 2 diabetic patients. sICAM-1 concentration was not significantly different. sE-selectin concentration correlated significantly with fasting glucose ($r = 0.23$, $P = .041$), postload glucose ($r = 0.39$, $P = .001$), and postload insulin ($r = 0.28$, $P = .023$). sVCAM-1 was significantly related to the postload glucose concentration ($r = 0.30$, $P = .009$). A significant correlation between sICAM-1 concentration and postload insulin was also observed ($r = 0.27$, $P = .025$). This would suggest that hyperglycemia increases sE-selectin and sVCAM-1 in plasma, which reflects excessive formation of atherosclerotic plaques in patients with disturbances of glucose metabolism.

CARDIOVASCULAR DISEASES are recognized as a main cause of mortality in type 2 diabetes mellitus. It is also well documented that diabetes mellitus is connected with a greater risk of myocardial infarction, stroke, and peripheral artery disease. The cause of accelerated atherogenesis in type 2 diabetes mellitus is still unclear. Some investigators suggest that metabolic disturbances connected with hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress may play a role. Endothelium participates in the first stage of contact with circulating leukocytes in the artery wall and thus plays a pivotal role in the initiation of atherogenesis.[1] Recruitment of leukocytes requires the expression on both the endothelium and leukocytes of various classes of adhesion molecules, eg, selectins, intercellular vascular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1).[2] Thus, the induction of cellular adhesion molecules, such as VCAM-1, ICAM-1, and selectins, to the endothelial surface appears to be an early event of atherogenesis.[2,3] The soluble (s) forms of cellular adhesion molecules circulate in the plasma and can be recognized as markers of endothelium activation.[4]

The United Kingdom Prospective Diabetes Study showed that more than 50% of patients with a recent onset of diabetes had macroangiopathy at the time of diagnosis.[5] On the other hand, a study performed in our center showed that about 50% of patients referred for coronary arteriography had unrecognized disturbances of glucose metabolism.[6] Moreover patients with diabetes mellitus had more pronounced atherosclerotic lesions in the coronary arteries (2- and 3-vessel disease).[6]

The aim of the present study was to investigate the concentrations of soluble forms of adhesion molecules in the plasma depending on glucose tolerance status in the patients with coronary heart disease (CHD) confirmed by coronary angiography.

MATERIALS AND METHODS

The study group consisted of 78 male patients referred to the Cardiology Department at the University Hospital of Medical Academy in Białystok, Poland for coronary arteriography. The protocol was approved by the Institutional Review Board and written informed consent was obtained from all participants before the study.

All patients had previously diagnosed clinical evidence of CHD, confirmed by a routine medical history, a positive exercise test, or a history of myocardial infarction. Coronary arteriography was performed in patients with symptoms of angina pectoris and positive exercise tests. The coronary arteriography was performed in the Department of Invasive Cardiology using Toshiba DFP-60A equipment (Shimoishigami, Japan). Patients who needed an urgent procedure based on the results of the coronary arteriography were not included.

The coronary angiograms were evaluated by an experienced cardiologist unaware of the glucose tolerance status of the patients. The degree of luminal narrowing was given in percentage of prestenotic diameter. Internal luminal narrowing of more than 70% was considered significant, except for the left coronary artery, where 50% stenosis was regarded as significant.

We examined the patients with no previous history of diabetes mellitus or other disturbances of carbohydrate metabolism. The clinical characteristics of the patients included age, duration of CHD, a history of myocardial infarction with Q-wave present, hypertension, and smok-

From the Department of Endocrinology, Diabetology, and Internal Medicine, and the Department of Cardiology, Medical Academy in Białystok, Poland.

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Address reprint requests to Irina Kowalska, MD, Department of Endocrinology, Diabetology, and Internal Medicine, Medical Academy in Białystok, 15-276 Białystok, ul.M.C.Skłodowskiej 24a, Poland.

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Table 1. Clinical Characteristics of Studied Group

Age (yr), mean ± SD	47.6 ± 7.0
BMI (kg/m^2), mean ± SD	28.4 ± 3.24
Hypertension (n)	25 (32%)
History of myocardial infarction (n)	45 (57.7%)
Family history of CHD (n)	36 (46.1%)
Smoker (n)	43 (55.1%)

ing status. The degree of obesity was expressed as a body mass index (BMI) according to Quetelet's formula. The clinical characteristics of the studied population are listed in Table 1.

Biochemical Investigations

After an overnight fast, all patients were given an oral glucose tolerance test (OGTT; 75-g glucose load) between 8 and 10 AM, with the subject in a sitting position. The blood samples for glucose and insulin determinations were taken at 0, 60, and 120 minutes. The diagnosis of diabetes or impaired glucose tolerance (IGT) was made according to World Health Organization criteria. Before the test, blood samples were taken for determination of sE-selectin, sVCAM-1, sICAM-1, $HbA_{1c,}$ total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) concentrations. Glucose concentrations were estimated immediately using the oxidaze method. Blood serum for insulin determinations was centrifuged and then frozen at -20°C until assayed. Plasma insulin concentrations were estimated using the immunoradiometric assay (IRMA) method (Polatom, Œwierk, Poland). HbA_{1c} levels in the serum were determined with high-performance liquid chromatography (HPLC variant; BioRad, Richmond, CA). The concentration of soluble forms of adhesion molecules was determined by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN). TC, HDL-C, and TG were determined by an enzymatic method using commercial kits produced by Analco (Warsaw,Poland). The concentration of low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's formula. The whole-body insulin sensitivity index was calculated from glucose and insulin measurements during OGTT as described by Matsuda and DeFronzo.[7]

Statistical analysis was performed using the Mann–Whitney *U* test and analysis of variance (ANOVA) followed by Tukey' s test in order to identify the differences between the groups. Pearson's correlation coefficient was performed to establish metabolic factors related to the adhesion molecules concentration. Data are expressed as the mean ± SD. *P* values less than .05 were considered statistically significant.

RESULTS

According to the results of the OGTT, 10.2% of the patients (n = 8) fulfilled the criteria for type 2 diabetes mellitus, and 44.9% (n = 35) for IGT.

Based on the results of the coronary angiography, 78.2% of the patients (n = 61) had significant stenoses in the coronary arteries: 32% (n = 25) had 1-vessel disease, 25.6% (n = 20) had 2-vessel disease, and 20.5% (n = 16) had 3-vessel disease. There were no significant changes in the coronary arteries in 21.8% (n = 17) of the patients. There were no significant differences in the prevalences of 1-, 2-, and 3-vessel disease dependent on the glucose tolerance status of the studied population (Table 2).

sE-selectin concentration was the highest in the patients with type 2 diabetes mellitus and was significantly different from the group without disturbances of glucose metabolism ($P = .0003$) and also from the group with IGT ($P = .002$) (Table 3). sVCAM-1 concentration increased progressively with glucose metabolism disturbances and remained the highest in the patients with diabetes mellitus. A statistically significant difference between the groups (type 2 diabetes mellitus *v* normal glucose tolerance) was observed ($P = .02$) (Table 3). sICAM-1 concentration was not statistically different between the groups (Table 3). After we excluded from analysis the patients without significant changes in the coronary arteries (n = 17), the observed differences in adhesion molecule concentrations persisted (Table 4). Analysis of the concentration of adhesion molecules depending on significant stenoses in the coronary arteries did not show any statistical differences (Table 5).

Table 2. Prevalence of CHD in Patients With Normal Glucose Tolerance and IGT/Diabetes Mellitus

	No Changes in Coronary Arteries (n = 17)	1-Vessel Disease (n = 25)	2-Vessel Disease (n = 20)	3-Vessel Disease (n = 16)
NGT				
No.	9	11	7	8
%	25.71%	31.43%	20.00%	22.86%
IGT/DM				
No.	8	14	13	8
%	18.60%	32.56%	30.23%	18.60%

Glucose and insulin concentrations during the OGTT are listed in Table 6. A statistically significant difference in glucose concentrations between the groups was noted (Table 6). Insulin concentrations were markedly higher at 120 minutes of the OGTT in patients with IGT and type 2 diabetes mellitus in comparison to the group with normal glucose tolerance ($P = .03$ and $P = 0.003$, respectively). A difference between the groups with diabetes mellitus and IGT was also observed ($P = .037$).

Lipid parameters remained the highest in patients with type 2 diabetes mellitus and differed significantly in comparison to the patients with IGT ($P = .025$ for TC, $P = .035$ for LDL-C, and $P = .015$ for TG) (Table 3).

sE-selectin concentration correlated significantly with

Table 3. Concentrations of Adhesion Molecules and Metabolic Parameters in Studied Groups

	Normal Glucose Tolerance (n = 35)	Impaired Glucose Tolerance (n = 35)	Diabetes Mellitus Type 2 (n = 8)
Age (yr)	47.5 ± 7.1	47.6 ± 6.8	48.7 ± 8.2
BMI(kg/m^2)	27.5 ± 3.4	28.9 ± 2.9	30.1 ± 3.1
sVCAM-1 (ng/mL)	637.27 ± 122.1	713.9 ± 134.8	828.8 ± 258.7*
sICAM-1 (ng/mL)	324.4 ± 71.8	350.3 ± 88.9	369.4 ± 78.7
sE-selectin (ng/mL)	58.9 ± 21.3	69.4 ± 30.9	96.6 ± 18.4†
HbA_{1c} (%)	5.6 ± 0.54	5.6 ± 0.5	5.8 ± 0.4
TC (mg/dL)	215.6 ± 28.9	189.9 ± 35.8	221.2 ± 35.1†
LDL-C (mg/dL)	136.2 ± 23.8	123.7 ± 30.7	143.0 ± 39.2†
HDL-C (mg/dL)	35.8 ± 13.3	34.3 ± 11.3	33.7 ± 12.8
TG (mg/dL)	179.3 ± 95.0	158.9 ± 88.0	273.7 ± 137.5†

*$P < .05$, diabetes mellitus group *v* group with normal glucose tolerance.

†$P < .05$, diabetes mellitus group *v* group with IGT.

Table 4. Concentrations of Adhesion Molecules After Exclusion of Patients Without Significant Coronary Artery Changes

	Normal Glucose Tolerance (n = 26)	Impaired Glucose Tolerance (n = 30)	Diabetes Mellitus Type 2 (n = 5)
sVCAM-1 (ng/mL)	634.4 ± 132.2	718.1 ± 130.6	903.4 ± 305.4*†
sICAM-1 (ng/mL)	324.6 ± 69.2	359.4 ± 91.5	409.1 ± 67.8
sE-selectin (ng/mL)	55.4 ± 21.4	69.7 ± 31.2	94.1 ± 18.2†

*$P < .05$, diabetes mellitus group *v* group with normal glucose tolerance.

†$P < .05$, diabetes mellitus group *v* group with IGT.

sICAM-1 ($r = 0.39$, $P = .001$), BMI ($r = 0.41$, $P = .001$), fasting glucose ($r = 0.23$, $P = .041$), postload glucose ($r = 0.39$, $P = .001$), and postload insulin ($r = 0.28$, $P = .023$). sVCAM-1 was significantly related to sICAM-1 ($r = 0.38$, $P = .001$) and postload glucose concentration ($r = 0.30$, $P = .009$). A significant correlation was alos observed between sICAM-1 concentration and postload insulin ($r = 0.27$, $P = .025$). All adhesion molecules concentrations correlated inversely with the index of insulin sensitivity (VCAM-1, $r = -0.2844$, $P = .02$; ICAM-1, $r = -0.2573$, $P = .036$; E-selectin, $r = -0.3249$, $P = .007$).

DISCUSSION

Different studies have observed an increased concentration of adhesion molecules in the plasma in patients with diabetes mellitus and symptoms of diabetic microangiopathy. Olsen et al showed that patients with type 1 diabetes mellitus and retinopathy had higher plasma sE-selectin and sVCAM-1 concentrations.[8] sICAM-1 concentrations did not differ from that of the control group. The same study noted a significant correlation between sE-selectin and HbA_{1c}. In another study, sE-selectin, sICAM-1, and sVCAM-1 concentrations were elevated in the corpus vitreous of patients with diabetic proliferative retinopathy.[9] An increased expression of E-selectin and P-selectin in kidney tissue of patients with diabetic nephropathy was observed in comparison to the patients with other types of glomerulopathy.[10] Clausen et al showed elevated plasma concentrations of sICAM-1 and sVCAM-1 in type 1 diabetic patients with microalbuminuria and macroalbuminuiria.[11] A higher expression of adhesion molecules was observed in in vitro studies with Schwann cells from patients with type 2 diabetes mellitus.[12]

In our group of patients with angiographically defined coronary artery disease, the concentrations of soluble forms of adhesion molecules were significantly elevated in the patients with type 2 diabetes mellitus. sE-selectin and sVCAM-1 concentrations were the highest in patients with type 2 diabetes mellitus, but the subjects with IGT also had increased concentrations of these adhesion molecules. Similar results were obtained by Bannan et al, who observed an increased sE-selectin level in type 2 diabetic patients and their first-degree relatives compared to that in the control group.[13] sVCAM-1 concentration was not statistically different in the studied groups. Contrary to our studies, patients did not have clinical evidence of vascular disease.[13] Kado and Nagata did not find any difference in sE-selectin concentration in their diabetic group in comparison to healthy volunteers.[14] There was also no difference between the patients with type 2 diabetes mellitus irrespective of the symptoms of diabetic macroangiopathy. Increased concentrations of sVCAM-1 and sICAM-1 were observed.[14] Albertini et al suggested that in patients with type 2 diabetes mellitus and coronary artery disease, sVCAM-1 level is elevated due to poor metabolic control irrespective of the atherosclerotic changes in coronary arteries.[15] A decrease in the L-selectin level was the only parameter that corresponded with the progression of atherosclerosis in the coronary vessels.[15] In other reports, sVCAM-1 concentration was significantly associated with the risk of cardiovascular mortality in type 2 diabetic patients.[16] An increase in the circulating forms of adhesion molecules (sICAM-1, sVCAM-1, sE-selectin) in the patients with diabetes mellitus and stroke has also been observed.[17]

Interestingly, in our study, there was a difference in sE-selectin concentration between the groups with diabetes mellitus and IGT. Ferri et al also reported a higher concentration of soluble E-selectin, ICAM-1, and VCAM-1 in patients with IGT and essential hypertension.[18] Thus, it seems that endothelial dysfunction is present in patients with "latent" disturbances of glucose metabolism. On the other hand, selectins are responsible for the initial contact of leukocytes with endothelial cells (tethering and rolling), and thus elevated levels of soluble E-selectin can be observed in patients with IGT.[1]

In the simple regression analysis, plasma E-selectin concen-

Table 5. Concentrations of Adhesion Molecules Depending on Significant Stenoses in the Coronary Arteries

	No Significant Changes in Coronary Arteries (n = 17)	Significant Changes in Coronary Arteries (n = 61)
sVCAM-1 (ng/mL)	668.7 ± 117.2	697.6 ± 165.0
sICAM-1 (ng/mL)	311.8 ± 66.6	348.7 ± 83.4
sE-selectin (ng/mL)	74.0 ± 25.8	65.6 ± 28.3

Table 6. Glucose and Insulin Concentrations During OGTT and Whole-Body Insulin Sensitivity Index in the Studied Groups

	Normal Glucose Tolerance (n = 35)	Impaired Glucose Tolerance (n = 35)	Diabetes Mellitus Type 2 (n = 8)
Glucose			
0 min	85.8 ± 10.6	93.6 ± 14.0*	104.0 ± 14.5†‡
60 min	139.9 ± 30.5	183.8 ± 38.9*	218.5 ± 31.9†‡
120 min	108.6 ± 17.6	161.6 ± 15.7*	232.1 ± 30.9†‡
Insulin			
0 min	9.3 ± 8.7	10.6 ± 8.9	12.8 ± 6.8
60 min	98.1 ± 80.7	103.1 ± 68.8	114.1 ± 73.3
120 min	52.7 ± 57.6	82.6 ± 78.5*	152.2 ± 127.2†‡
Insulin sensitivity index	7.8 ± 4.6	4.8 ± 2.3*	2.8 ± 1.5†

*$P < .05$, IGT group *v* group with normal glucose tolerance.

†$P < .05$, diabetes mellitus group *v* group with normal glucose tolerance.

‡$P < .05$, diabetes mellitus group *v* group with IGT.

tration correlated significantly with glucose concentration during OGTT and sVCAM-1 with postload glucose level. Other studies have also found a significant correlation between sE-selectin and HbA_{1c}.[8] This is partially in agreement with the hypothesis that adhesion molecule concentration is elevated in diabetic patients with poor metabolic control[19,20] and is normalized after improvement of glucose concentration.[19,20] However, in the present study the increased concentrations of sE-selectin and sVCAM-1 were observed in patients with no previous history of diabetes, and diabetes was diagnosed after the OGTT. In addition, the HbA_{1c} concentration in the diabetic group was below 6%. The results obtained by Yudkin et al do not support this hypothesis.[21] They did not observe any changes in adhesion molecule concentrations after 16 weeks of diabetes treatment (insulin or sulfonyloureas) that did normalize glucose concentration.[21] Very interesting is the suggestion by Smulders et al that short-term and chronic hyperglycemia each cause an independent effect on the endothelium, followed by an elevation of different markers in the plasma.[22] They suggest that an increase in sE-selectin levels reflects short-term effects of glucose on the endothelium, that sVCAM-1 concentration reflects advanced glycation end products (AGEs) action on the endothelial cells, and that vonWillebrand factor is released during short glycemic changes as well as during chronic hyperglycemia.[22]

It should be stressed that all the patients in this study had angiographically defined coronary artery disease, but only the patients with disturbances of glucose metabolism had significantly higher concentrations of the estimated adhesion molecules. The possible explanation of this finding is that adhesion molecules are not constantly present on the surface of endothelium and their expression is upregulated by risk factors, eg, hyperglycemia. The parameter that differentiated the studied groups was hyperglycemia. We therefore conclude that in patients with atherosclerosis the higher glucose level could be an additional factor responsible for elevated sE-selectin and sVCAM-1 concentrations, which reflect excessive formation of atherosclerotic plaques in patients with disturbances of glucose metabolism.

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