Chronic Cigarette Smoking Is Associated With Increased Plasma Circulating Intercellular Adhesion Molecule 1 Levels in Young Type 1 Diabetic Patients

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OBJECTIVE — The purposes of this study were to compare plasma concentrations of circulating intercellular adhesion molecule 1 (cICAM-1), a marker of endothelial dysfunction, in nondiabetic subjects and type 1 diabetic patients and to evaluate whether chronic cigarette smoking had a deleterious effect on plasma cICAM-1 levels in type 1 diabetic patients.

RESEARCH DESIGN AND METHODS — Plasma cICAM-1 concentrations were measured in 54 young type 1 diabetic patients without clinical macroangiopathy and in 20 healthy control subjects who were matched for age, sex, BMI, and smoking habit.

RESULTS — Type 1 diabetic patients had significantly higher plasma levels of cICAM-1 than control subjects (280.4 ± 59 vs. 224 ± 53.6 ng/ml, respectively) ($P < 0.001$). After stratification by smoking status, diabetic smokers had values for age, sex, BMI, lipids, blood pressure, glycemic control, diabetes duration, and chronic complications of diabetes that were superimposable on their nonsmoking counterparts. Nevertheless, plasma cICAM-1 levels were markedly elevated in type 1 diabetic smokers (321.4 ± 64.2 vs. 257.3 ± 41.5 ng/ml, respectively) ($P < 0.001$) in a dose-dependent fashion ($P < 0.001$ by analysis of variance when subjects were categorized by number of cigarettes smoked per day).

CONCLUSIONS — Chronic cigarette smoking has a deleterious effect on plasma cICAM-1 levels in young type 1 diabetic patients, which further supports the clinical importance of discouraging the initiation of smoking and promoting its cessation in people with type 1 diabetes.

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Morbidity and mortality in diabetes is mainly associated with atherosclerotic cardiovascular diseases and late complications (1,2). In these pathological processes, endothelial dysfunction seems to be an early event (3). Several studies have suggested that plasma circulating adhesion molecules (cAMs) may be used as markers of endothelial dysfunction (4–7). Recently, it has been reported that elevated levels of cAM, particularly elevated levels of circulating intercellular adhesion molecule 1 (cICAM-1), are associated with an increased risk of cardiovascular diseases in apparently healthy men (8). Elevated cAM levels have been demonstrated in type 1 diabetic patients compared with nondiabetic subjects, which thus supports the presence of underlying endothelial dysfunction (9,10). However, in type 1 diabetic patients, other concomitant coronary risk factors such as cigarette smoking may play a role in worsening vascular damage (11–14). Indeed, although higher levels of cAM, particularly cICAM-1, have been found in nondiabetic smokers compared with nonsmokers (8,15), we know of no data available regarding the effect of chronic cigarette smoking on plasma cICAM-1 levels in young adults with type 1 diabetes. Examining the effect of chronic smoking in cICAM-1 may be useful in explaining underlying mechanisms and may be of clinical importance in developing preventive and therapeutic strategies. Thus, the main aim of this study was to evaluate whether chronic cigarette smoking had an adverse effect on plasma cICAM-1 levels in a group of young type 1 diabetic patients with no clinical evidence of macrovascular diseases.

RESEARCH DESIGN AND METHODS

— A total of 54 young type 1 diabetic patients who regularly attend the Division of Endocrinology and Metabolic Diseases, University of Verona, were selected for the study. A total of 20 healthy volunteers (recruited from hospital staff members and relatives) matched for age, sex, BMI, and smoking habit formed the control group. All participants underwent a medical history and a physical examination. None of the participants had a history of recent acute diseases or had clinical evidence of any cardiovascular events or kidney or liver diseases. Type 1 diabetic patients had been treated with insulin and diet and had stable metabolic control; none of the participants, including the control subjects, were taking any other medications. Blood pressure was measured with a standard mercury sphygmomanometer by a trained staff member. Information on smoking habit (smokers vs. nonsmokers) was obtained from all subjects via a questionnaire. Information on the number of cigarettes smoked per day was available only for diabetic patients.

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Abbreviations: AER, albumin excretion rate; ANOVA, analysis of variance; cAM, circulating adhesion molecule; cICAM-1, circulating intercellular adhesion molecule 1.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Main clinical characteristics of type 1 diabetic patients and healthy control subjects*

	Type 1 diabetic subjects	Control subjects	*P* value
n	54	20	—
Age (years)	31.6 ± 8.9	31.8 ± 5.3	NS
Sex (M/F)	30/24	12/8	NS
BMI (kg/m^2)	23.8 ± 2.9	23.3 ± 3.0	NS
Smokers (*n*)	20	10	NS
cICAM-1 (ng/ml)	280.4 ± 59.0	224.0 ± 53.6	<0.001
HbA_{1c} (%)	6.7 ± 1.14	—	—
Diabetes duration (years)	14.8 ± 1.14	—	—

Data are means ± SD for continuous variables and absolute frequency for categorical variables.

Venous blood was drawn in the morning (between 8:00 and 8:30 A.M.) after an overnight fast and after at least 8 h of abstaining from smoking. Plasma glucose, creatinine, triglyceride, and total cholesterol concentrations were determined by using an automatic colorimetric method (DAX 96; Bayern Diagnostics, Milan, Italy). HbA_{1c} was assayed by using high-performance liquid chromatography (16); normal values in our laboratory ranged from 3.0 to 5.5%. Plasma samples of cICAM-1 were stored at −80°C for no longer than 6 weeks. Determination of plasma cICAM-1 concentrations was performed in duplicate with an enzyme-linked immunosorbent assay according to the supplied instruction manual (Bender MedSystem, Vienna, Austria) with both inter- and intra-assay coefficients of variation of <9%. Urinary albumin excretion rate (AER) was determined as the mean of at least three 24-h urine collections by using a radioimmunoassay method after excluding proteinuria resulting from urinary tract infection. AER was defined as normal when <20 μg/min and was defined as elevated when ≥20 μg/min. Elevated AER was also categorized as microalbuminuria (20–200 μg/min) or macroalbuminuria (>200 μg/min). A total of 10 diabetic subjects were microalbuminuric, but most of the subjects (82%) had normal AER values. Presence of retinopathy was diagnosed via fundoscopy by a single ophthalmologist after pupillary dilation. About two-thirds of patients had no diabetic retinopathy, whereas 19 patients (35.2%) had background (*n* = 12) or preproliferative (*n* = 7) retinopathy.

Statistical analyses were performed with SPSS software (Chicago). The following statistical tests were conducted: Student's *t* test for unpaired data, Pearson's product-moment correlation, one-way analysis of variance (ANOVA), analysis of covariance, and χ^2 test (for categorical variables). When the distribution of continuous variables was skewed, logarithmic transformations were carried out. Because the differences in the results were extremely small, we included only the statistical analyses that involved untransformed variables. Nonparametric statistical tests were also performed, but because the results obtained with parametric and nonparametric statistical procedures were similar, only the parametric procedures are presented. Data are means ± SD. $P < 0.05$ was considered statistically significant.

RESULTS — Type 1 diabetic patients had significantly higher (~25%) plasma levels of cICAM-1 compared with control subjects. The glycemic control of diabetic patients was fairly good (mean HbA_{1c} 6.7%), and the mean duration of diabetes was 14.8 years (Table 1).

In Table 2, the clinical and biochemical characteristics of diabetic patients are shown subdivided into groups according to smoking status. The subjects were predominantly nonobese, normolipidemic, and normotensive. Although diabetic smokers had slightly higher plasma triglyceride levels than nonsmokers, the two groups of patients were comparable regarding other potential confounders. In particular, there were no significant differences in sex, age, BMI, plasma total cholesterol concentration, glycemic control, blood pressure, diabetes duration, and chronic diabetes complications. Nevertheless, plasma cICAM-1 concentrations were markedly higher in type 1 diabetic patients who smoked than in patients who did not smoke without any significant difference between sexes (292.1 ± 69.0 vs. 265.5 ± 39.8 ng/ml, men vs. women) ($P = 0.15$). The results remained substantially unchanged when an allowance was made for plasma triglycerides (data not shown). Similarly to diabetic subjects, cigarette smoking also had a deleterious effect on plasma cICAM-1 levels in control subjects (smokers vs. nonsmokers: 247.0 ± 52.6 vs. 200.9 ± 46.0 ng/ml) ($P = 0.05$). As shown in Fig. 1, the relationship between the plasma levels of cICAM-1 and the number of cigarettes smoked per day was strictly dose dependent, and the relationship maintained statistical significance even after adjustment for all potential confounders such as age, sex, BMI, plasma triglycerides, blood pressure, HbA_{1c}, diabetes duration, and complications status ($F = 5.1$; $P = 0.006$ by analysis of covariance). Because the diabetic group included individuals with microvascular complications (i.e., the presence of clinical retinopa-

Table 2—*Clinical and biochemical characteristics of type 1 diabetic patients grouped according to smoking status*

	Nonsmokers	Smokers
n	34	20
Age (years)	30.0 ± 7.6	34.0 ± 10.5
Sex (M/F)	18/16	12/8
BMI (kg/m^2)	23.5 ± 3.0	24.4 ± 2.8
Systolic blood pressure (mmHg)	125 ± 15	130 ± 19.0
Diastolic blood pressure (mmHg)	77 ± 15	75 ± 19
Total cholesterol (mmol/l)	4.6 ± 0.7	5.0 ± 0.8
Triglycerides (mmol/l)	0.8 ± 0.2	1.4 ± 0.9*
HbA_{1c} (%)	6.6 ± 1.2	6.9 ± 0.9
cICAM-1 (ng/ml)	257.3 ± 41.5	321.4 ± 64.2†
Diabetes duration (years)	15.0 ± 6.7	14.3 ± 10.0
Retinopathy (*n*)	10	9
Urinary AER (*n*)	5	5

Data are means ± SD. *$P = 0.01$; †$P < 0.001$; all other differences were not statistically significant.

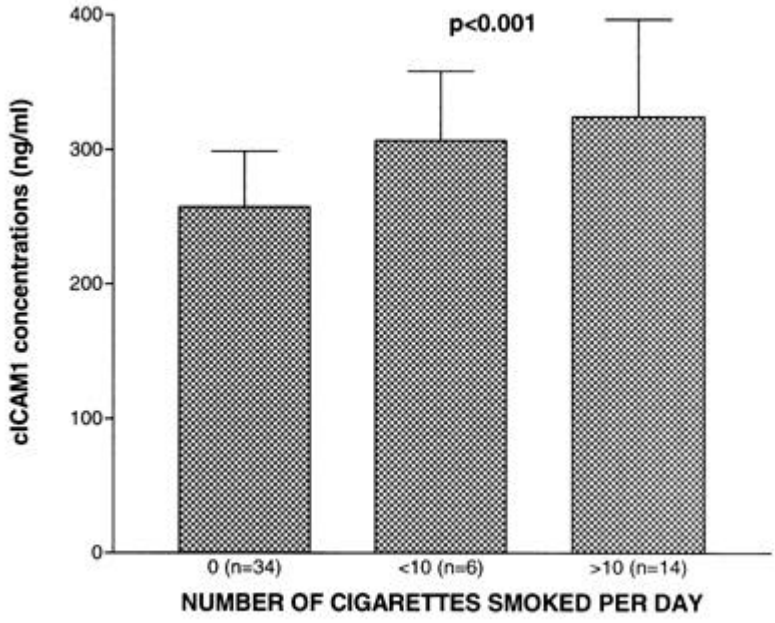

Figure 1—*Plasma cICAM-1 concentrations in relation to the number of cigarettes smoked per day in young type 1 diabetic patients. Data are means ± SD. Statistical analysis was carried out by one-way ANOVA.*

thy and/or microalbuminuria), conditions that may alter the plasma levels of cICAM-1, a statistical analysis excluding these participants (n = 21) was performed. The results did not substantially change. Plasma cICAM-1 concentrations were significantly higher in type 1 diabetic smokers than in their nonsmoking counterparts (319.2 ± 52.6 vs. 245.1 ± 43.2 ng/ml, respectively) ($P < 0.001$). When type 1 diabetic patients were considered all together, plasma cICAM-1 levels did not significantly correlate with any of the study variables except for a borderline significance with glycemic control. When diabetic subjects were grouped according to smoking status, plasma cICAM-1 levels were significantly correlated with glycemic control in diabetic nonsmokers (r = 0.41, P = 0.013) but not in smokers.

CONCLUSIONS — Type 1 diabetic patients are at higher risk for developing macrovascular complications than nondiabetic individuals (17,18). In this article, we found that plasma cICAM-1 levels are significantly higher in young adults with type 1 diabetes than in a well-matched group of healthy control subjects. We likewise found that chronic cigarette smoking had an adverse effect on plasma cICAM-1 levels in both type 1 diabetic patients and control subjects. Additionally, when diabetic patients were categorized according to the number of cigarettes smoked per day, the potentially deleterious effect of smoking on plasma cICAM-1 levels was dose dependent.

In this study we evaluated the effect of smoking on plasma cICAM-1 levels in young diabetic patients without clinical evidence of macrovascular complications, which would have complicated the interpretation of data. Moreover, that the two groups of diabetic patients (smokers and nonsmokers) were comparable for several factors known to adversely affect plasma cICAM-1 levels enhances the validity of our findings. According to our results, the differences in plasma cICAM-1 levels between the two groups are secondary to cigarette smoking and theoretically may be caused by the direct effects of nicotine, carbon monoxide, or other components of tobacco smoke.

The two groups of type 1 diabetic patients differed in their plasma triglyceride concentrations. Thus, the difference in plasma cICAM-1 concentrations we observed between smokers and nonsmokers may be partially dependent on plasma triglyceride levels. However, a role of cigarette smoking independent of plasma triglycerides is supported by the dose-response relationship we observed between the number of cigarettes smoked per day and plasma cICAM-1 levels and by the results of our multivariate analyses, including plasma triglyceride levels.

Therefore, the evidence from this and other studies (9,19) suggests that type 1 diabetic patients have elevated plasma cICAM-1 concentrations compared with healthy subjects and that smoking itself may play a role in the elevation of plasma cICAM-1 levels in both diabetic patients and healthy individuals. Furthermore, because diabetic nonsmokers had significantly higher plasma cICAM-1 concentrations than healthy control subjects (both smokers and nonsmokers), the increase in plasma cICAM-1 concentrations we observed in patients with type 1 diabetes may only be partially explained by cigarette smoking, and other specific and diabetes-related mechanisms may be involved such as hyperglycemia. For example, hyperglycemia may accelerate oxidation processes and cause endothelial damage by oxidized LDLs and increased formation of free radicals by glucose autooxidation (20–22). In this regard, that we found a significant correlation between glycemic control and plasma cICAM-1 concentrations only in diabetic nonsmokers is interesting. This suggests that hyperglycemia may be involved in the processes that lead to endothelial damage and that, more importantly, cigarette smoking may reduce the advantages obtained by reaching and maintaining good glycemic control of endothelial function.

The relatively small number of subjects examined did not allow us to conduct complete analyses by complications status. Nevertheless, plasma cICAM-1 levels tended to be higher in diabetic individuals with microvascular complications (presence of clinical retinopathy and/or microalbuminuria) than in patients without clinical evidence of microvascular complications (287.6 ± 58 vs. 270.2 ± 51 ng/ml, respectively). This finding agrees with data reported by Fashing et al. (19), who demonstrated increased plasma cICAM-1 levels in type 1 diabetic subjects with microvascular complications, which suggests a role for endothelial dysfunction in the development of diabetes complications. On the other hand, although the evidence of a relationship between smoking status and diabetic complications is still conflicting, several studies have reported a strong relationship between smoking and microvascular diseases (23,24).

Our data are cross-sectional and cannot provide substantial support for the hypothesis of a cause-and-effect relationship. Nevertheless, our results suggest that

chronic cigarette smoking has a deleterious effect on plasma cICAM-1 levels in young type 1 diabetic subjects without clinical macroangiopathy, which further supports the clinical importance of discouraging the initiation of smoking and promoting its cessation in people with type 1 diabetes.

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