Risk Factors for Asymptomatic Atherosclerosis in Japanese Type 2 Diabetic Patients Without Diabetic Microvascular Complications

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Atherosclerotic vascular diseases are frequently associated with diabetes mellitus. There has been increasing evidence showing that the atherosclerotic diseases in diabetic patients are distinct from diabetic microvascular complications as to their pathophysiology and epidemiology. However, we have no information on the prevalence of asymptomatic atherosclerosis in diabetic patients before the onset of microvascular diseases. In the present investigation, we aimed to evaluate risk factors for the atherosclerosis in type 2 diabetic patients without the microvascular diseases. For this purpose, we evaluated atherosclerotic change of carotid arteries in 125 Japanese type 2 diabetic patients who had neither atherosclerotic vascular diseases nor diabetic microvascular complications. When atherosclerotic change was defined as the mean intima-media thickness (IMT) of ≥ 1.1 mm and/or the presence of plaque lesion, 50% of patients had atherosclerosis of the carotid arteries. Risk factors for the carotid atherosclerosis were age, low-density lipoprotein (LDL)-cholesterol, hypertension, and diabetes treatment. Age and LDL-cholesterol were associated with mean IMT. Age, diabetes treatment, LDL-cholesterol, and hypertension were positively associated with plaque lesion, while high-density lipoprotein (HDL)-cholesterol was negatively associated with it. Fasting plasma glucose, glycosylated hemoglobin (HbA_{1c}), and known diabetes duration remained unassociated with any parameters of asymptomatic atherosclerosis of the carotid arteries. These results indicate that glycemic control is unrelated with asymptomatic atherosclerosis in type 2 diabetic patients without diabetic microvascular complications. Conventional risk factors and diabetes treatment are independently associated with atherosclerosis of the carotid arteries in these patients.

ATHEROSCLEROTIC VASCULAR diseases, such as cerebrovascular disease (CVD), coronary heart disease (CHD), and peripheral artery disease (PAD) are frequently associated with diabetes mellitus.[1-4] Several studies[5-7] have shown that these macrovascular complications of diabetes are distinct from diabetic microvascular complications as to their pathophysiology and epidemiology. The Wisconsin Epidemiologic Study of Diabetic Retinopathy[5] has shown that a 1% increase in glycosylated hemoglobin (HbA_{1c}) resulted in a 70% increase in proliferative retinopathy, but only a 10% increase in CHD events. Large-scale clinical trials in type 1 and type 2 diabetic patients[6,7] have demonstrated that intensive treatment of hyperglycemia significantly reduced microvascular complications, but did not significantly decrease the events by macrovascular complications. These studies indicate that although hyperglycemia cannot be neglected as a risk factor for atherosclerotic vascular diseases, it should not be the only risk factor in diabetic patients.[4] Recent clinical studies[8-11] have shown that blood glucose levels seem to be a risk factor for CHD events even within a range below the diabetic threshold separating those subjects who are at substantially increased risk for diabetic microvascular complications.[12] Thus, atherosclerotic vascular diseases in diabetic patients are indicated to be caused by different pathogenesis from diabetic microvascular complications, besides hyperglycemia.

Previous studies have mainly focused on evaluating the association of confounding variables with CHD in diabetic patients.[13-15] These studies included patients with diabetic microvascular complications. Such analyses may produce a bias, because a complicated relationship probably exists between atherosclerotic vascular diseases and diabetic microvascular complications. In fact, several studies[16-18] showed an excess mortality due to CHD in patients with type 2 diabetes mellitus who have microalbuminuria or proteinuria. We previously demonstrated that diabetic nephropathy is the most predictive risk factor for atherosclerotic vascular diseases in type 2 diabetic patients.[19] However, we have no information on the prevalence of asymptomatic atherosclerosis in diabetic patients before the onset of microvascular complications, and it remains undetermined which risk factors are involved in asymptomatic atherosclerosis. Thus, in the present study, we aimed to clarify these issues. For the diagnosis of asymptomatic atherosclerosis, we used ultrasound high-resolution B-mode imaging of the carotid arteries, because this method is accepted as a noninvasive and early detection method of atherosclerotic changes.[20-23]

PATIENTS AND METHODS

Study Patients

Of Japanese patients with type 2 diabetes mellitus who attended Osaka University Hospital from April 2001 to March 2002, 125 patients who had neither symptomatic atherosclerotic vascular diseases nor diabetic microvascular complications (retinopathy, nephropathy, neuropathy), were randomly enrolled for this study. The diagnosis of type 2 diabetes was based on the criteria by the American Diabetes Association's diagnostic guidelines.[24]

The diagnosis of atherosclerotic vascular diseases (CHD, CVD, and PAD) was based on laboratory tests sufficient to prove the disease status (eg, electrocardiography, magnetic resonance imaging of brain,

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Table 1. Clinical Characteristics of Type 2 Diabetic Patients Who Had Neither Symptomatic Atherosclerotic Vascular Diseases nor Diabetic Microvascular Complications

No.	125
Age (yr)	59 ± 11
Sex (M/F)	72/53
BMI (kg/m^2)	24.0 ± 4.1
Known diabetes duration (yr)	8 ± 7
Diabetes treatment (diet/OHA/insulin) (%)	29/50/21
Fasting plasma glucose (mmol/L)	8.8 ± 3.2
HbA_{1c} (%)*	7.4 ± 1.8
LDL-cholesterol (mmol/L)	3.13 ± 0.85
HDL-cholesterol (mmol/L)	1.42 ± 0.41
Hypertension (%)	37
Current smoker (%)	46

NOTE. Data are means ± SD, no. or %.
Abbreviation: OHA, oral hypoglycemic agents.
*Control ranges: 4.4% to 5.8%.

ankle blood pressure measurement, and ultrasonic velocity detector). Diabetic retinopathy was diagnosed based on fundus examination by ophthalmologists. Diabetic nephropathy was diagnosed when the patients had microalbuminuria or overt albuminuria on the basis of the criteria by Krolewski et al.[25] Diabetic neuropathy was diagnosed when the patients had peripheral sensorimotor neuropathy plus either abnormal nerve conduction in at least 2 peripheral nerves or abnormal nerve tests.[26]

Blood pressure was measured with a mercury sphyngmomanometer, after a supine rest of 5 minutes. Hypertension was diagnosed when either the systolic blood pressure was consistently ≥ 140 mm Hg or the diastolic pressure ≥ 90 mm Hg or when at least 1 antihypertensive drug, such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers, calcium channel blockers, or α- or β-adrenergic antagonists, was currently being used. Some patients with hypercholesterolemia were given pravastatin, simvastatin, or atorvastatin.

Ultrasonographic Evaluation of Carotid Arteries

We performed high-resolution B-mode imaging of carotid arteries[20-22] using an echotomographic system (SSA-380A; Toshiba Medical, Tokyo, Japan) with a 7.5-MHz transducer to evaluate early atherosclerosis. The axial resolution on this system was at least 0.3 mm. Scanning of the common carotid, the internal carotid, and the external carotid arteries was performed bilaterally as described.[19,27] Intima-media thickness (IMT) was the distance between the lumen-intima interface and the media-adventitia interface.[20,21] IMT was measured at 3 differential sites: the greatest thickness and 2 other points, 1 cm upstream and 1 cm downstream from the site of the greatest thickness. The mean of the 3 determinations of right and left IMT was defined as mean IMT as described previously.[19,27] The plaque lesion was defined when a distinct area with 50% greater IMT compared with neighboring sites was identified.[23]

Laboratory Tests

Fasting plasma glucose, HbA_{1c}, serum total cholesterol, serum high-density lipoprotein (HDL)-cholesterol, and serum triglyceride levels were measured by standard laboratory assays. Serum low-density lipoprotein (LDL)-cholesterol levels were calculated by the equation of Friedwald et al.[28]

Statistics

All data are shown as mean ± SD. The statistical analyses of the data between 2 groups were performed with use of unpaired *t* test. The prevalence of sex, current smoker, treatment of diabetes, and hypertension was compared by chi-square test or Fisher's exact test, as appropriate. To evaluate the relationship between mean IMT and different variables, single linear univariate regression analyses and stepwise multivariate regression analyses were performed. In the stepwise multivariate regression analyses, the *F* value for the inclusion of the variables was set at 4.0. To analyze the effects of different variables on the presence of atherosclerotic changes of carotid arteries, we performed logistic regression analyses. These analyses were performed using StatView computer program (Abacus Concepts, Berkeley, CA).

RESULTS

Clinical characteristics of the study patients are shown in Table 1. The averaged age was 59 years, and known duration of diabetes was 8 years. The average HbA_{1c} was 7.4%, fasting plasma glucose 8.8 mmol/L, LDL-cholesterol 3.13 mmol/L, and HDL-cholesterol 1.42 mmol/L. Thirty-seven percent of the

Table 2. Comparison of Clinical Characteristics in Type 2 Diabetic Patients With and Without Early Atherosclerosis of the Carotid Arteries

	Patients With Atherosclerosis	Patients Without Atherosclerosis	*P* Value
No.	63	62	
Mean IMT (mm)	1.11 ± 0.23	0.87 ± 0.12	<.0001
Plaque lesion (%)	73	0	<.0001
Age (yr)	63 ± 10	55 ± 9	<.0001
Sex (M/F)	39/24	33/29	.368
BMI (kg/m^2)	23.7 ± 4.1	24.3 ± 4.2	.444
Known diabetes duration (yr)	10 ± 8	7 ± 7	.025
Diabetes treatment (diet/OHA/insulin) (%)	27/48/25	31/53/16	.114
Fasting plasma glucose (mmol/L)	8.4 ± 3.1	9.2 ± 3.3	.157
HbA_{1c} (%)	7.2 ± 1.8	7.6 ± 1.9	.216
LDL-cholesterol (mmol/L)	3.31 ± 0.75	2.94 ± 0.90	.024
HDL-cholesterol (mmol/L)	1.42 ± 0.39	1.42 ± 0.47	.890
Hypertension (%)	37	18	.026
Current smoker (%)	48	45	.858

NOTE. Data are means ± SD, no. or %. Statistical analyses were performed by unpaired Student's *t* test, Fisher's exact test, or χ^2 test, as appropriate.
Abbreviations: IMT, intima-media thickness of carotid arteries, OHA, oral hypoglycemic agents.

Table 3. Logistic Regression Analysis of the Effects of Confounding Variables on Early Atherosclerosis of the Carotid Arteries

Variables	Risk Ratio	95% CI	*P* Value
Age (yr)	1.145	1.075-1.221	<.0001
LDL-cholesterol (mmol/L)	3.093	1.629-5.873	.0006
Hypertension	3.980	1.248-12.699	.020
Diabetes treatment	2.485	1.097-5.628	.029

NOTE. Hypertension was 0, −; 1, +; diabetes treatment was 0, diet; 1, oral hypoglycemic agents; 2, insulin. Other independent variables were sex (0, female; 1, male), BMI (kg/m^2), known diabetes duration (yr), fasting plasma glucose (mmol/L), HbA_{1c} (%), HDL-cholesterol (mmol/L), and current smoker (0, −; 1, +).

Abbreviation: CI, confidence interval.

Table 5. Logistic Regression Analysis of the Effects of Confounding Variables on Plaque Lesions of the Carotid Arteries

Variables	Risk Ratio	95% CI	*P* Value
Age (yr)	1.146	1.072-1.225	<.0001
Diabetes treatment	4.394	1.771-10.905	.0014
LDL-cholesterol (mmol/L)	2.727	1.357-5.482	.0049
Hypertension	3.736	1.114-12.528	.033
HDL-cholesterol (mmol/L)	0.205	0.046-0.911	.037

NOTE. Diabetes treatment was 0, diet; 1, oral hypoglycemic agents; 2, insulin; hypertension was 0, −; 1, +. Other independent variables were sex (0, female; 1, male), BMI (kg/m^2), known diabetes duration (yr), fasting plasma glucose (mmol/L), HbA_{1c}) (%), HDL-cholesterol (mmol/L), and current smoker (0, −; 1, +).

patients had hypertension, and 46% were current smokers. Mean IMT of the carotid arteries was 0.99 ± 0.22 mm in the study patients. Forty-six patients (37%) had plaque lesions. When atherosclerotic change of the carotid arteries was defined as the mean IMT of ≥ 1.1 mm and/or the presence of plaque lesions,[23] 63 patients (50%) were found to have atherosclerosis of the carotid arteries. The patients with carotid atherosclerosis were older and had longer diabetes duration (Table 2). They had a higher prevalence of hypertension than those without atherosclerosis. In addition, serum LDL-cholesterol levels were significantly higher in patients with atherosclerosis than those without it. Sex, body mass index (BMI), diabetes treatment, smoking status, fasting plasma glucose, HbA_{1c}, and HDL-cholesterol were not different between both groups (Table 2). Logistic regression analysis showed that age, LDL-cholesterol, hypertension, and diabetes treatment were significantly associated with early atherosclerosis of the carotid arteries in the study patients (Table 3).

Next, we analyzed the effects of confounding variables on mean IMT values of the carotid arteries in 125 study patients. By univariate regression analysis, age was only associated with mean IMT value (Table 4). The other variables were not associated with it. Multivariate regression analysis demonstrated that the risk factors for the mean IMT were age (F = 21.30, $P < .0001$) and LDL-cholesterol (F = 7.85, P = .017). In Table 5, we show logistic regression analysis concerning the effects of confounding variables on the presence of plaque lesions in carotid arteries. The results demonstrated that age, diabetes treatment, LDL-cholesterol, and hypertension were associated with plaque lesions, while HDL-cholesterol was negatively associated with it. Fasting plasma glucose, HbA_{1c}, and known diabetes duration were not associated with any parameters of atherosclerosis of the carotid arteries.

DISCUSSION

The present study provides data for asymptomatic atherosclerosis of carotid arteries in Japanese type 2 diabetic subjects who had no diabetic microvascular complication. We evaluated mean IMT and plaque lesions of the carotid arteries, which are generally accepted markers for early atherosclerosis and progression of atherosclerotic vascular diseases.[20-23,29-31] There have been many investigations demonstrating high prevalence of asymptomatic atherosclerosis in type 2 diabetes.[19,32-34] Risk factors for carotid atherosclerosis in diabetic subjects, however, were not the same among these studies. One reason for such discrepancies may be a difference in clinical phenotypes of the study patients as to diabetes duration, glycemic controls, and diabetic microvascular complications. In this relationship, we previously found an association of diabetic nephropathy with atherosclerotic vascular diseases.[19] So far, there is no information available on asymptomatic atherosclerosis of type 2 dia-

Table 4. Univariate and Multivariate Regression Analyses on Mean IMT of the Carotid Arteries

	Univariate Regression Analysis		Multivariate Regression Analysis		
Variables	Correlation Coefficient	*P* Value	Partial Regression Coefficient	*F*	*P* Value
Age (yr)	0.249	.005	.007	21.296	<.0001
LDL-cholesterol (mmol/L)	0.095	.304	0.052	7.845	.017
Sex	0.057	.524	-	-	-
BMI (kg/m^2)	−0.034	.703	-	-	-
Known diabetes duration (yr)	0.109	.228	-	-	-
Diabetes treatment	0.001	.992	-	-	-
Fasting plasma glucose (mmol/L)	0.054	.548	-	-	-
HbA_{1c} (%)	0.045	.620	-	-	-
HDL-cholesterol (mmol/L)	0.134	.143	-	-	-
Hypertension	0.098	.279	-	-	-
Current smoker	−0.030	.736	-	-	-

NOTE. R^2 = .183, F = 12.77, and $P < .0001$ in the multivariate regression analysis. Sex was 0, female; 1, male; diabetes treatment was 0, diet; 1, oral hypoglycemic agents; 2, insulin; hypertension was 0, −; 1, +; current smoker, 0, −; 1, +.

betic patients before the onset of microvascular complications. Therefore, we aimed to perform the studies on restricted type 2 diabetic patients with neither diabetic microvascular complications nor atherosclerotic vascular diseases.

Of 125 study patients with a mean age of 59 years, 50% had atherosclerotic changes of the carotid arteries according to the definition by Salonen et al.[23] These subjects with asymptomatic atherosclerosis were older and had longer diabetes duration. By logistic regression analysis, age, LDL-cholesterol, hypertension, and diabetes treatment were found to be risk factors for asymptomatic atherosclerosis, whereas other parameters including BMI, known diabetes duration, fasting plasma glucose, and HbA_{1c} were not. Risk factors for plaque lesions of the carotid arteries were age, LDL-cholesterol, hypertension, diabetes treatment, and decreased concentration of HDL-cholesterol. By contrast, the variables associated with mean IMT of the carotid arteries were only age and LDL-cholesterol by multivariate regression analysis. These results indicate that plaque as a localized thickening lesion and mean IMT as a diffuse thickening lesion are independently defined by contributing risk factors.

Our studies demonstrated that fasting plasma glucose, HbA_{1c}, and known diabetes duration failed to be involved in any parameters of asymptomatic atherosclerosis of the carotid arteries. Many of the previous studies, including type 2 diabetic patients with diabetic microvascular and macrovascular complications, also found no significant association of fasting plasma glucose and HbA_{1c} with IMT,[19,31,33] while fasting glucose levels were weakly associated with IMT of the common carotid arteries ($P < .05$), but not IMT of the internal carotid arteries in the Insulin Resistance Atherosclerosis Study (IRAS).[33] In a study on 71 patients with newly detected type 2 diabetes,[35] fasting plasma glucose and HbA_{1c} did not predict IMT of the common carotid arteries. In addition, fasting plasma glucose and HbA_{1c} were not associated with IMT in 582 individuals aged 40 to 70 years and at risk for type 2 diabetes.[36] The latter 2 studies evaluated subjects in the early diabetic stage. We, in the present investigations, studied type 2 diabetic patients with relatively longer duration, but not with diabetic microvascular complications. Taken together, glycemic controls are indicated not important factors defining asymptomatic atherosclerosis in type 2 diabetic patients with early stage, as well as those with no diabetic microvascular complication. There has been increasing evidence that postprandial hyperglycemia is a strong risk factor for the development of macrovascular complications in diabetes[10,13] and even in subjects with impaired glucose tolerance.[8,11] Postchallenge plasma glucose and glycemic spikes have been shown to be strongly associated with IMT in individuals at risk for type 2 diabetes.[36] At present, we have no data as to whether or not postprandial plasma glucose is associated with asymptomatic atherosclerosis in type 2 diabetic patients with no microvascular complication. Further studies should be performed to clarify this issue.

We showed here that in type 2 diabetic patients with no microvascular complication, LDL-cholesterol, hypertension, age, and diabetes treatment were important risk factors for asymptomatic atherosclerosis, in contrast to fasting plasma glucose and HbA_{1c}. In a prospective study including 1,059 patients with type 2 diabetes,[14] dyslipidemia and hyperglycemia were found to predict CHD. In another study of 1,539 type 2 diabetic patients, age, hypertension, cigarette smoking, and total/HDL cholesterol ratio, but not HbA_{1c}, were associated with CVD, CHD, and/or PAD.[15] In the United Kingdom prospective diabetes study (UKPDS: 23),[37] risk factors for CHD were increased concentrations of LDL-cholesterol, decreased concentrations of HDL-cholesterol, elevated blood pressure, hyperglycemia, and smoking. In that study, the estimated hazard ratio was highest for LDL-cholesterol. Thus, conventional atherosclerotic vascular disease risk factors, rather than hyperglycemia, are much more important for both asymptomatic and symptomatic atherosclerosis.

Much evidence has been accumulated indicating that atherosclerosis can precede the development of diabetes mellitus.[38-41] From our data, about half of type 2 diabetic patients had asymptomatic atherosclerosis before the onset of diabetic microvascular diseases. Increased LDL-cholesterol and the presence of hypertension, in addition to aging and diabetes treatment, were convincing risk factors for atherosclerosis. Intensive management of these risk factors may be a more promising target for intervention to reduce atherosclerotic complications in type 2 diabetes.

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