Porphyromonas Gingivalis Infection Is Associated With Carotid Atherosclerosis in Non-Obese Japanese Type 2 Diabetic Patients

Ataru Taniguchi, Fusanori Nishimura, Yoji Murayama, Shoichiro Nagasaka, Mitsuo Fukushima, Masahiko Sakai, Satoru Yoshii, Akira Kuroe, Haruhiko Suzuki, Yoshihiro Iwamoto, Yoshihiko Soga, Takahide Okumura, Masahito Ogura, Yuichiro Yamada, Yutaka Seino, and Yoshikatsu Nakai

The aim of the present study was to investigate whether non-obese Japanese type 2 diabetic patients with porphyromonas gingivalis infection have atherosclerotic vascular diseases. A total of 134 non-obese Japanese type 2 diabetic patients (96 men and 38 women, aged 36 to 84 years, body mass index [BMI] 20.1 to 26.9 kg/m^2) were studied. In conjunction with BMI, glycosylated hemoglobin (HbA_{1c}), fasting glucose, and serum lipids (triglycerides, total cholesterol, high-density lipoprotein [HDL] cholesterol, low-density lipoprotein [LDL] cholesterol) were measured. LDL cholesterol was calculated using the Friedewald formula. Using high-resolution B-mode ultrasound scan, we measured intimal medial thickness (IMT) in plaque-free segments of bilateral common carotid arteries, and the mean of IMT in 2 vessels was used for the analysis. Furthermore, we calculated the degree of stenosis in plaque segments of bilateral common carotid arteries. The degree of carotid atherosclerosis was expressed as a percentage ratio between the area of plaque and that of the lumen using the formula (Lumen Area Residual − Lumen Area)/Lumem Area × 100. Both the areas were automatically measured by the system on a frozen transverse scanning plane at the site of maximal narrowing. When 2 or more plaques were present in the vessel, only that causing the greatest degree of stenosis was considered for analysis. Values represent mean±SEM unless otherwise stated. Immunoglobulin G (IgG) titer against porphyromonas gingivalis was 245 ± 65 (mean ± 2 SD) in nondiabetic healthy subjects. In contrast, there was a wide variation in IgG titer against porphyromonas gingivalis in type 2 diabetic patients studied (range, 16 to 26,800). Thus, we classified our type 2 diabetic patients into 2 subpopulations according to the value of mean ± 2 SD (= 310) of nondiabetic healthy subjects: one with high IgG titer against porphyromonas gingivalis (>310) (1,422 ± 408) and the other with normal IgG titer against porphyromonas gingivalis (<310) (152 ± 10, *P* = .002). The populations did not differ with respect to age, sex, BMI, fasting glucose, HbA_{1c}, serum triglycerides, total, HDL, and LDL cholesterol levels. Although the mean IMT in plaque-free segments was not different between the 2 groups (0.73 ±0.03 *v* 0.68 ± 0.02 mm, *P* = .098), the degree of stenosis in plaque segments was significantly higher in the high IgG titer group (12.0% ± 2.2%) than in normal one (5.5% ± 1.4%, *P* = .009). From these results, it can be concluded that porphyromonas gingivalis infection, although still a subclinical infection, is associated with atherosclerotic vascular disease in non-obese Japanese type 2 diabetic patients.

THE MAJOR CLINICAL consequence of type 2 diabetes is mortality and morbidity from atherosclerotic vascular disease. Regarding the risk factors responsible for the evolution of atherosclerosis in diabetic patients, Bierman[1] previously estimated that typical risk factors including smoking, cholesterol, and blood pressure (BP) can account for no more than 25% to 30% of excess cardiovascular risk factors in diabetic patients. This suggests that other factors might play a key role in the progression of atherosclerosis in diabetes. The degree of atherosclerosis can be evaluated by high-resolution B-mode ultrasound scan. Carotid atherosclerosis is important in view of its relationship to cerebrovascular ischemic diseases and coronary atherosclerosis.[2]

Whereas insulin resistance is established to be one of the risk factors for the evolution of coronary heart disease (CHD),[3] there are some data suggesting that subclinical inflammation is hypothesized to be associated with CHD events in man.[4-7] Elevated levels of C-reactive protein (CRP), although still for the most part in the healthy reference range, have been shown to be associated with increased risk of future CHD events.[4-6] Some cross-sectional and case-control studies have reported elevated antibody titers directed against Chlamydia pneumoniae, Helicobacter pyroli, and Cytomegalovirus among those with prevalent heart disease.[7]

Type 2 diabetic patients are known to have a high prevalence of atherosclerosis and periodontal disease.[8] Porphyromonas gingivalis has been shown to play an important role in the periodontis of type 2 diabetic patients.[9] Thus, periodontis especially porphyromonas gingivalis infection is hypothesized to be associated with atherosclerosis in type 2 diabetic patients. To the best of our knowledge, however, the relationship between porphyromonas gingivalis infection and the degree of atherosclerosis has not been fully clarified in type 2 diabetic patients. In this regard, a major problem is that the degree of being overweight or of hyperglycemia per se affects atherosclerosis in man. To overcome this difficulty, we recruited non-obese well-controlled unique Japanese type 2 diabetic patients who had no evidence of cardiovascular disease, ischemic

From the Division of Diabetes, Kansai-Denryoku Hospital, Osaka; Department of Patho-physiology/Periodontal Science, Okayama University Graduate School of Medicine and Dentistry, Okayama; Division of Endocrinology and Metabolism, Jichi Medical School, Tochigi; and the Department of Metabolism and Clinical Nutrition, Graduate School of Medicine and College of Medical Technology, Kyoto University, Kyoto, Japan.

Submitted January 14, 2002; accepted July 30, 2002.

Address reprint requests to Ataru Taniguchi, MD, Division of Diabetes, Kansai-Denryoku Hospital, 2-1-7 Fukushima, Fukushima-ku, Osaka-City, Osaka 553-0003, Japan.

0026-0495/03/5202-0040$35.00/0
doi:10.1053/meta.2003.50001

stroke, or chronic renal failure and investigated the relationships between porphyromonas gingivalis infection and the degree of carotid atherosclerosis. This is the first description that porphyromonas gingivalils infection is associated with carotid atherosclerosis in non-obese Japanese type 2 diabetic patients.

SUBJECTS AND METHODS

One hundred and 34 non-obese Japanese type 2 diabetic patients who visited Kansai-Denryoku Hospital were enrolled for the present study. Type 2 diabetes mellitus was diagnosed based on the criteria of the World Health Organization (WHO).[10] They had no evidence of current acute illness including clinically significant infectious disease. The duration of diabetes was 11.2 ± 0.7 years (mean ± SEM). Ninty-three of 134 diabetic patients were taking sulfonylureas (gliclazide) to control their diabetes and the rest with diet alone. None of the patients have received insulin therapy. All subjects had ingested at least 150 g of carbohydrates for the 3 days preceding the study. None of the subjects had significant renal, hepatic, or cardiovascular disease. They did not consume alcohol or perform heavy exercise for at least 1 week before the study.

The blood was drawn in the morning after a 12-hour fast. Plasma glucose was measured with the glucose oxidase method. Triglycerides, total and high-density lipoprotein (HDL) cholesterol were measured. The low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.[11]

Serum immunoglobulin G (IgG) antibody titer against porphyromonas gingivalis was assayed by enzyme-linked immunosorbent assay as described previously.[12] Porphyromonas gingivalis SU 83 was used as an antigen. A soluble fraction of whole bacterial sonicates was used as an antigen and was prepared as described previously.[12]

A carotid sonography was performed with high-resolution B-mode scanning equipment (Logic 500 GE Yokogawa, Milwaukee, WI) with a 7.5-MHz sector scanner probe.[13] The common carotid arteries of both sides were examined with longitudinal and transverse scans, because we could not analyze the internal and external carotid arteries fully in all patients. The coefficient of variation (CV) for interobserver variability was found to be 8.5% and the CV for intraobserver variability was 6.0%. The intimal plus medial thickness (IMT) of the common carotid artery was measured in plaque-free segments as the distance from the leading edge of the first echogenic line to that of the second echogenic line. The mean of IMT in plaque-free segments of bilateral common carotid arteries was used for the analysis. The degree of stenosis was also measured in the plaque segments of bilateral common carotid arteries. It was calculated as a percentage ratio between the area of the plaque and that of the lumen using the formula (Lumen Area − Residual Lumen Area)/Lumen Area × 100. Both the areas were automatically measured by the system on a frozen transverse scanning plane at the site of maximal narrowing. When 2 or more plaques were present in the vessel, only that causing the greatest degree of stenosis was considered for analysis.

Statistical Analysis

The statistical analysis was performed with the StatView 5 system (Statview, Berkeley, CA). The differences of mean were determined by the Student's *t* test, taking a value of $P < .05$ as significant. Data were expressed as the mean ± SEM unless otherwise stated.

RESULTS

The subjects studied were all Japanese type 2 diabetic patients (96 men and 38 women) with an age range of 36 to 84 years (60.8 ± 0.8) and a body mass index (BMI) of 20.1 to 26.9 kg/m^2 (23.3 ± 0.2). They were all non-obese.[14] The fasting plasma glucose was 147 ± 3 mg/dL and glycosylated hemoglobin (HbA_{1c}) was 7.1% ± 0.1%. Serum triglycerides, total, and HDL cholesterol levels were 129 ± 7 mg/dL and 203 ± 3 mg/dL, and 52 ± 1 mg/dL, respectively. Serum LDL concentration was 125 ± 3 mg/dL. Mean IMT in plaque-free segments was 0.71 ± 0.02 mm. In contrast, the degree of carotid stenosis in plaque segments was 8.9% ± 1.4%.

Table 1. Clinical Characteristics in High and Normal IgG Titer Against Porphyromonas Gingivalis

Characteristic	High Titer	Normal Titer	*P*
Antibody titers	>310	<310	—
Antibody titers	1,422 ± 408	152 ± 10	.002
No. of subjects studied	70	64	—
Male/Female	48/22	48/16	.056
Degree of carotid stenosis (%)	12.0 ± 2.2	5.5 ± 1.4	.009
Mean IMT (mm)	0.73 ± 0.03	0.68 ± 0.02	.098
Triglycerides (mg/dL)	130 ± 9	127 ± 10	.393
Total cholesterol (mg/dL)	206 ± 4	200 ± 4	.148
LDL cholesterol (mg/dL)	128 ± 3	121 ± 5	.100
HDL cholesterol (mg/dL)	51 ± 2	53 ± 2	.234
BMI (kg/m^2)	23.3 ± 0.2	23.3 ± 0.2	.475
Fasting glucose (mg/dL)	148 ± 4	147 ± 5	.452
HbA_{1c} (%)	7.2 ± 0.1	7.4 ± 0.2	.198
Age (yr)	62 ± 1	60 ± 1	.150
Systolic blood pressure	129 ± 2	131 ± 2	.253
Diastolic blood pressure	75 ± 1	76 ± 1	.247

IgG titer against porphyromonas gingivalis was 245 ± 65 (mean ± 2 SD) in nondiabetic healthy subjects. In contrast, there was a wide variation in IgG titer against porphyromonas gingivalis in type 2 diabetic patients studied (range, 16 to 26,800). Thus, we classified our type 2 diabetic patients into 2 subpopulations according to the value of mean ± 2 SD of nondiabetic healthy subjects (= 310): one with a high IgG titer against porphyromonas gingivalis (> 310) (1,422 ± 408) and the other with a normal IgG titer against porphyromonas gingivalis (< 310) (152 ±10, $P = .002$). There was no overlap in IgG titer between the 2 populations. Seventy-one percent (50/70) of the patients with a high titer and 68% (43/64) of those with a normal titer were treated with sulfonylurea. The rests were treated with diet alone. No significant difference was observed in medication status between the 2 groups. When the patients were divided into high and normal IgG titer subgroups, the degree of carotid stenosis was significantly higher in high titer subgroups than in normal titer subgroups (12.0% ± 2.2% *v* 5.5% ± 1.4%, $P = .009$). The mean IMT in plaque-free segments was higher in the high IgG titer group than in the normal one, but was not statistically significant (0.73 ± 0.03 *v* 0.68 ± 0.02 mm, $P = .098$). Serum triglycerides, total, and LDL cholesterol levels were higher in the patients with a high IgG titer than in those with a normal IgG titer, but they were not statistically significant between the 2 groups. No significant difference was observed in HDL cholesterol, BMI, fasting glucose, HbA_{1c}, age, and systolic and diastolic BP between the 2 subgroups (Table 1).

DISCUSSION

Our main observation in the present study is that 70 of 134 patients (52%) had high IgG titer against porphyromonas gin-

givalis (IgG titer > 310), and that the degree of carotid stenosis, but not IMT in plaque-free segments, characterized the patients with a high IgG titer against porphyromonas gingivalis as compared with those with normal a IgG titer. The patients studied were unique in that they were non-obese and were well controlled in terms of HbA_{1c} (mean $HbA_{1c,}$7.1%) and BP (mean BP, 130/76 mm Hg). Moreover, they had no evidence of CHD, ischemic stroke, or chronic renal failure. Of particular interest is that the degree of carotid stenosis was 8.9% ±1.4% and that the comparison was made between the groups with 12.0% and 5.5% (Table 1). To the best of our knowledge, however, carotid ultrasonography was not reported in type 2 diabetic patients with very early stages of carotid atherosclerosis, such as in our present study. Thus, the mechanism by which the very early stages of carotid atherosclerosis occurs in type 2 diabetic patients is not known, but it may be hypothesized that porphyromonas gingivalils infection is one of the factors responsible for the early onset of carotid atherosclerosis in non-obese Japanese type 2 diabetic patients. It is well known that carotid atherosclerosis is important in view of its relationship to cerebrovascular ischemic diseases and coronary atherosclerosis.[2]

Whereas porphyromonas gingivalis infection is a subclinical inflammation, there are some findings suggesting that subclinical infection is associated with atherosclerosis in man. Elevated levels of CRP, although still for the most part in the healthy reference range, have been shown to be associated with an increased risk of future CHD events.[4-6] Some cross-sectional and case-control studies have reported elevated antibody titers directed against *Chlamydia pneumoniae*, *Helicobacter pyroli*, and cytomegalovirus among those with prevalent heart disease.[7]

Thus, porphyromonas gingivalis infection seems to be associated with the early stage of atherosclerosis in non-obese Japanese type 2 diabetic patients.

Finally, carotid atherosclerosis was not significantly associated with an abnormal lipid profile, such as high concentrations of LDL cholesterol in the present study. The reason is unclear, but it may be due to the clinical characteristics studied. Our patients were all non-obese Japanese type 2 diabetic patients. Indistinct from white populations, non-obese Japanese type 2 diabetic patients are unique in that they are divided into 2 variants: one with normal insulin sensitivity and the other with insulin resistance.[15-19] Non-obese Japanese type 2 diabetic patients with insulin resistance are characterized by higher BMI, higher triglycerides, higher remnant-like particle cholesterol, and lower HDL cholesterol levels as compared with those with normal insulin sensitivity. Our patients studied were unique in that they were well controlled in terms of BMI, HbA_{1c}, BP, LDL cholesterol, triglycerides, total cholesterol, and HDL cholesterol. Furthermore, our patients had no evidence of cardiovascular disease, ischemic stroke, or chronic renal failure.[20] Therefore, an association between carotid atherosclerosis and conventional risk factors including LDL cholesterol would be more significant among obese type 2 diabetic patients who has abnormal lipid profile and/or an evidence of cardiovascular disease, ischemic stroke, or chronic renal failure. Alternatively, the diabetic state per se is such a powerful factor on carotid atherosclerosis that the effect of other risk factors is masked. Mohan et al[21] recently demonstrated that diabetes and age, but not conventional risk factors, are the most important risk factors associated with increased IMT in South Indian diabetic patients with a BMI of 24.5 kg/m^2.

In summary, although our present study was performed among the limited patients who were well controlled in terms of BMI, HbA_{1c}, BP, LDL cholesterol, triglycerides, total cholesterol, and HDL cholesterol, porphyromonas gingivalis infection seems to be associated with an early stage of atherosclerosis in non-obese Japanese type 2 diabetic patients.

ACKNOWLEDGMENT

We are very grateful to Chiaki Taniguchi, Nina Hayasaki, Dr Satoshi Teramura, Dr Keiichi Marumoto, Dr Kenichi Hama, Dr Ikuko Yoshioka, Dr Noriko Yamadori, Dr Toshiki Watanabe, Dr Tsuyoshi Oguma, Dr Ryuji Hayashi, Dr Akihisa Fukuda, Dr Hiroyuki Kishimoto, Division of Diabetes, Kansai-Denryoku Hospital, Dr Shinji Okamoto, Dr Chieko Kudo, Dr Yukiko Ogino, Dr Yukari Kubo, Dr Naoki Takahashi, Department of Patho-physiology/Periodontal Science, Okayama University Graduate School of Medicine and Dentistry, and Shionogi Biomedical Laboratory in Osaka, Japan, for encouraging this study.

REFERENCES

1. Bierman EL: George Lyman Duff Memorial Lecture. Atherogenesis in diabetes. Arterioscler Thromb 12:647-656, 1992

2. Craven TE, Ryu JE, Espeland MA, et al: Evaluation of the associations between carotid artery atherosclerosis and coronary artery stenosis: A case control study. Circulation 82:1230-1242, 1990

3. Kuller L, Tracy R, Shaten J, et al: Relationship of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Am J Epidemiol 144:537-547, 1996

4. Ridker PM, Cushman M, Stampfer MJ, et al: Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 336:973-979, 1997

5. Koenig W, Sund M, Frolich M, et al: C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men. Results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Ausburg Cohort Study, 1984 to 1992. Circulation 99:237-242, 1999

6. Tracy R, Lemaitre R, Psaty B, et al: Relationship of C-reactive protein to risk of cardiovascular disease in the elderly: Results from the Cardiovascular Health Study and the Rural Health Promotion Project. Arterioscler Thromb Vasc Biol 17:1121-1127, 1997

7. Danesh J, Collins R, Peto R: Chronic infections and coronary heart disease: Is there a link? Lancet 350:430-436, 1997

8. Nishimura F, Terranova V, Foo H, et al: Glucose-mediated alteration of cellular function in human periodontal ligament cells. J Dent Res 75:1664-1671, 1996

9. Yuan K, Chang CJ, Hsu PC, et al: Detection of putative periodontal pathogens in non-insulin-dependent diabetes mellitus and non-diabetes mellitus by polymerase chain reaction. J Periodontal Res 36:18-24, 2001

10. World Health Organization: Diabetes Mellitus. 1985 Report of a WHO Study Group. Geneva, Switzerland, World Health Org (Tech Rep Ser, no.727)

11. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499-502, 1972

12. Murayama Y, Nagai A, Okumura K, et al: Serum immunoglobulin G antibody to periodontal bacteria. Adv Dent Res 2:339-345, 1988

13. Taniguchi A, Nakai Y, Fukushima M, et al: Ultrasonographycally assessed carotid atherosclerosis in Japanese type 2 diabetic patients: Role of non-esterified fatty acids. Metabolism 51:539-543, 2002

14. Taniguchi A, Nakai Y, Doi K, et al: Insulin sensitivity, insulin secretion, and glucose effectiveness in obese subjects: A minimal model analysis. Metabolism 44:1397-1400, 1995

15. Haffner SM, D'Agostino R Jr, Mykkanen L, et al: Insulin sensitivity in subjects with type 2 diabetes. Relationship to cardiovascular risk factors: The Insulin Resistance Atherosclerosis Study. Diabetes Care 22:562-568, 1999

16. Taniguchi A, Nakai Y, Fukushima M, et al: Pathogenic factors responsible for glucose tolerance in patients with NIDDM. Diabetes 41:1540-1546, 1992

17. Nagasaka S, Tokuyama K, Kusaka I, et al: Endogenous glucose production and glucose effectiveness in type 2 diabetic subjects derived from stable-labeled minimal model approach. Diabetes 48:1054-1060, 1999

18. Taniguchi A, Fukushima M, Sakai M, et al: The role of the body mass index and triglyceride levels in identifying insulin-sensitive and insulin-resistant variants in Japanese non-insulin-dependent diabetic patients. Metabolism 49:1001-1005, 2000

19. Taniguchi A, Fukushima M, Sakai M, et al: Remnant-like particle cholesterol, triglycerides, and insulin resistance in nonobese Japanese type 2 diabetic patients. Diabetes Care 23:1766-1769, 2000

20. Matsumoto K, Miyake S, Yano M, et al: Insulin resistance and classic risk factors in type 2 diabetic patients with different subtypes of ischemic stroke. Diabetes Care 22:1191-1195, 1999

21. Mohan V, Ravikumar R, Rani SS, et al: Intimal medial thickness of the carotid artery in South Indian diabetic and nondiabetic subjects: The Chennai Urban Population Study (CUPS). Diabetologia 43:494-499, 2000