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Helicobacter pylori infection and arterial stiffness in patients with type 2 diabetes mellitus

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Abstract

Epidemiologic studies have suggested possible atherogenic roles for such pathogens as *Chlamydia pneumoniae*, *Helicobacter pylori* (Hp), cytomegalovirus, and herpes simplex virus. The aim of the present study was to examine the relationship between seropositivity of antibodies to Hp (Hp infection) and arterial stiffness determined by pulse wave velocity (PWV) in 130 patients (73 men and 57 women) with type 2 diabetes mellitus without a history of cardiovascular disease. The prevalence of Hp infection in patients with type 2 diabetes mellitus was 53.8%. Age (66.7 ± 11.3 vs 60.0 ± 12.2 years, $P = .0014$) and systolic blood pressure (138 ± 19 vs 131 ± 22 mm Hg, $P = .0420$) were significantly higher in patients with Hp infection than in those without. Serum C-reactive protein was higher in patients with Hp infection than in those without, although it did not reach statistical significance (0.23 ± 0.27 vs 0.18 ± 0.20 mg/dL, $P = .2205$). Pulse wave velocity was significantly higher in patients with Hp infection than in those without (1877 ± 550 vs 1585 ± 331 cm/s, $P = .0005$). Multiple regression analysis demonstrated that age ($\beta = .388$, $P < .0001$), mean arterial pressure ($\beta = .289$, $P = .0006$), hypertensive treatment ($\beta = .185$, $P = .0282$), and presence of Hp infection ($\beta = .169$, $P = .0220$) were independent determinants of PWV. In conclusion, Hp infection is associated with arterial stiffness determined by PWV in patients with type 2 diabetes mellitus.

1. Introduction

Chronic infections and inflammations by *Chlamydia pneumoniae*, cytomegalovirus, hepatitis B and C, and herpes simplex virus have been reported to be linked to systemic atherosclerosis and the occurrence of vascular disease [1,2]. *Helicobacter pylori* (Hp) infection also has been reported to be associated with the development and progression of atherosclerosis [3-6]. There are several investigations concerning the association between the prevalence of Hp infection and duration of diabetes, the daily dosage of insulin, or glycemic control (levels of hemoglobin A_{1c} [HbA_{1c}]) in diabetic patients [7,8]. Most studies have demonstrated that the prevalence of Hp infection is higher in patients with diabetes mellitus than in those without [9].

Cardiovascular disease (CVD) is the primary cause of mortality and morbidity in patients with type 2 diabetes mellitus; and several risk factors, including smoking, hypertension, and dyslipidemia, have been shown to accelerate the progression of CVD [10,11]. Pulse wave velocity (PWV), a simple, noninvasive marker of atherosclerosis, measures arterial stiffness to serve as an indicator of future outcome of atherosclerotic vascular disease [12]. To our knowledge, no previous studies have investigated the relationship between Hp infection and atherosclerosis in patients with type 2 diabetes mellitus. In the present study, we have examined the relationship between Hp infection and arterial stiffness determined by PWV in patients with type 2 diabetes mellitus.

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2. Subjects and methods

2.1. Subjects

Serum immunoglobulin G (IgG) antibodies to Hp were measured in 130 (73 men and 57 women) consecutive patients with type 2 diabetes mellitus recruited from outpatient clinics of the Matsushita Memorial Hospital. We then evaluated relationships of PWV to serum IgG antibodies to Hp as well as to major cardiovascular risk factors, including age, blood pressure, serum lipid concentration, HbA_{1c}, and body mass index (BMI).

Serum IgG antibodies to Hp were measured using an enzyme immunoassay (E plate; Eiken Chemical, Tokyo, Japan) [13]; an assay value of at least 10 U/mL was considered as positive. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods. Hemoglobin A_{1c} was assayed using high-performance liquid chromatography. Serum C-reactive protein (CRP) was measured by a modified latex-enhanced immunoturbidimetric assay (reference range: <0.3 mg/dL).

Type 2 diabetes mellitus was diagnosed according to the "Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus" [14]. Blood pressure was measured with subjects seated after a 5-minute rest. Retinopathy was graded as follows: no diabetic retinopathy, simple diabetic retinopathy, or proliferative diabetic retinopathy. Nephropathy was graded as follows: normoalbuminuria, urinary albumin excretion less than 30 mg/g creatinine (Cr); microalbuminuria, 30 to 300 mg/g Cr; or macroalbuminuria, more than 300 mg/g Cr. Mean values for biochemical parameters obtained during the previous year in patients with type 2 diabetes mellitus were used for statistical analysis. Smoking status was recorded as nonsmoker, past smoker, or current smoker according to a self-administered questionnaire. *Cardiovascular disease* was defined as the presence of angina pectoris, positive coronary catheterization, previous myocardial infarction, or cerebral infarction based on the clinical history or physical examination. Patients were excluded if they had a history of CVD. Furthermore, we excluded patients with peripheral artery disease because obstructive arterial disease of lower limbs would delay the arrival of pressure wave at the ankle and increase the time transit, which artifactually decrease PWV. Peripheral artery disease was diagnosed by a value of ankle-brachial index (ABI) less than 0.9 according to the conventional cutoff [15]. Approval for the study was obtained from the local Research Ethics Committee, and informed consent was obtained from all participants.

2.2. Measurement of PWV

Brachial-ankle (ba) PWV and ABI were measured by an automatic waveform analyzer (model BP-203RPEII; Colin, Komaki, Japan), which simultaneously measures pulse volumes in the brachial and ankle arteries using an oscillometric method together with bilateral arm and ankle blood pressure. Subjects were examined in the supine position after 5 minutes of bed rest. The baPWV was calculated by time phase as distance/time (in centimeters per second). The time delay between the arrival of the pulse wave at the brachium and ankle at each side was measured automatically by gating the pulse wave to the peak of the R wave of the electrocardiogram. The distance between the brachium and ankle at each side was estimated based on body height and adjusted for average Japanese body composition. Details of the method have been described elsewhere [16]. After bilateral determination of baPWV, the higher value was taken as representative for each subject. The ABI was calculated bilaterally as the ratio of systolic pressure in the ankle to systolic pressure in the arm, with the lower value considered representative for each subject.

2.3. Statistical analysis

Means and frequencies of potential confounding variables were calculated. Unpaired Student t tests or χ^2 tests were conducted to assess statistical significance of differences between patients with Hp infection and those without using Stat View software (version 4.0; SAS, Cary, NC). Analysis of variance was conducted to assess statistical significance of differences between groups. All continuous variables are presented as the mean ± SD. Multiple regression analysis was performed to assess the combined association of variables that are known and

Table 1
Clinical characteristics of patients with diabetes mellitus

	Hp positive	Hp negative
n	70	60
Sex (male/female)	40/30	33/27
Age (y)	66.7 ± 11.3*	60.0 ± 12.2
Duration of diabetes (y)	11.5 ± 10.1	9.4 ± 9.2
BMI (kg/m^2)	24.2 ± 4.8	25.1 ± 4.4
HbA_{1c} (%)	8.2 ± 1.9	7.9 ± 1.4
CRP (mg/dL)	0.23 ± 0.27	0.18 ± 0.20
Systolic blood pressure (mm Hg)	138 ± 19†	131 ± 22
Diastolic blood pressure (mm Hg)	79 ± 11	76 ± 11
Heart rate	67 ± 9	68 ± 10
Total cholesterol (mg/dL)	203 ± 31	210 ± 35
Triglyceride (mg/dL)	154 ± 84	152 ± 94
HDL cholesterol (mg/dL)	50 ± 13	53 ± 14
Hypertensive treatment (CCB/ARB and/or ACE-I)	17/30	15/23
Hyperlipidemic treatment (statin/fibrate)	19/3	22/3
Retinopathy (NDR/SDR/PDR)	28/33/9	40/12/8
Nephropathy (normo-/micro-/macroalbuminuria)	31/30/9	38/14/8
Smoking status (none/past/current)	34/5/31	34/3/23

Data are mean ± SD or number (percentage). CCB indicates calcium channel blocker; ARB, angiotensin II receptor blocker; ACE-I, angiotensin-converting enzyme inhibitor; NDR, no diabetic retinopathy; SDR, simple diabetic retinopathy; PDR, proliferative diabetic retinopathy.

* $P = .0014$ vs Hp negative.

† $P = .0420$ vs Hp negative.

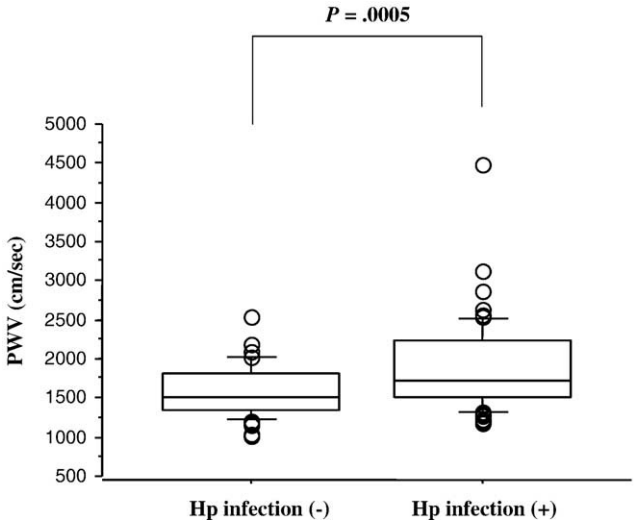

Fig. 1. Correlation between Hp infection and PWV in patients with type 2 diabetes mellitus. Data are presented as medians, 25th and 75th percentiles (boxes), and 10th and 90th percentiles (whiskers).

potential cardiovascular risk factors on PWV. To examine the associations of various factors on PWV, age, sex, duration of diabetes, BMI, HbA_{1c}, mean arterial pressure, heart rate, serum total cholesterol and HDL cholesterol concentrations, serum CRP, smoking status, hypertensive treatment, hyperlipidemic treatment, and presence of Hp infection were considered as independent variables. A P value less than .05 was considered statistically significant.

3. Results

Clinical characteristics of the 130 patients with type 2 diabetes mellitus in this study are shown in Table 1. The prevalence of Hp infection in patients with type 2 diabetes mellitus was 53.8%. Age (66.7 ± 11.3 vs 60.0 ± 12.2 years, $P = .0014$) and systolic blood pressure (138 ± 19 vs 131 ± 22 mm Hg, $P = .0420$) were significantly higher in patients with Hp infection than in those without. Serum CRP was higher in patients with Hp infection than in those without, although it did not reach statistical significance (0.23 ± 0.27 vs 0.18 ± 0.20 mg/dL, $P = .2205$). Pulse wave velocity was significantly higher in patients with Hp infection than in those without (1877 ± 550 vs 1585 ± 331 cm/s, $P = .0005$; Fig. 1). Multiple regression analysis demonstrated that age ($\beta = .388$, $P < .0001$), mean arterial pressure ($\beta = .289$, $P = .0006$), hypertensive treatment ($\beta = .185$, $P = .0282$), and presence of Hp infection ($\beta = .169$, $P = .0220$) were independent determinants of PWV (Table 2). However, sex ($\beta = -.069$, $P = .4446$), duration of diabetes ($\beta = .115$, $P = .1406$), BMI ($\beta = -.073$, $P = .4080$), HbA_{1c} ($\beta = -.048$, $P = .5381$), heart rate ($\beta = .021$, $P = .7635$), total cholesterol ($\beta = -.020$, $P = .8011$), HDL cholesterol ($\beta = .116$, $P = .1412$), CRP ($\beta = .080$, $P = .3047$), hyperlipidemic treatment ($\beta = .080$, $P = .2710$), and smoking status ($\beta = .040$, $P = .6367$) were not.

Table 2
Independent determinants of PWV

	β	P
Age	.388	<.0001
Mean arterial pressure	.289	.0006
Hypertensive treatment	.185	.0282
Presence of Hp infection	.169	.0220

4. Discussion

We evaluated relationships between arterial stiffness determined by PWV and Hp infection in patients with type 2 diabetes mellitus. Pulse wave velocity was significantly higher in patients with Hp infection than in those without. A stronger correlation was found when the threshold for Hp seropositivity was set for greater than 50 U/mL (1955 ± 600 vs 1625 ± 356 cm/s, $P = .0002$, in patients with value of Hp antibodies greater than 50 and less than 50, respectively). Multiple regression analysis also demonstrated that presence of Hp infection was an independent determinant of PWV.

Several reports have implicated Hp infection in CVD, especially when more virulent strains are involved (eg, the CagA strain) [17,18]; and Hp seropositivity has been postulated to be an independent risk factor for ischemic stroke [19]. Kinjo et al [20] reported that Hp infection is associated with acute myocardial infarction independent of the classic coronary risk factors in younger individuals in Japan. On the other hand, Wald et al [21] found no association between Hp seropositivity and ischemic heart disease in a population of 21 520 professional men attending routine medical examination. A possible explanation for the different results among countries is a large degree of genomic and allelic diversity of Hp. The severity of Hp-related disease is associated with the presence of the *cag* pathogenicity island [22,23]. In Japan, almost all of the strains possess CagA [24], which is considered to be a marker of the presence of the *cag* pathogenicity island [25]; and the severity of Hp-related disease in Japan is higher compared with that in Western countries [26]. Kowalski [27] demonstrated the reduction in restenosis of coronary vessels after Hp eradication, which indicates the involvement of Hp infection in the progression of CVD induced by a local inflammatory process.

A causal relationship between Hp infection and atherosclerosis is still unknown. Davies et al [28] reported that expression of adhesion molecules in the endothelium and smooth muscle is a key component of the inflammatory response in atherosclerotic lesions. Ameriso et al [29] demonstrated that Hp is detected not only in gastric mucosa but also in human atherosclerotic plaque and that the expression of intercellular adhesion molecule–1 is higher in plaques with Hp than in those without. *Helicobacter pylori*

infection stimulates the production of proinflammatory cytokines such as tumor necrosis factor, interleukin-6, and interleukin-8, which are atherogenic [30,31]; however, we could not measure those cytokines in the present study. Furthermore, the association of Hp infection with increased serum CRP and fibrinogen concentrations has been reported [4,32]. Serum CRP was higher in patients with Hp infection than in those without in the present study, although it did not reach statistical significance. Pulse wave velocity in patients with positive Hp infection and elevated CRP, positive Hp infection and normal CRP, negative Hp infection and elevated CRP, and negative Hp infection and normal CRP were 1957 ± 727, 1853 ± 482, 1667 ± 296, and 1571 ± 340 cm/s, respectively. Pulse wave velocity was significantly higher in patients with positive Hp infection and elevated CRP ($P = .0037$) and positive Hp infection and normal CRP ($P = .0033$) than that in patients with negative Hp infection and normal CRP. *Helicobacter pylori* infection has been reported to decrease serum HDL cholesterol concentration [33,34], which has anti-inflammatory effects, inhibits low-density lipoprotein oxidation, and reduces inflammatory cytokines and vascular leukocyte adhesion molecules. Serum HDL cholesterol was lower in patients with Hp infection than in those without in the present study, although it did not reach statistical significance. Limitations of our study include a cross-sectional design and a very small number of subjects, which can only be hypothesis generating. Ongoing treatments for diabetes, hypertension, and hyperlipidemia necessarily complicate analyses of patients with type 2 diabetes mellitus. *Helicobacter pylori* infection is common, and this study demonstrated a borderline association between Hp infection and arterial stiffness. However, type 2 diabetes mellitus is associated with a considerably increased risk for development of CVD [35,36]. Aggressive risk factor management is important for reducing cardiovascular morbidity and mortality in this patient group. Therefore, detection of additional risk factors, including Hp infection, for arterial stiffness might be useful in patients with type 2 diabetes mellitus. Large prospective trials and intervention studies are needed to better assess influences of Hp infection on the progression of atherosclerosis in patients with type 2 diabetes mellitus. In conclusion, Hp infection is associated with arterial stiffness determined by PWV in patients with type 2 diabetes mellitus.

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