

## Original Article

# Associations Between Cardio-Ankle Vascular Index and Microvascular Complications in Type 2 Diabetes Mellitus Patients

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**Aim:** Type 2 diabetes mellitus (T2DM) is a risk factor for increased arterial stiffness. We evaluated associations between the cardio-ankle vascular index (CAVI) and carotid plaque, intima media thickness (IMT), and diabetic microvascular complications in Korean T2DM patients.

**Methods:** We conducted a retrospective, cross-sectional study of 320 Korean T2DM patients without a history of macrovascular disease or macroalbuminuria. We measured 24-hour urinary albumin excretion (UAE) levels and performed funduscopic and neuropathy examinations to assess the extent of diabetic microvascular complications. Arterial stiffness was assessed using CAVI. We also measured the ankle-brachial index (ABI), common carotid artery IMT, and carotid beta stiffness index.

**Results:** Among the 320 patients enrolled in this study, 64 (20%) had increased CAVI ( $\geq 9$ ). We found that CAVI was correlated with systolic blood pressure, pulse pressure, IMT, carotid beta stiffness index, log-transformed UAE, and total cholesterol. In multiple logistic regression analysis, mean IMT and the presence of carotid plaque were independently associated with increased CAVI ( $\geq 9$ ) (OR=5.77,  $P<0.01$ ; OR=5.36,  $P<0.001$ , respectively). Furthermore, an increased CAVI was associated with peripheral neuropathy (OR=2.03,  $P=0.03$ ) and microalbuminuria (OR=2.47,  $P<0.01$ ) after adjusting for possible confounding variables.

**Conclusions:** The results of this study suggest that increased CAVI is associated with the presence of arterial plaque, increased IMT, and microvascular complications, such as nephropathy and neuropathy, in T2DM patients.

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**Key words;** Arterial stiffness, Cardio-ankle vascular index, Type 2 diabetes mellitus, Diabetic neuropathy, Microalbuminuria

## Introduction

Cardiovascular disease is the leading cause of death in diabetes mellitus patients<sup>1</sup>, but the pathophysiological mechanisms underlying this relationship

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are not completely understood. Since increased arterial stiffness commonly occurs in patients with diabetes, it may be an important factor linking diabetes to increased cardiovascular risk. Indeed, increased arterial stiffness predicts the development of cardiovascular disease and mortality in type 2 diabetes mellitus patients<sup>2</sup>. Furthermore, aortic stiffness itself has been demonstrated to predict cardiovascular morbidity and mortality beyond other risk factors such as dyslipidemia and hypertension<sup>3</sup>. Even so, other clinical factors associated with increased arterial stiffness in diabetic patients have not yet been completely explored,

especially diabetic microvascular complications. Because diabetic microvascular complications are powerful predictors of cardiovascular mortality<sup>4, 5</sup>, we hypothesized that increased arterial stiffness may be closely related with microvascular complications, even in type 2 diabetes mellitus (T2DM) patients without any macrovascular complications.

Arterial stiffness can be evaluated by measuring pulse wave velocity (PWV) on both sides of the arterial tree; however, little is known about the direct association between the presence of diabetes-related microvascular complications and vascular stiffness in T2DM patients. Although previous studies have reported that the ankle-brachial index (ABI)<sup>6</sup> and PWV<sup>7</sup> are related to chronic diabetic complications, there were some limitations. ABI does not completely reflect arterial stiffness and PWV is influenced by several factors, including blood pressure (BP), body weight, high fasting serum glucose levels, and autonomic nerve function; therefore, PWV does not solely reflect arterial stiffness in hypertensive diabetic patients.

Recently, CAVI, which reflects the stiffness of the aorta, femoral artery, and tibial artery, and involves the measurement of PWV and BP, has been investigated in several populations<sup>8-11</sup>. CAVI was developed by combining two indices: stiffness parameter  $\beta$  and Bramwell-Hill's formula<sup>12</sup>. Because CAVI uses stiffness parameter  $\beta$ , which is independent of BP, CAVI reflects the degree of arteriosclerosis and is superior to brachial-ankle PWV (baPWV) as an index of arterial stiffness<sup>9</sup>. Based on previous studies, we hypothesized that CAVI could become a useful diagnostic tool in predicting plaque formation and microvascular complications. Therefore, we investigated the association between the presence of carotid plaque, increased IMT, microvascular complications, and increased arterial stiffness in Korean T2DM patients using CAVI, a novel blood pressure-independent arterial wall stiffness parameter<sup>10</sup>.

## Methods

### Patients and Research Design

This study was a retrospective, cross-sectional study on 320 type 2 diabetic patients who were registered on the Severance Hospital Diabetes Complications Registry between January 2007 and August 2009. We recruited patients who met the following criteria: (1) between 30 and 70 years of age; (2) had undergone blood sampling and diabetic complication work-ups [carotid intima media thickness (IMT), funduscopy, CAVI, ABI], one of any neuropathy exami-

nations [nerve conduction velocity (NCV) or current perception threshold (CPT)], and 24-hour urine collection to test for albuminuria and proteinuria on the same day. Exclusion criteria included overt macroalbuminuria, defined as 24-hour urinary albumin excretion (UAE)  $\geq 300$  mg/day, documented concomitant atherosclerotic vascular disease, such as angina, myocardial infarction or cerebrovascular accidents, peripheral vascular disease, and an ABI less than 0.9.

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Hypertension was defined as two documented measurements  $\geq 140/90$  mmHg or patients already being treated with antihypertensive drugs. Dyslipidemia was defined as serum concentrations of total cholesterol of  $\geq 6.5$  mmol/L, triglyceride concentrations exceeding 2.3 mmol/L, or patients already being treated with lipid-lowering agents.

Diabetic nephropathy was defined as microalbuminuria (30-299 mg/day) based on UAE testing. Retinopathy was confirmed by an ophthalmologist based on funduscopy findings. Neuropathy was defined as a positive result of a neuropathy test (NCV, CPT, or autonomic function test (AFT)). This study was approved by the independent institutional review board of Severance Hospital, which waived the requirement of informed consent.

### Measurement of IMT and CAVI

All measurements were conducted over a 4-hour period in a quiet room kept at a constant temperature. Ultrasound examinations were performed by two sonographers who used an Aloka ProSound ALPHA 10 with a 13 MHz linear probe. We measured IMT, carotid beta stiffness index, pulse pressure, and the luminal diameter of the common carotid artery (CCA). All measurements were performed with the patient in a supine position, with their head elevated up to 45°, and tilted to either side at 30°, depending on the side being examined. We performed B mode and M mode examinations at 1.5 cm proximal to the carotid bifurcation, on the far wall of the CCA, on both sides. The carotid beta stiffness index for both carotid arteries was measured as previously described<sup>9</sup>. We measured the minimum dimension ( $D_d$ ) and maximum dimension ( $D_s$ ) of the carotid artery, and then calculated the systolic dimensional change ( $\Delta D = D_s - D_d$ ) on the M-mode echogram. The leading edge of near gain in the carotid artery is inside IMT. Stiffness parameter  $\beta$  was calculated as  $\ln(P_s/P_d) \times (D_d/\Delta D)$  and used to express the stiffness of the carotid arterial wall. Based on multiple images, plaque formation was identified where vessel wall thickness was greater than 1.1 mm

and where it appeared to be at least 50% greater than the thickness of the surrounding wall<sup>13</sup>.

We calculated CAVI using a conventional PWV measurement with the formula:  $a[q/DP[In Ps/Pd]ca-PWV^2]+b$ , where  $a$  and  $b$  are constants,  $q$ =blood density,  $DP$ =difference between systolic and diastolic pressure,  $Ps$ =systolic blood pressure,  $Pd$ =diastolic blood pressure, and  $ca-PWV$ =cardio-ankle pulse wave velocity. CAVI was measured with a VaSera CAVI instrument (Fukuda Denshi Co. Ltd, Tokyo) using the methods described previously. Electrocardiograph (ECG), phonocardiograph (PCG), and pressures and waveforms of the brachial and ankle arteries were measured, and  $ca-PWV$  and subsequently CAVI were calculated automatically<sup>10</sup>. The mean arterial pressure (MAP) was calculated as diastolic blood pressure (DBP) + 1/3 [systolic blood pressure (SBP) – DBP].

### Statistical Analyses

In this analysis, UAE measurements were log-transformed for correlation analysis as they showed a highly skewed distribution. Data are presented as the median and 25th and 75th percentile values. All categorical variables were summarized as frequencies and percentages. Differences between the two groups were analyzed by unpaired Student's *t*-test, chi-square test, or by Fisher's exact probability test. Simple (Spearman's rank) correlation coefficients between CAVI and various parameters were calculated. Correlations were reported unadjusted, and age and pulse pressures were adjusted.

To examine the differences in the frequency of diabetic microvascular complications between patients with and without increased arterial stiffness, multivariate logistic regression analysis was performed. Models were first adjusted for age and pulse pressure, and then for all possible confounding covariates. Finally, all microvascular complications were simultaneously used in one fully-adjusted model. An alpha level of 0.05 was accepted as significant for all statistical procedures. All analyses were performed using SAS software (SAS Institute, Cary, NC, USA).

## Results

### Baseline Characteristics and Bivariate Comparisons Between Patients With and Without Increased Arterial Stiffness

Among the 320 patients in the study, 64 (20%) had increased aortic stiffness (CAVI  $\geq 9$ ). As shown in **Table 1**, median age, gender, BMI, and past medical history were similar between patients with and without increased arterial stiffness. Furthermore, there

were no significant differences between the two groups with respect to administered medications such as insulin (34 vs. 32%), oral hypoglycemic agents (66 vs. 68%), and antiplatelet agents (60 vs. 65%). Patients with increased CAVI were more frequently smokers (7.6% vs. 6.4%,  $P<0.05$ ), and had a longer duration of diabetes (3 years vs. 7 years,  $P<0.001$ ). Diastolic BP was not significantly different between the two groups; however, resting systolic BP was higher (130.0 mmHg vs. 138.0 mmHg,  $P<0.001$ ) and pulse pressure was greater (48.0 mmHg vs. 56.0 mmHg,  $P<0.001$ ) in the group with increased arterial stiffness. Both mean and maximum IMT, and the carotid beta stiffness index were significantly higher in patients with increased CAVI. Similarly, the prevalence of carotid plaque was significantly higher in patients with increased CAVI. No diabetes-related variables were associated with increased arterial stiffness in bivariate comparisons, resulting in comparable homeostasis model assessment indexes (HOMA-IR). With regard to lipid metabolism, levels of serum triglycerides, low density lipoprotein (LDL), and high density lipoprotein (HDL) cholesterol showed no significant differences between the groups, whereas serum total cholesterol levels were slightly higher in the group with increased CAVI ( $P=0.049$ ). Patients with increased CAVI had a higher prevalence of diabetes-related microvascular complications, such as neuropathy and microalbuminuria, than patients with normal CAVI; however, no difference was noted in the prevalence of retinopathy.

### Independent Correlates of Arterial Stiffness

**Table 2** presents the unadjusted and age- and pulse pressure-adjusted correlates of continuous CAVI. In univariate linear regression analyses, the correlates of CAVI were age ( $P<0.01$ ), SBP ( $P<0.01$ ), pulse pressure ( $P<0.001$ ), mean and maximum IMT ( $P<0.001$ ), carotid beta stiffness index ( $P<0.001$ ), and UAE ( $P<0.01$ ). No diabetes-related variables were independently associated with CAVI. Serum total cholesterol ( $P<0.01$ ) and HDL cholesterol ( $P<0.05$ ) were independently associated with aortic stiffness; however, LDL cholesterol, duration of diabetes, dyslipidemia, and the number of antihypertensive drugs in use were not associated with CAVI. After adjusting for age and pulse pressure, CAVI was associated only with mean IMT ( $P<0.001$ ), maximum IMT ( $P<0.001$ ), carotid beta stiffness index ( $P<0.05$ ), and total cholesterol ( $P<0.05$ ).

**Table 1.** Clinical Characteristics of the Study Participants

| Variables                            | Patients with CAVI < 9<br>(n = 256) | Patients with CAVI ≥ 9<br>(n = 64) | P-value |
|--------------------------------------|-------------------------------------|------------------------------------|---------|
| Age (years)                          | 58.0 (49.0-66.0)                    | 61.0 (51.8-70.2)                   | NS      |
| Male gender (%)                      | 54                                  | 52                                 | NS      |
| Body mass index (kg/m <sup>2</sup> ) | 24.9 (22.0-27.0)                    | 25.0 (23.0-27.0)                   | NS      |
| Smoking status (%)                   |                                     |                                    |         |
| Current/Past/Never                   | 6.4/31.4/62.2                       | 7.6/54.8/37.6                      | < 0.05  |
| Diabetes duration (years)            | 3.0 (0.3-6.0)                       | 7.0 (0.9-10.0)                     | < 0.001 |
| Drug and Medical History (%)         |                                     |                                    |         |
| Oral hypoglycemic agent              | 66                                  | 68                                 | NS      |
| Insulin                              | 34                                  | 32                                 | NS      |
| Antiplatelet use                     | 60                                  | 65                                 | NS      |
| Hypertension                         | 60                                  | 59                                 | NS      |
| Dyslipidemia                         | 67                                  | 69                                 | NS      |
| Blood pressure (mmHg)                |                                     |                                    |         |
| Systolic blood pressure              | 130.0 (118.0-141.0)                 | 138.0 (126.0-151.5)                | < 0.001 |
| Diastolic blood pressure             | 82.0 (76.0-89.0)                    | 84 (77.0-89.5)                     | NS      |
| Pulse pressure                       | 48.0 (40.0-54.0)                    | 56.0 (47.0-65.5)                   | < 0.001 |
| ABI                                  | 1.09 (1.02-1.15)                    | 1.08 (1.02-1.13)                   | NS      |
| IMT mean (mm)                        | 0.70 (0.61-0.82)                    | 0.77 (0.70-0.87)                   | < 0.001 |
| IMT max (mm)                         | 0.82 (0.72-0.97)                    | 0.94 (0.82-1.07)                   | < 0.001 |
| Carotid beta stiffness index         | 5.7 (2.6-9.2)                       | 6.9 (3.8-14.7)                     | < 0.001 |
| Carotid plaque (%)                   |                                     |                                    |         |
| Lt/Rt/Total                          | 48/52/67                            | 79/79/89                           | < 0.001 |
| Laboratory variables                 |                                     |                                    |         |
| Fasting plasma glucose (mmol/L)      | 7.3 (5.9-9.3)                       | 7.4 (5.9-9.2)                      | NS      |
| Glycated hemoglobin (%)              | 7.4 (6.7-8.9)                       | 7.4 (6.4-8.7)                      | NS      |
| HOMA-IR                              | 2.9 (1.8-5.3)                       | 3.5 (1.6-7.1)                      | NS      |
| Serum cholesterol (mmol/L)           | 4.2 (3.6-4.8)                       | 4.6 (3.9-5.3)                      | < 0.05  |
| HDL-cholesterol (mmol/L)             | 1.1 (1.0-1.4)                       | 1.1 (0.9-1.3)                      | NS      |
| LDL-cholesterol (mmol/L)             | 2.3 (2.0-3.1)                       | 2.5 (2.1-3.2)                      | NS      |
| Serum triglycerides (mmol/L)         | 1.5 (1.0-2.2)                       | 1.5 (0.9-2.3)                      | NS      |
| C-reactive protein (ug/mL)           | 0.89 (0.48-1.83)                    | 1.13 (0.47-2.87)                   | NS      |
| 24-hour UAE (mg/24h)                 | 13.3 (6.6-38.0)                     | 24.4 (8.1-46.1)                    | < 0.05  |
| Serum creatinine (mg/dL)             | 0.88 (0.752-1.01)                   | 0.98 (0.80-1.15)                   | < 0.05  |
| Microvascular complications (%)      |                                     |                                    |         |
| Retinopathy                          | 73                                  | 83                                 | < 0.01  |
| Neuropathy                           | 31                                  | 35                                 | NS      |
| Albuminuria                          | 36                                  | 47                                 | < 0.05  |
| Albuminuria                          | 33                                  | 52                                 | < 0.01  |

ABI, ankle brachial index; CAVI, carotid ankle vascular index; GFR, glomerular filtration rate; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; IMT, intima media thickness; LDL, low density lipoprotein; Lt, left; Rt, right; NS, not significant; UAE, urine albumin excretion. Range of values shown in parentheses

### Independent Associations Between Diabetic Microvascular Complications and Increased Arterial Stiffness

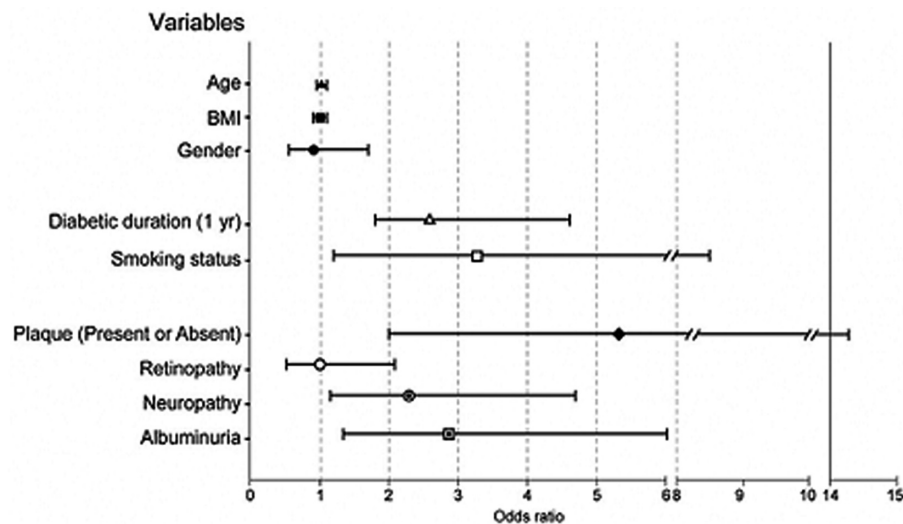
**Fig. 1** shows the results of logistic regression analysis for variables independently associated with increased CAVI. In multivariate logistic regression analyses, the variables independently associated with an

increased CAVI ( $\geq 9$ ) were pulse pressure (OR=1.04, 95% CI; 1.01-1.06,  $P<0.01$ , for increments of 10 mmHg), duration of diabetes (OR=2.62, 95% CI; 1.90-3.63,  $P<0.01$ ), presence of carotid plaque (OR = 5.36, 95% CI; 2.01-14.34,  $P<0.01$ ), and current smoking status (OR=3.26, 95% CI; 1.24-8.56,  $P<0.01$ ). Again, no diabetes-related variables were corre-

**Table 2.** Correlation with CAVI and Other Clinical Parameters

|                              | CAVI (unadjusted)<br>(n=320) |         | CAVI (adjusted for age and pulse pressure)<br>(n=320) |         |
|------------------------------|------------------------------|---------|---|---------|
|                              | Coefficient                  | P-value | Coefficient   | P-value |
| Age (years)                  | 0.162                        | <0.01   | -   | -       |
| BMI (kg/m <sup>2</sup> )     | 0.027                        | NS      | 0.007   | NS      |
| SBP (mmHg)                   | 0.154                        | <0.01   | 0.013   | NS      |
| DBP (mmHg)                   | 0.035                        | NS      | 0.055   | NS      |
| MAP (mmHg)                   | 0.098                        | NS      | 0.106   | NS      |
| Pulse pressure (mmHg)        | 0.229                        | <0.001  | -   | -       |
| IMT mean (mm)                | 0.378                        | <0.001  | 0.288   | <0.001  |
| IMT max (mm)                 | 0.36                         | <0.001  | 0.268   | <0.001  |
| ABI                          | 0.015                        | NS      | 0.012   | NS      |
| Carotid beta stiffness index | 0.421                        | <0.001  | 0.264   | <0.05   |
| Glucose (mg/dL)              | 0.047                        | NS      | -0.045  | NS      |
| Glycated hemoglobin (%)      | 0.057                        | NS      | -0.093  | NS      |
| Total cholesterol (mmol/L)   | 0.172                        | <0.01   | 0.101   | <0.05   |
| HDL (mmol/L)                 | -0.143                       | <0.05   | -0.08   | NS      |
| CRP ( $\mu$ g/mL)            | 0.099                        | NS      | 0.084   | NS      |
| 24-hour UAE (mg/24h)         | 0.156                        | <0.01   | 0.049   | NS      |

ABI, ankle brachial index; BMI, body mass index; CAVI, carotid ankle vascular index; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; IMT, intima media thickness; LDL, low density lipoprotein; MAP, mean arterial pressure; SBP, systolic blood pressure; TG, triglyceride; NS, not significant; UAE, urine albumin excretion.



**Fig. 1.** Multiple Logistic Regression for Variables Independently Associated with Increased Arterial Stiffness [cardio-ankle vascular index (CAVI)  $\geq$  9] (dependent variable).

lated with increased CAVI. Patients with diabetic retinopathy had almost no chance of having increased arterial stiffness, whereas those with peripheral neuropathy had a 2-fold greater likelihood of having increased CAVI. Patients with microalbuminuria had a nearly

3-fold greater chance of having increased CAVI.

**Table 3** shows the results of multivariate logistic regression analysis for independent associations between microvascular complications and increased CAVI. Only microalbuminuria and neuropathy were

**Table 3.** Multivariate Logistic Regression Analysis for the Associations Between Diabetic Microvascular Complications and Increased Carotid Ankle Vascular Index (the dependent variable)

| Microvascular Complications      | Model 1 <sup>†</sup> | Model 2 <sup>§</sup> | Model 3 <sup>‡</sup> |
|----------------------------------|----------------------|----------------------|----------------------|
| Retinopathy (present vs. absent) | 1.00 (0.49-2.03)     | 0.81 (0.37-1.78)     | 0.62 (0.26-1.47)     |
| Neuropathy (present vs. absent)  | 1.80 (1.03-2.98)*    | 2.13 (1.14-4.83)*    | 2.03 (1.04-3.97)*    |
| Albuminuria (present vs. absent) | 1.93 (1.04-3.55)*    | 2.08 (1.05-4.10)*    | 2.47 (1.29-4.07)**   |

<sup>†</sup>Model 1 adjusted for age and pulse pressure. <sup>§</sup>Model 2 adjusted for age, gender, pulse pressure, body mass index, diabetes duration, smoking status, IMT mean, plaque status. <sup>‡</sup>Model 3 same as Model 2 with the three microvascular complications entered simultaneously into the model. Values are odds ratios (95% confidence intervals) with range of values shown in parentheses. \* $p < 0.05$ . \*\* $p < 0.01$ .

significantly associated with increased CAVI, following adjustment for age and pulse pressure (Model 1), after full statistical adjustment for potential confounding covariates (Model 2), and in fully adjusted analysis where all the four microvascular complications were entered in the same run (Model 3).

## Discussion

The present study demonstrates a significant association between CAVI and diabetes-related microvascular complications in T2DM patients without any macrovascular complications. This study has three main findings: first, it verifies that CAVI is significantly related to IMT and the presence of carotid plaques in T2DM patients; second, it demonstrates that the presence of diabetic neuropathy and nephropathy (microalbuminuria) are associated with increased arterial stiffness, independent of other established determinants of arterial stiffness such as aging, BP, and other cardiovascular risk factors; and third, it confirms that other predictors of increased arterial stiffness in T2DM patients are smoking, longer duration of diabetes, and greater pulse pressure.

Recently, Cardoso *et al.* reported that increased central arterial stiffness measured by PWV was associated with microvascular complications<sup>7</sup>. With respect to this result, our findings partially coincide with this study; however, the method of measuring arterial stiffness and patient baseline characteristics differed as we used CAVI, a novel and blood pressure-independent diagnostic tool, instead of PWV. PWV may be overestimated in patients with hypertension or autonomic dysfunction<sup>9, 10, 14</sup>. PWV can especially deviate from the actual value in diabetic patients with renal insufficiency or macrovascular complication due to various mechanisms, including activation of the renin-angiotensin system, sodium retention, and abnormal sympathetic nervous system. Therefore, CAVI, which is less affected by such mechanisms, is thought to be needed for accurate assessment of arterial stiffness in

type 2 DM patients. We excluded all patients who had any history or evidence of macroalbuminuria and diabetic macrovascular complications. Furthermore, the median duration of diabetes in our patients with increased arterial stiffness was about 7 years. The patients in Cardoso's study had a mean duration of diabetes of 13 years, and since the duration of diabetes is closely related to diabetic microvascular complications, this could be the major cause of the discrepancy.

It has been reported that CAVI is related to IMT, the carotid beta stiffness index, and carotid plaques<sup>8, 9, 15</sup>; however, few studies have been performed on T2DM patients. Takaki *et al.* reported that CAVI was significantly related to the carotid beta stiffness index in patients with chest pain syndrome. In that study, however, only 20% of the 130 patients enrolled had diabetes<sup>9</sup>. Izuhara *et al.* reported that the severity of carotid arteriosclerosis and the aortic stiffness parameters were significantly correlated with arteriosclerotic risk factors such as age, sex, and DM<sup>15</sup>. Our study reveals that CAVI is associated with IMT, the carotid beta stiffness index, and carotid plaque in T2DM patients. Considering that diabetes is a significant risk factor for increased arterial stiffness, CAVI could serve as a clinical marker for the progression of carotid arteriosclerosis, even in T2DM patients.

This study demonstrates that diabetic nephropathy and neuropathy are associated with increased CAVI. Few previous studies<sup>7, 16-20</sup> have investigated the relationships between diabetic microvascular complications and arterial stiffness, as assessed by the carotid-femoral PWV (cfPWV). One study reported that the presence of microalbuminuria is correlated to arterial stiffness only if BP variables are excluded from multivariate analysis. This finding suggests that the association is mediated by increased BP levels<sup>21</sup>. Meanwhile, another study found independent associations between arterial stiffness and BP variables<sup>19, 22</sup>. This dissonance may have been caused by the small number of patients with increased arterial stiffness ( $n=64$ ) and biased patient selection. A comparative study to

evaluate the correlation between microalbuminuria and arterial stiffness using both baPWV and CAVI showed a significant correlation between CAVI and the albumin-to-creatinine ratio (ACR); however, they could not find a relationship between baPWV and ACR<sup>23</sup>). This result supported the idea that different methods may cause discrepancies in microalbuminuria. Diabetic neuropathy, a known risk factor for sudden cardiac death<sup>24</sup>), also correlated with increased CAVI in our study. Because we did not perform a cardiac autonomic function test, we cannot conclude that increased CAVI is associated with cardiac autonomic neuropathy; however, the presence of peripheral neuropathy, which is diagnosed using CPT and NCV in T2DM patients, does appear to be closely related to CAVI.

Regarding retinopathy, Cardoso *et al.* reported that diabetic retinopathy was the most strongly related microvascular complication<sup>7</sup>). Aso *et al.* also demonstrated that baPWV is closely related to diabetic retinopathy<sup>20</sup>); however, our study produced conflicting results. As mentioned earlier, different patient baseline characteristics and methods to measure arterial stiffness may have caused this discrepancy. The differences in the number of patients, ethnic diversity, diabetic duration, macrovascular complications, diagnostic tool (CAVI vs. PWV), and methods used to diagnose retinopathy all may have contributed to the discrepancy. Another possible explanation involves the possibility of an additional mechanism other than the increase in arterial stiffness in patients with a short duration of diabetes and no macrovascular complications, which may affect diabetic retinopathy. This issue requires further investigation.

Much evidence supports the concept of increased arterial stiffness in T2DM patients<sup>25-27</sup>). These findings support that macrovascular disease associated with T2DM begins in an early stage, even in the prediabetic state<sup>28, 29</sup>). This means that even a patient who has had T2DM for a short duration may have risk factors that may cause increased arterial stiffness. Microalbuminuria and peripheral neuropathy in patients with T2DM are thought to indicate generalized endothelial damage<sup>30</sup>). There are many reports about the mechanism of the association between arterial stiffness and albuminuria<sup>21, 31</sup>). Among them, endothelial dysfunction may be one of the important mechanisms as it aggravates renal disease, and the progression of renal dysfunction may worsen atherosclerosis; therefore, aggravated renal dysfunction could, in turn, trigger vascular injury and increase arterial stiffness<sup>32-34</sup>).

Research on diabetic neuropathy has also focused on the pathogenic link between insulin resistance syn-

drome and endothelial function<sup>35</sup>). Endothelial dysfunction impairs microvascular blood flow and causes endoneurial hypoxia, which is considered to play a major role in causing diabetic neuropathy in human and animal models<sup>36</sup>). This would also increase the inflammatory fibro-proliferative response, which damages the endothelium and smooth muscle of the arterial wall. Since the above phenomena reflect the same pathophysiology as diabetes, an increase in CAVI due to endothelial dysfunction may be associated with both diabetic nephropathy and peripheral neuropathy.

This study has some methodological issues which need to be addressed. We did not directly measure insulin resistance. The homeostasis model assessment used is less appropriate in diabetic patients who are taking anti-diabetic medications, particularly insulin, so insulin resistance could be one confounding factor in the relationship between microvascular complications and increased CAVI. Also, this study was performed in a tertiary-care hospital and the patients were predominantly middle-aged to elderly individuals who were referred by their primary care physician.

In summary, we demonstrated independent associations between aortic stiffness measured by CAVI, a novel method to assess arterial stiffness without the potential influence of BP, and the presence of diabetic microvascular complications in T2DM patients without a history of macrovascular complications and macroalbuminuria. In the clinical setting, it is difficult to investigate diabetic complications in all patients. It is especially difficult to screen for diabetic complications in patients with a short duration of diabetes with no other accompanying disease. In this case, evaluating arterial stiffness using CAVI, which is unaffected by blood pressure, may be a useful index that predicts the occurrence of microvascular complications.

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## Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the reported research.

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**Appendix 1.** Multiple Logistic Regression for the Variables Independently Associated with Increased Arterial Stiffness (CAVI  $\geq$  9) (dependent variable)

|                            | Odds Ratio | 95% CI     | P-value |
|----------------------------|------------|------------|---------|
| Age (10 years)             | 1.01       | 0.98-1.03  | NS      |
| BMI                        | 1.01       | 0.92-1.11  | NS      |
| Gender                     | 0.89       | 0.47-1.71  | NS      |
| Pulse pressure (10 mmHg)   | 1.04       | 1.01-1.06  | <0.01   |
| IMT mean                   | 5.77       | 1.15-29.05 | <0.01   |
| Plaque (present or absent) | 5.36       | 2.01-14.34 | <0.01   |
| Diabetic duration (1 year) | 2.62       | 1.90-3.63  | <0.01   |
| Smoking status             | 3.26       | 1.24-8.56  | <0.01   |
| Retinopathy                | 0.99       | 0.47-2.09  | NS      |
| Neuropathy                 | 2.31       | 1.14-4.69  | <0.01   |
| Albuminuria                | 2.88       | 1.36-6.09  | <0.01   |

BMI, body mass index; CAVI, carotid ankle vascular index; IMT, intima media thickness; ns, not significant.