Original Article

Arterial Wall Elasticity Measured Using the Phased Tracking Method and Atherosclerotic Risk Factors in Patients with Type 2 Diabetes

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***Aim*: The aim of this study was to investigate the relationship between atherosclerotic manifestations and brachial and radial arterial wall elasticity (AWE) measured using the phased tracking method in patients with type 2 diabetes mellitus (T2DM).**
***Methods*: This study included T2DM patients (n=220, mean age 59 years) without a history of stroke or coronary artery disease. The brachial AWE, radial AWE, carotid mean intima-media thickness (IMT), max-IMT and flow-mediated vasodilation (FMD) were measured. The patients were classified according to the number of atherosclerotic risk factors, including obesity, dyslipidemia and hypertension. Group 1 included T2DM patients only, group 2 included patients with two risk factors, group 3 included patients with three risk factors and group 4 included patients with four risk factors. The patients were also divided into two groups according to microangiopathic complications, including retinopathy and nephropathy. The between-group differences were analyzed.**
***Results*: The brachial AWE (548, 697, 755 and 771 kPa for groups 1, 2, 3 and 4, respectively) and radial AWE (532, 637, 717 and 782 kPa for groups 1, 2, 3 and 4, respectively) significantly increased in association with an increasing number of risk factors. The brachial AWE and radial AWE were significantly higher in the patients with microangiopathic complications than in those without microangiopathic complications (brachial AWE 797 and 694 kPa and radial AWE 780 and 660 kPa, respectively). Receiver operating characteristic curve analyses revealed that, for brachial AWE and radial AWE, the area under the curve was equal to the max-IMT and higher than the mean-IMT and FMD.**
***Conclusions*: Upper limb AWE measurement can reflect the degree of atherosclerosis risk overload and may be useful for evaluating vascular complications in T2DM patients.**

J Atheroscler Thromb, 2013; 20:678-687.

Key words: Ultrasonography, Carotid intima-media thickness, Flow-mediated vasodilation

Introduction

Type 2 diabetes mellitus (T2DM) is associated with atherosclerosis, which leads to various vascular complications, including macroangiopathies, such as coronary artery disease and strokes, and microangiopathies, such as retinopathy and nephropathy[1-5]. The addition of obesity, dyslipidemia and hypertension to T2DM accelerates the progression of atherosclerosis and increases the risk of cardiovascular disease[3, 6-8]. Managing these atherosclerotic risk factors can prevent atherosclerotic progression[9]; therefore, it is important to evaluate a patient's atherosclerotic risk overload prior to the occurrence of cardiovascular events[10, 11]. Several atherosclerotic markers, such as the carotid intima-media thickness (IMT) and flow-mediated

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Received: October 18, 2012
Accepted for publication: March 10, 2013

vasodilation (FMD), are useful for assessing atherosclerotic risks, although they do not always predict cardiovascular risks among T2DM patients[12, 13].

Recently, a noninvasive, transcutaneous, ultrasonic technique using the phased tracking method was developed to evaluate arterial wall elasticity (AWE). This method is believed to be able to detect atherosclerotic changes in association with regional tissue composition[14-16]. During a single heartbeat, the displacement of each point of the arterial wall from the luminal surface to the adventitia is tracked along an ultrasonic beam, and minute changes in the thickness of different layers can be detected, allowing the elastic modulus to provide information on regional tissue composition[14-16]. The AWE is expressed as the mean elastic modulus of the entire arterial wall of a regional artery. Because it is an easy-to-use and noninvasive method, measuring the AWE using the phased tracking method is suitable as an atherosclerotic screening test.

The phased tracking method has been used to demonstrate that soft tissue, such as soft plaques with a lipid core within the arteries have a low AWE, while a high AWE is seen in stiff tissue, such as lesions where calcification and proliferation of vascular smooth muscle and collagen exist[14]. The carotid AWE has been reported to be significantly associated with atherosclerotic markers, such as the carotid max-IMT and pulse wave velocity, in T2DM patients[15], whereas the radial AWE is significantly associated with the serum C-reactive protein level, a chronic inflammatory index, in healthy subjects[16, 17]. Therefore, measuring the AWE may be useful for evaluating the degree of atherosclerotic overload. However, currently, only a few studies have evaluated the AWE measured according to the phased tracking method in different arteries or different populations[15, 16]; therefore, further clinical data are required to establish the clinical utility of AWE measurement using this method. For this purpose, we investigated the relationship between the degree of atherosclerotic risk overload and the AWE in addition to the differences in AWE between patients with and without microangiopathic complications in order to determine whether upper limb AWE measurement can be used as new approach for evaluating atherosclerosis.

Aim

The brachial AWE, radial AWE and other ultrasonic atherosclerosis-related markers, including the carotid IMT and FMD, and conventional atherosclerotic risk factors were measured in T2DM patients without a history of stroke or coronary artery disease. This study investigated whether brachial and radial AWE values are correlated with the number of atherosclerotic risk factors in T2DM patients. Furthermore, we investigated the brachial and radial AWE in patients with microangiopathic complications because patients with diabetic microangiopathic complications, particularly those in the advanced stage, constitute a special population with a high risk for cardiovascular events[18-20].

Methods

Subjects

A total of 220 patients with T2DM (men, 57%; mean ± standard deviation [SD] age, 59 ± 11 years) were sequentially included in this cross-sectional hospital-based study. The Ethics Committee at Jichi Medical University approved the study, and each patient gave their informed consent. Patients with a history of stroke or coronary artery disease were excluded. All of the study patients were stable and receiving antidiabetic treatments, such as diet therapy, oral medications and insulin. T2DM was diagnosed based on the World Health Organization (WHO) and American Diabetes Association (ADA) criteria: a fasting plasma glucose level of ≥ 126 mg/dL and/or a 2-hour plasma glucose level after a 75-g oral glucose tolerance test of ≥ 200 mg/dL[21, 22].

The diagnosis of retinopathy was determined by a trained ophthalmologist using indirect ophthalmoscopic examinations based on the presence of clinical features in the fundus in both eyes[23]. Advanced-stage retinopathy was defined as proliferative retinopathy, severe nonproliferative retinopathy, post vitreous surgery retinopathy or post panretinal photocoagulation[23]. The renal function was assessed using the estimated glomerular filtration rate (eGFR), which was calculated according to the glomerular filtration rate equation for Japanese subjects[24]. Patients with an eGFR of ≤ 30 mL/min/1.73 m^2 and/or macroalbuminuria (a urinary albumin-to-creatinine ratio [ACR] of ≥ 300 mg/g creatinine [Cr]) were classified as having advanced stage disease[25-27]. Nephropathy was defined as an eGFR of ≤ 60 mL/min/1.73 m^2 and/or microalbuminuria (ACR ≥ 30 mg/g Cr)[26, 27].

The patients were divided into four groups based on the number of atherosclerotic risk factors, including obesity, dyslipidemia and hypertension[3, 6]. Group 1 included patients with T2DM only, group 2 included patients with two risk factors, group 3 included patients with three risk factors and group 4 included patients with all four risk factors. The patients were further classified into two groups: those with and

without microangiopathic complications, including advanced-stage retinopathy or nephropathy.

Physical Examinations

Smoking habits were determined in interviews conducted by doctors, and smokers were defined as current smokers. The body mass index (BMI) was calculated as the weight divided by the square of the body height while wearing light clothes. Obesity was defined as a BMI of ≥ 25.0 kg/m^2 based on the Japan Society for the Study of Obesity criteria[28]. Blood pressure was measured twice in the supine position with a minimum of five minutes between measurements, and the mean value was recorded. Hypertension was defined as a systolic blood pressure of ≥ 140 mmHg and/or a diastolic blood pressure of ≥ 90 mmHg and/or the current use of antihypertensive agents[29].

Laboratory Measurements

Blood samples were drawn in the morning after a 12-hour fast. The level of hemoglobin A1c (HbA1c) was measured using high-performance liquid chromatography. The present study used the value of HbA1c (%) estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) (HbA1c [Japan Diabetes Society: JDS] + 0.4%)[30]. The levels of blood glucose, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides and Cr were determined enzymatically. Dyslipidemia was defined as an LDL-C level of ≥ 140 mg/dL, an HDL-C level of < 40 mg/dL, a triglyceride level of ≥ 150 mg/dL and/or the current use of statins or fibrates[31]. The ACR in spot urine was also assessed, and values of albuminuria surpassing the sensitivity of the ACR measurement were recorded as 300 mg/g Cr.

Using B-mode ultrasound imaging with a 5- to 10-MHz linear transducer (ALOKA, SSD-α10, Tokyo, Japan), the mean-IMT and max-IMT were evaluated bilaterally at the common carotid artery, bifurcation and internal carotid arteries[32, 33]. The mean-IMT was measured in the bilateral common carotid arteries (excluding the bulbus), and the levels at two or more measurement points were averaged[32, 33]. The max-IMT was measured at the greatest IMT in the bilateral common carotid arteries, the bulbus and the internal carotid arteries[32].

For the FMD measurements, the patients fasted and did not use tobacco for at least eight hours before measurement[34, 35]. The FMD was assessed in the patient's right brachial artery above the antecubital fossa in the supine position in a quiet, temperature-controlled room. The diameter of the brachial artery was measured continuously using B-mode ultrasound. A cuff was placed on the forearm and, after measuring the baseline diameter, arterial occlusion was induced by cuff inflation to a pressure of 200 mmHg or 50 mmHg above the systolic blood pressure for five minutes. When the cuff was released, the FMD was calculated as the maximum percent increase in the diameter during hyperemia compared with the baseline diameter.

Measurement of Arterial Wall Elasticity Using the Phased Tracking Method

The brachial AWE and radial AWE were evaluated using the phased tracking method[14-16]. The brachial and radial arteries were assessed above the antecubital fossa and approximately 5 cm proximal to the wrist with the palm turned upward, respectively, in the supine position in a quiet, temperature-controlled room. The arteries were scanned in the longitudinal plane using B-mode ultrasound imaging with an 8- to 16-MHz linear array transducer (DIASUS, Dynamic Imaging Ltd., Livingston, UK). Ultrasound was used to record changes in the thickness of the artery during a single heartbeat. The arterial wall was divided into layered blocks with a depth of 312 μm and a width of 200 μm for the entire wall, and the elastic modulus of each layer (E_θ [Pascal: Pa]) was calculated using the equation $E_\theta = (1/2) \times (r_0/h_0 + 1) \times (\Delta P_{max}/\varepsilon_r)$, where $\varepsilon_r = \Delta h_{max}/h_0$, Δh_{max} is the maximum decrease in the thickness of the 312-μm layer during one heartbeat, ΔP_{max} is the pulse pressure and h_0 and r_0 are the initial thicknesses of the layer and radius of the vessel at end-diastole, respectively. The elastic modulus was determined for each 312-μm layer, and the AWE was expressed as the mean level of all layers. The AWE was determined in the bilateral brachial and radial arteries, and the bilateral averages of each artery were used for all analyses. All examinations were performed by the same trained physician. The intraobserver coefficients of variation for the brachial AWE and radial AWE values were 9.3% and 10.9%, respectively.

Statistical Analyses

The data are presented as the mean ± SD, median (interquartile range) or number (%). The between-group differences of each parameter were compared using a one-way analysis of variance (ANOVA) with a multiple comparison test or the chi-square test. ANOVA adjusted for confounding factors, such as age, sex, the smoking status, the Cr level and ACR, was performed to determine the between-group differences in atherosclerotic parameters, such as the brachial AWE, radial AWE, mean-IMT, max-IMT and FMD. The between-group differences in atherosclero-

Table 1. Patient profiles for each group based on the number of atherosclerotic risk factors

Parameter	All (n=220)	Group 1 (n=22)	Group 2 (n=65)	Group 3 (n=76)	Group 4 (n=57)	p-value
Male (%)	126 (57%)	13 (59%)	40 (62%)	40 (53%)	33 (58%)	0.76
Age (years)	59 ± 11	57 ± 14	61 ± 9	60 ± 11	58 ± 10	0.32
Body mass index (kg/m^2)	25 ± 6	20 ± 2	23 ± 3*	26 ± 6$^{*, \S}$	30 ± 4$^{*, \S, \dagger}$	<0.01
Smoking (%)	47 (21%)	6 (27%)	14 (22%)	18 (24%)	9 (16%)	0.62
SBP (mmHg)	128 ± 15	117 ± 13	125 ± 15	129 ± 15*	134 ± 14$^{*, \S}$	<0.01
DBP (mmHg)	77 ± 9	72 ± 8	76 ± 9	77 ± 9	80 ± 9$^{*, \S}$	<0.01
Anti-HT agents (%)	112 (51%)	0 (0%)	15 (23%)	45 (59%)$^{*, \S}$	52 (91%)$^{*, \S, \dagger}$	<0.01
RAS inhibitors (%)	104 (47%)	0 (0%)	13 (20%)	42 (55%)$^{*, \S}$	49 (86%)$^{*, \S, \dagger}$	<0.01
CCB (%)	69 (31%)	0 (0%)	8 (12%)	27 (36%)$^{*, \S}$	34 (60%)$^{*, \S, \dagger}$	<0.01
β blockers (%)	11 (5%)	0 (0%)	1 (2%)	7 (9%)	3 (5%)	0.13
Diuretics (%)	28 (13%)	0 (0%)	4 (6%)	11 (14%)$^{*, \S}$	13 (23%)$^{*, \S, \dagger}$	0.01
Glucose (mg/dL)	135 ± 42	127 ± 54	134 ± 43	136 ± 42	139 ± 38	0.67
Hemoglobin A1c (%)	7.9 ± 2.0	7.9 ± 2.3	7.9 ± 2.3	8.0 ± 2.0	7.7 ± 1.6	0.87
Antidiabetic agents (%)	158 (72%)	13 (59%)	41 (63%)	57 (75%)	47 (82%)	0.05
OHA (%)	109 (50%)	7 (32%)	29 (45%)	43 (57%)	30 (53%)	0.16
Insulin treatment (%)	49 (22%)	6 (27%)	12 (18%)	14 (18%)	17 (30%)	0.34
Retinopathy (%)	80 (36%)	2 (9%)	20 (31%)	30 (39%)*	28 (49%)$^{*, \S}$	0.01
Non-advanced stage (%)	42 (19%)	1 (5%)	12 (18%)	13 (17%)	16 (21%)	0.10
Advanced stage (%)	38 (17%)	1 (5%)	8 (12%)	17 (22%)	12 (21%)	0.14
LDL-cholesterol (mg/dL)	109 ± 32	106 ± 22	109 ± 31	114 ± 33	104 ± 33	0.37
HDL-cholesterol (mg/dL)	57 ± 19	78 ± 25	57 ± 18*	56 ± 16*	52 ± 18*	<0.01
Triglycerides (mg/dL)	108 (81-160)	58 (50-84)	104 (65-126)*	110 (83-171)*	153 (100-194)$^{*, \S}$	<0.01
Anti-HL agents (%)	87 (40%)	0 (0%)	17 (26%)	34 (45%)$^{*, \S}$	36 (63%)$^{*, \S, \dagger}$	<0.01
Statins (%)	79 (36%)	0 (0%)	15 (23%)	33 (43%)$^{*, \S}$	31 (54%)$^{*, \S}$	<0.01
Fibrates (%)	8 (4%)	0 (0%)	2 (3%)	1 (1%)	5 (9%)	0.10
Cr (mg/dL)	0.68 (0.59-0.80)	0.66 (0.53-0.80)	0.70 (0.59-0.79)	0.69 (0.59-0.84)	0.66 (0.57-0.79)	0.71
eGFR (mL/min/1.73 m^2)	83 ± 24	88 ± 25	84 ± 23	80 ± 26	84 ± 21	0.45
ACR (mg/g Cr)	16 (8-86)	10 (7-23)	17 (10-87)	17 (9-84)	21 (9-143)	0.08
Nephropathy (%)	100 (45%)	5 (23%)	27 (42%)	39 (51%)	29 (51%)	0.08
Non-advanced stage (%)	70 (32%)	4 (18%)	17 (26%)	29 (38%)	20 (35%)	0.21
Advanced stage (%)	30 (14%)	2 (9%)	10 (15%)	10 (13%)	9 (16%)	0.58
Mean-IMT (mm)	0.72 ± 0.12	0.70 ± 0.13	0.70 ± 0.09	0.74 ± 0.14	0.74 ± 0.11	0.15
Max-IMT (mm)	1.60 ± 0.84	1.31 ± 0.74	1.53 ± 0.70	1.68 ± 0.92	1.70 ± 0.89	0.21
FMD (%)	2.4 ± 1.9	3.4 ± 2.9	2.4 ± 1.5	2.4 ± 1.9	2.1 ± 1.6	0.05
Brachial AWE (kPa)	721 ± 217	548 ± 141	697 ± 200*	755 ± 222*	771 ± 221*	<0.01
Radial AWE (kPa)	692 ± 222	532 ± 149	637 ± 207	717 ± 206*	782 ± 238$^{*, \S}$	<0.01

SBP: systolic blood pressure; DBP: diastolic blood pressure; Anti-HT: antihypertensive; RAS: renin-angiotensin system; CCB: calcium channel blockers; OHA: oral antihyperglycemic agents; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Anti-HL: antihyperlipidemic; Cr: creatinine; eGFR: estimated glomerular filtration rate; ACR: urinary albumin-to-Cr ratio; IMT: intima-media thickness; FMD: flow-mediated vasodilation; AWE: arterial wall elasticity; Pa: Pascal. The patients were divided into four groups based on the number of the following atherosclerotic risk factors: diabetes mellitus, obesity, dyslipidemia and hypertension. Group 1 included patients with diabetes mellitus only, group 2 included patients with two risk factors, group 3 included patients with three risk factors and group 4 included patients with all four risk factors. The data are presented as the mean ± standard deviation, median (interquartile range) or number (%). The levels of triglycerides and Cr and the ACR values were log-transformed due to their skewed distributions. The p-values were determined using a one-way analysis of variance with multiple comparison tests or the chi-square test with residual tests. Significance level: $^{*}p<0.05$ vs. group 1; $^{\S}p<0.05$ vs. group 2; $^{\dagger}p<0.05$ vs. group 3.

sis parameters adjusted for antidiabetic medications, antihypertensive medications, antihyperlipidemic medications, retinopathy and nephropathy, in addition to age, sex, the smoking status and the ACR, were also analyzed. Additionally, the groups with and without microangiopathic complications were compared using

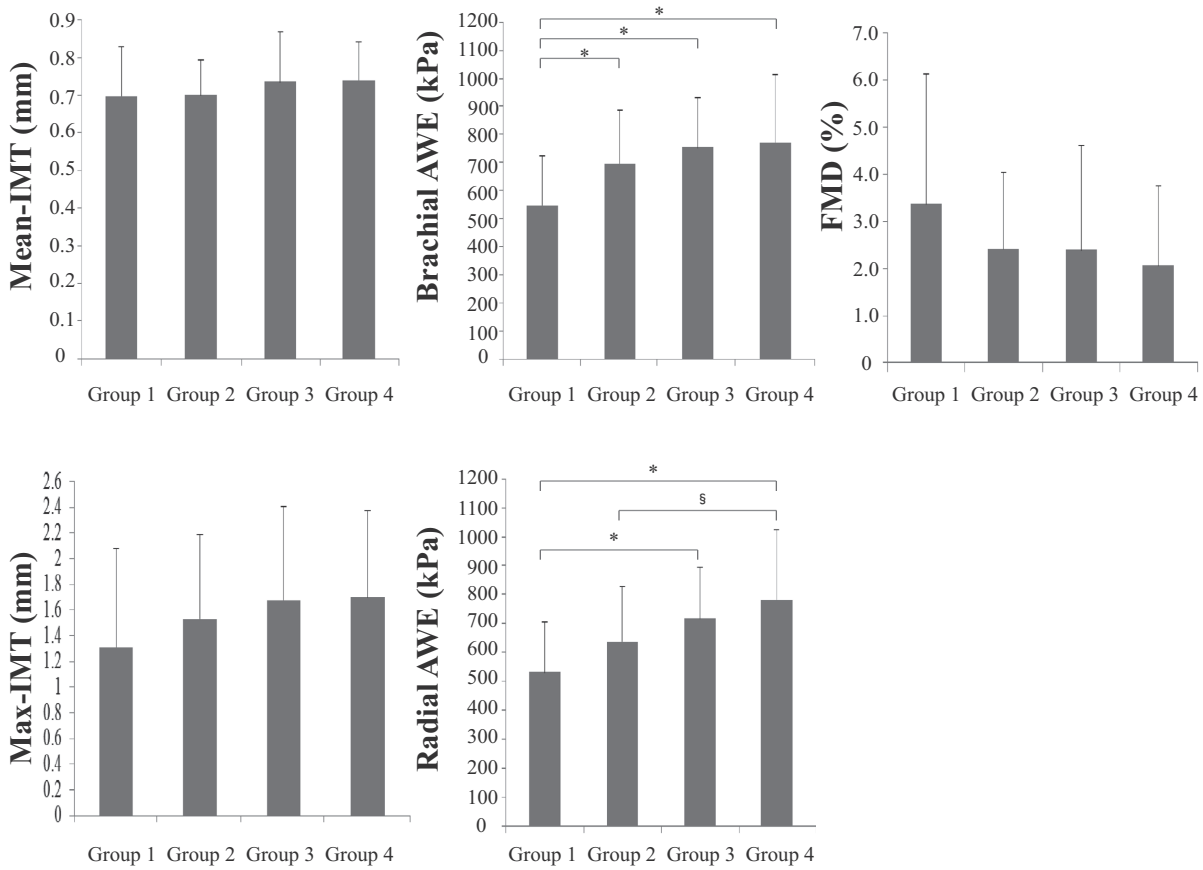

Fig. 1. Differences between the groups classified by the number of atherosclerotic risk factors. Group 1 included patients with diabetes mellitus only, group 2 included patient with two risk factors, group 3 included patients with three risk factors and group 4 included patients with all four risk factors. IMT: intima-media thickness; AWE: arterial wall elasticity; Pa: Pascal; FMD: flow-mediated vasodilation. Significance level: $^{*}p<0.05$ vs. group 1; $^{\S}p<0.05$ vs. group 2; $^{\dagger}p<0.05$ vs. group 3.

Student's *t*-test and the chi-square test. Receiver-operating characteristic (ROC) curve analyses of the mean-IMT, max-IMT, brachial AWE, radial AWE and FMD in the patients with microangiopathic complications were performed, and the areas under the curve (AUCs) were compared[36].

In all analyses, the log-transformed values of the levels of triglycerides and Cr and the ACR were used due to their skewed distributions. A value of $p<0.05$ was considered to be significant. All statistical analyses, except for the comparisons of the AUC, were performed using the Dr. SPSS II version 11 software program (SPSS Inc., Tokyo, Japan). The AUC comparisons were made using the StatFlex version 6 software package (Artech, Co., Ltd., Osaka, Japan).

Results

The patient characteristics of each group based on the number of atherosclerotic risk factors are shown in **Table 1** and **Fig. 1**. As the number of atherosclerotic risk factors increased, the number of patients with retinopathy and nephropathy tended to increase. In addition, the mean-IMT and max-IMT values showed a tendency to increase from groups 1 to 4. The FMD tended to decrease from groups 1 to 4.

The brachial AWE and radial AWE values significantly increased from groups 1 to 4. The brachial AWE value was significantly higher in groups 2, 3 and 4 than in group 1. The radial AWE value was significantly higher in group 4 than in groups 1 and 2, and the radial AWE value was significantly higher in group 3 than in group 1.

After adjusting for age, sex, the smoking status, the Cr level and the ACR, significant between-group differences were found in brachial AWE ($p<0.01$) and radial AWE ($p<0.01$), and a marginally significant between-group difference was found in FMD ($p=$

Table 2. Patient profiles for the groups with and without microangiopathic complications

Parameter	All (n=220)	Without complications (n=162)	With complications (n=58)	p-value
Male (%)	126 (57%)	92 (57%)	34 (59%)	0.81
Age (years)	59±11	60±11	58±10	0.17
Body mass index (kg/m^2)	25±6	25±6	26±5	0.70
Smoking (%)	47 (21%)	34 (21%)	13 (22%)	0.82
SBP (mmHg)	128±15	127±15	131±14	0.04
DBP (mmHg)	77±9	76±10	79±9	0.04
Anti-HT agents (%)	112 (51%)	73 (45%)	39 (67%)	<0.01
RAS inhibitors (%)	104 (47%)	67 (41%)	37 (64%)	<0.01
CCB (%)	69 (31%)	47 (29%)	22 (38%)	0.21
β blockers (%)	11 (5%)	8 (5%)	3 (5%)	0.94
Diuretics (%)	28 (13%)	17 (10%)	11 (19%)	0.10
Glucose (mg/dL)	135±42	133±41	142±45	0.19
Hemoglobin A1c (%)	7.9±2.0	7.6±1.9	8.5±2.2	<0.01
Antidiabetic agents (%)	158 (72%)	109 (67%)	49 (84%)	0.01
OHA (%)	109 (50%)	85 (52%)	24 (41%)	0.15
Insulin treatment (%)	49 (22%)	24 (15%)	25 (43%)	<0.01
Retinopathy (%)	80 (36%)	33 (20%)	47 (81%)	<0.01
Non-advanced stage (%)	42 (19%)	33 (20%)	9 (16%)	0.42
Advanced stage (%)	38 (17%)	0 (0%)	38 (66%)	<0.01
LDL-cholesterol (mg/dL)	109±32	110±31	106±34	0.47
HDL-cholesterol (mg/dL)	57±19	60±20	51±14	<0.01
Triglycerides (mg/dL)	108 (81-160)	102 (70-146)	120 (86-175)	0.01
Anti-HL agents (%)	87 (40%)	63 (39%)	24 (41%)	0.74
Statins (%)	79 (36%)	57 (35%)	22 (38%)	0.71
Fibrates (%)	8 (4%)	6 (4%)	2 (3%)	0.93
Cr (mg/dL)	0.68 (0.59-0.80)	0.68 (0.57-0.80)	0.70 (0.61-0.86)	0.19
eGFR (mL/min/1.73 m^2)	83±24	84±22	80±28	0.57
ACR (mg/g Cr)	16 (8-86)	13 (8-34)	239 (28-300)	<0.01
Nephropathy (%)	100 (45%)	55 (34%)	45 (78%)	<0.01
Non-advanced stage (%)	70 (32%)	55 (34%)	15 (26%)	0.26
Advanced stage (%)	30 (14%)	0 (0%)	30 (52%)	<0.01
Mean-IMT (mm)	0.72±0.12	0.72±0.12	0.73±0.12	0.59
Max-IMT (mm)	1.60±0.84	1.48±0.65	1.96±1.15	<0.01
FMD (%)	2.4±1.9	2.5±1.9	2.1±1.6	0.17
Brachial AWE (kPa)	721±217	694±213	797±212	<0.01
Radial AWE (kPa)	692±222	660±207	780±243	<0.01

SBP: systolic blood pressure; DBP: diastolic blood pressure; Anti-HT: antihypertensive; RAS: renin-angiotensin system; CCB: calcium channel blockers; OHA: oral antihyperglycemic agents; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Anti-HL: antihyperlipidemic; Cr: creatinine; eGFR: estimated glomerular filtration rate; ACR: urinary albumin-to-Cr ratio; IMT: intima-media thickness; FMD: flow-mediated vasodilation; AWE: arterial wall elasticity; Pa: Pascal. Microangiopathic complications included advanced-stage retinopathy and nephropathy. The patients were divided into groups with and without microangiopathic complications. The data are presented as the mean±standard deviation, median (interquartile range) or number (%). The levels of triglycerides and Cr and the ACR values were log-transformed due to their skewed distributions. The p-values were determined using Student's t-test or the chi-square test. Significance level: $p<0.05$.

0.05). Furthermore, there were significant between-group differences in brachial AWE ($p<0.01$) and radial AWE ($p<0.01$), but not in the FMD values ($p=0.65$), after adjusting for antidiabetic medications, antihypertensive medications, antihyperlipidemic medications, retinopathy and nephropathy, in addition to age, sex, the smoking status and the ACR.

The patients' characteristics in the groups with and without microangiopathic complications are shown in **Table 2**. The max-IMT, brachial AWE and

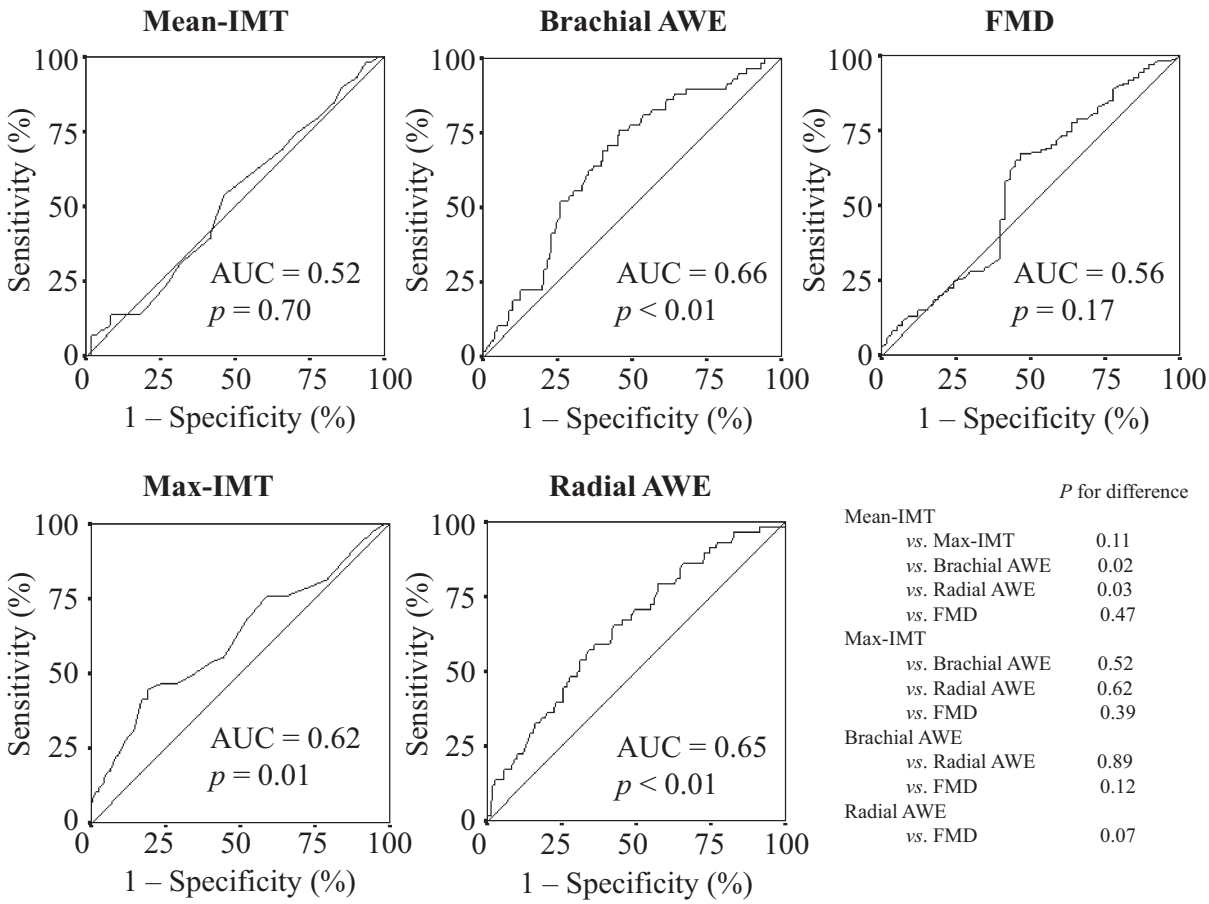

Fig. 2. ROC curve analyses of the mean-IMT, max-IMT, brachial AWE, radial AWE and FMD for microangiopathic complications were performed, and the AUCs were measured. ROC: receiver operating characteristic; IMT: intima-media thickness; AWE: arterial wall elasticity; FMD: flow-mediated vasodilation; AUC: area under the receiver operating characteristic curve. Significance level: $p<0.05$.

radial AWE values were significantly higher in the group with microangiopathic complications than in the group without microangiopathic complications. The ROC curves of the mean-IMT, max-IMT, brachial AWE, radial AWE and FMD regarding microangiopathic complications are shown in **Fig. 2**. The AUCs of the mean-IMT, max-IMT, brachial AWE, radial AWE and FMD were 0.52 (95% confidence interval [CI] 0.43-0.60, $p=0.70$), 0.62 (95% CI 0.53-0.71, $p=0.01$), 0.66 (95% CI 0.58-0.74, $p<0.01$), 0.65 (95% CI 0.57-0.73, $p<0.01$) and 0.56 (95% CI 0.47-0.65, $p=0.17$), respectively. When comparing the AUCs of the mean-IMT, max-IMT, brachial AWE, radial AWE and FMD, no differences were found among the max-IMT, brachial AWE, radial AWE or FMD. The AUCs of the brachial and radial AWE were significantly higher than that of the mean-IMT (**Fig. 2**).

Discussion

As the number of atherosclerotic risk factors increased, the brachial AWE and radial AWE values increased in the T2DM patients without a history of stroke or coronary artery disease. Although the mean-IMT and max-IMT tended to increase while the FMD decreased, the brachial and radial AWE produced more clear findings of atherosclerotic risk, to some extent, than the other ultrasonic atherosclerosis-related markers. When comparing the groups with and without microangiopathic complications, the max-IMT, brachial AWE and radial AWE values were higher in the group with microangiopathic complications than in the group without microangiopathic complications. Although the AUCs of the brachial and radial AWE did not exhibit a high accuracy, the AUCs of the brachial and radial AWE were approximately equal to that of the max-IMT and higher than

those of the mean-IMT and FMD. Considering that ultrasonic markers for atherosclerosis are not always established[5, 13, 37-40] and the clinical relevance of max-IMT for atherosclerosis has been indicated[41] in T2DM patients, it appears meaningful to note that the brachial and radial AWE may reflect atherosclerotic risks, similar to the max-IMT and superior to the mean-IMT and FMD, in this population.

As atherosclerotic disease progresses, endothelial dysfunction leads to secretory disorders of physiologically active substances, such as nitric oxide derived from the endothelium, inhibition of vascular smooth muscle relaxation and reductions in vascular tone[17, 42, 43]. This can partly induce the proliferation of vascular smooth muscle cells and reduce the vascular smooth muscle function[17, 42, 43]. Histopathological data obtained using the phased tracking method indicate high AWE values in tissue with proliferation of vascular smooth muscle cells[14]. Another study showed that the radial AWE is significantly and positively correlated with the serum C-reactive protein level, an inflammatory index[16]. Inflammation induced by endothelial dysfunction is the primary pathological basis for atherosclerosis[17]. Therefore, AWE measurement can be used to assess the degree of atherosclerotic overload and vascular complications.

FMD is the preferred method for evaluating the endothelial function[34, 35, 44], and the measurements of AWE and FMD are thought to partially overlap. In this study, the FMD tended to decrease in association with an increased number of atherosclerotic risk factors; however, the degree was not large compared with that of brachial and radial AWE. This may be because the patients in our study had poor glycemic control and multiple atherosclerotic risk factors, and the mean FMD value was decreased, even in group 1 (normal range 5-10%[45, 46]).

The carotid artery is a common site of atherosclerosis, and atherosclerotic changes in the carotid artery often reflect systemic atherosclerosis[37, 47, 48]. A large number of studies have demonstrated that the carotid IMT is higher in patients with atherosclerotic risk factors than in healthy subjects[33, 49], while other studies have showed that the carotid IMT is weakly correlated with cardiovascular disease in T2DM patients[13, 38]. Previous studies of the association between the IMT and microangiopathic complications have reported that the mean-IMT is weakly correlated with microangiopathic complications, while the max-IMT is positively correlated with microangiopathic complications[37, 41]. In our study, the carotid IMT did not clearly increase in association with increased atherosclerotic risk factors, and the max-IMT was increased significantly in the patients with microangiopathic complications. Our results support the findings of previous studies[13, 37-39], and we think that the weak correlation observed between the carotid mean-IMT and atherosclerotic risks may be partly due to our study population containing T2DM patients.

The present study is associated with several limitations. The study design was cross-sectional, and cardiovascular event outcomes were not evaluated. Prospective evaluations are needed to confirm the results of our study.

Conclusions

As the number of atherosclerotic risk factors increased, the brachial and radial AWE values increased in T2DM patients without a history of stroke or coronary artery disease. The brachial and radial AWE used to detect microangiopathic complications were approximately equal to the max-IMT. These results indicate that the brachial and radial AWE can reflect the degree of atherosclerotic overload and may be useful for detecting vascular complications. Measuring the upper limb AWE would be useful for assessing the degree of subclinical atherosclerosis and lead to new approaches for evaluating atherosclerosis.

Acknowledgments

This study was supported in part by Jichi Medical University.

Conflict of Interest Statement

The authors declare that there are no financial or other conflicts of interest.

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