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Original Article

Association of Advanced Glycation End Products with coronary Artery Calcification in Japanese Subjects with Type 2 Diabetes as Assessed by Skin Autofluorescence

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***Aim*: Advanced glycation end products (AGE) are considered to be among the critical pathogenic factors involved in the progression of diabetic complications. Skin autofluorescence (AF), a noninvasive measurement of AGE accumulation, has been recognized as a useful and convenient marker for diabetic vascular diseases in Caucasians. This study aimed to evaluate the association of tissue AGE, assessed using skin AF, with coronary artery calcification in Japanese subjects with type 2 diabetes.**
***Methods*: In total, 122 Japanese subjects with type 2 diabetes enrolled in this cross-sectional study underwent multi-slice computed tomography for total coronary artery calcium scores (CACS) estimation and examination with a skin AF reader.**
***Results*: Skin AF positively correlated with age, sex, diabetes duration, pulse wave velocity, systolic blood pressure, serum creatinine, and CACS. In addition, skin AF results negatively correlated with BMI, eGFR, and serum C-peptide concentration. According to multivariate analysis, age and systolic blood pressure showed strong positive correlation and eGFR showed negative correlation with skin AF values. Multiple linear regression analyses revealed a significant positive correlation between skin AF values and logCACS, independent of age, sex, diabetes duration, HbA1c, BMI, IMT, and blood pressure. However, skin AF showed no association with serum levels of AGE, such as Nε-(carboxymethyl) lysine and 3-deoxyglucosone.**
***Conclusion*: Skin AF results positively correlated with CACS in Japanese subjects with type 2 diabetes. This result indicates that AGE plays a role in the pathogenesis of diabetic macrovascular disease. Measurement of skin AF values may be useful for assessing the severity of diabetic complications in Japanese subjects.**

Key words: Advanced glycation end products, Coronary artery calcium scores, Type 2 diabetes, Surrogate marker

Introduction

Atherosclerotic diseases are the leading cause of death in individuals with type 2 diabetes[1)]. In a Japanese prospective population study, patients with type 2 diabetes had approximately 2.0 to 3.5 times higher risk of cardiovascular disease (CVD), including ischemic stroke and coronary heart disease, than nondiabetic subjects[2)]. Thus, detection and treatment of subclinical atherosclerosis in the diabetic population may prevent CVD events and substantially reduce the risk of cardiovascular death.

The coronary artery calcification score (CACS) measured by multi-detector computed tomography (MDCT) is reportedly a better predictor of CVD than traditional noninvasive surrogate markers, such as

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carotid intima-media thickness (IMT)[3]. Therefore, CACS is well-accepted and recommended in asymptomatic individuals with an intermediate risk, as assessed according to the Framingham Risk Score, in Western countries[4]. CACS measurement has also been regarded as being clinically useful for evaluating coronary atherosclerosis in Japan[5] where the incidence of coronary heart disease is much lower than in Western countries. In a previous study, we showed CACS to be positively associated with biological markers of oxidative stress[6] and to predict the morbidity of CVD in Japanese subjects with type 2 diabetes[7].

The risks of diabetic vascular complications are not fully represented by the currently established risk factors, such as HbA1c. Advanced glycation end products (AGE) are the irreversible products of nonenzymatic glycation, resulting from long-term hyperglycemia[8]. A large number of studies clarified that AGE mainly contributes to the development and progression of vascular complications in diabetes[9]. Tissue AGE accumulation exerts deleterious effects. One of the mechanisms underlying these effects involves changing the three-dimensional structure of proteins. Another involves the receptor for AGE-mediated activation of oxidative stress and inflammation pathways[10]. There is accumulating evidence of the relationships between serum AGE levels and vascular disease, but current serum AGE concentrations are not consistently related to diabetic complications according to several studies[11]. AGE accumulation, as assessed by skin biopsy specimens, reportedly shows a positive association with the presence of vascular disease[12]. These results raise the possibility that the pathogenic effects of AGE on vascular disease are exerted via tissue accumulation over many years[13]. A noninvasive technique for evaluating tissue accumulation of several types of fluorescent AGE by measuring skin autofluorescence (AF) was developed[14]. On the basis of a number of studies conducted mainly in Western countries, skin AF has now been recognized as a predictor of vascular complications in subjects with chronic kidney disease (CKD)[15, 16]. In addition, the relationships of skin AF with diabetic micro- and macroangiopathy have been intensively examined in subjects with both type 1 and type 2 diabetes[17-19]. However, the effectiveness of the measurement instrument, the AGE Reader, in diabetic patients has not been sufficiently evaluated in non-Caucasian, including Japanese, populations[20, 21]. Therefore, we designed this cross-sectional study to clarify the validity of skin AF for predicting the severity of atherosclerosis assessed using baPWV (brachial ankle pulse wave velocity), carotid IMT, and CACS in Japanese subjects with type 2 diabetes who were free of renal dysfunction.

Subject and Methods

Study Subjects

The subjects were 122 type 2 diabetes patients who visited Iwate Medical University Hospital during the period from April 2013 to December 2014. These patients ranged in age from 20 to 80 years. Patients with skin reflectance (R%) below 6% were excluded because of the limitation of the instrument in measuring skin AF accurately in non-Caucasians with relatively dark skin[22, 23]. Patients were excluded if they had renal dysfunction [estimated glomerular filtration rate (eGFR) below 30 mL min^{-1} 1.73 m^{-2}], any malignancy, an infectious disorder, or a past history of stroke or coronary artery disease. Written informed consent was obtained from all study participants. This study was approved by the Institutional Review Board of Iwate Medical University (Approval number: H25-25).

Measurement of Skin AF

Skin AF was assessed by an autofluorescence reader (AGE reader; Diagnoptics, Groningen, The Netherlands) as previously described[14]. AF measurement was defined as the average light intensity of the excitation spectrum between 420 nm and 600 nm, divided by the average light intensity of the emission spectrum between 300 nm and 420 nm and multiplied by one hundred and expressed in arbitrary units (AU). Skin AF was measured on the volar surface of the lower arm, approximately 10−15 cm below the elbow fold, with the patient in a seated position. The coefficient of variation for intraindividual measurements repeated over a few days was 5.82% ($n=5$).

Measurement of Coronary Artery Calcification

A VCT 240 slice MDCT (Aquilion ONE, Toshiba Medical, Tokyo, Japan) was used to obtain plain multislice CT scans. The calcium score analyses of coronary arteries were performed with a 0.5 mm collimation width, a gantry rotation speed of 0.4 s/rotation, 120 kV, and 300 mA using prospective ECG-gated axial scanning. Calcium plaque was defined as reaching a threshold of 130 HU and covering an area of at least 0.51 mm^2. The total CACS were determined on a workstation (ZIO Station, ZIO Soft, Inc., Tokyo, Japan) using a software program for coronary artery calcification according to the Agatston method[24]. The subjects were divided into three groups according to their CACS; the CAC 0, CAC 1−399, and CAC >400 groups.

Measurements of ABI, baPWV, and Carotid Artery IMT

ABI (ankle brachial pressure index) and baPWV were measured using an automatic waveform analyzer (BP-203RPE; Colin Co., Komaki, Japan), as described previously[25]. IMT of the carotid arteries was measured using ultrasound diagnostic equipment (LOGIQ 500, GE Yokogawa Medical Systems Corp., Hino, Tokyo, Japan) with an electrical linear transducer (mid-frequency of 7.5 MHz). The common carotid artery, carotid bulb, and portions of the internal and external carotid arteries on both sides were scanned with the subject in the supine position[6]. We defined the max IMT as the thickest portion detected in the scanned regions. The scans were performed by a trained sonographer.

Biochemical Measurements

Laboratory values were measured employing routine techniques on blood and urine samples obtained after a 12-h overnight fast. Plasma levels of Nε-(carboxymethyl) lysine, 3-deoxyglucosone, and malondialdehyde low density lipoprotein (MDA-LDL) as well as urinary levels of 8-hydroxy-2′-deoxyguanosine (OHdG) and 8-isoprostane were measured by SRL, Inc. (Tokyo, Japan).

Statistical Analysis

Quantitative data are presented as means ± standard deviation (SD). Variables were compared using Spearman's rank-order correlation analysis. We performed multivariate regression analysis using the force entry method to analyze variables independently related to skin AF. A multiple linear regression analysis adjusted for age, gender, body mass index (BMI), diabetes duration, history of smoking, systolic blood pressure, eGFR, skin AF, HbA1c, and max IMT was performed to evaluate parameters independently showing significant correlations with CACS. CACS plus one value were logarithmically converted. Linear regression analysis was performed with the step-down procedure to examine the grade of CACS and skin AF. Differences among the three groups were calculated employing the Kruskal−Wallis test for continuous variables and the Chi-square test for categorical variables. Receiver operating characteristics (ROC) curve analyses were drawn, and the areas under the curve (AUC) were then calculated. The level of significance was set at $p<0.05$. SPSS version 21 (SPSS Japan Inc., Tokyo, Japan) was used for all analyses.

Results

The clinical characteristics of our 122 subjects are shown in **Table 1**. Mean age was 61.0 years, mean diabetes duration was 10.7 years, and 59.0% of the subjects were men. Nearly half (49%) of the subjects were currently smoking or had a smoking history. The mean skin AF value was 2.42 (AU), compatible with that in a previous report on East Asian subjects[26].

The skin AF value positively correlated with age ($r=0.375$, $p<0.001$), diabetes duration ($r=0.338$, $p<0.001$), systolic blood pressure ($r=0.233$, $p=0.01$), serum creatinine levels ($r=0.206$, $p=0.023$), baPWV ($r=0.335$, $p<0.001$), and logCACS ($r=0.344$, $p<0.001$) (**Table 2**). In addition, AF showed negative correlations with BMI ($r=-0.203$, $p=0.025$), eGFR

Table 1. Baseline characteristics of the study participants

	$n=122$
Gender (male/female)	72/50
Age (years)	61.0 ± 13.0
BMI (kg/m^2)	26.4 ± 5.1
Diabetes duration (years)	10.7 ± 9.3
Hypertension, n (%)	73 (60)
Dislipidemia, n (%)	89 (73)
SBP (mmHg)	131.7 ± 17.7
DBP (mmHg)	75.1 ± 12.0
History of smoking (%)	60 (49)
Total cholesterol (mg/dL)	187.4 ± 34.5
Triglyceride (mg/dL)	138.3 ± 70.2
HDL cholesterol (mg/dL)	52.2 ± 16.3
LDL cholesterol (mg/dL)	110.0 ± 30.0
eGFR (ml/min per 1.73 m^2)	77.4 ± 23.6
Fasting blood glucose (mg/dL)	153.6 ± 51.1
HbA1c (%)	8.57 ± 2.33
Urinary 8-isoprostane (pg/mgCr)	265.2 ± 264.2
Urinary 8-OHdG (ng/mgCr)	11.4 ± 7.9
MDA-LDL (U/dl)	133.0 ± 42.3
Nε-(carboxymethyl) lysine (μg/ml)	3.49 ± 0.96
3-deoxyglucosone (ng/ml)	24.37 ± 14.2
max IMT (mm)	1.64 ± 0.70
mean baPWV (cm/s)	1569.4 ± 311.4
mean ABI	1.13 ± 0.96
Coronary artery calcification score, (AU)	197.03 ± 412.78
Skin autofluorescence (AU)	2.42 ± 0.417
Metformin, n (%)	52 (43)
DPP-4 inhibitors, n (%)	53 (44)
Statins, n (%)	50 (41)
RAS inhibitors, n (%)	61 (50)

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobinA1c; OHdG, hydroxydeoxyguanosine; MDA-LDL, malondialdehyde modified low density lipoprotein; IMT, intima-media thickness; baPWV, brachial ankle pulse wave velocity; ABI, ankle brachial index; DPP, dipeptidyl peptidase; RAS, renin-angiotensin system

Table 2. Correlations of skin AF with clinical parameters

Variable	Correlation coefficient	*P* value
Age, years	0.375	< 0.001
Sex	− 0.07	0.936
History of smoking	0.039	0.67
Body mass index, kg/m^2	− 0.203	0.025
Diabetes duration, years	0.338	< 0.001
Systolic blood pressure, mmHg	0.233	0.01
Diastolic blood pressure, mmHg	0.073	0.429
HbA1c, %	− 0.038	0.677
Fasting blood glucose, mg/dl	− 0.013	0.886
eGFR (ml/min per 1.73 m^2)	− 0.321	< 0.001
Serum creatinine, mg/dl	0.206	0.023
LDL-C , mg/dl	0.031	0.733
HDL-C, mg/dl	− 0.076	0.403
TG, mg/dl	− 0.096	0.294
Urinary 8-isoprostane, pg/mgCr	− 0.121	0.193
Urinary 8-OHdG, ng/mgCr	− 0.081	0.374
MDA-LDL-C, U/dl	− 0.01	0.915
max IMT, mm	0.159	0.08
mean baPWV, cm/s	0.335	< 0.001
mean ABI	− 0.66	0.47
log CACS, AU	0.344	< 0.001
C-peptide, ng/ml	− 0.23	0.011
Nε-(carboxymethyl) lysine, μg/ml	0.086	0.377
3-deoxyglucosone, ng/ml	− 0.147	0.13
High-sensitivity C-reactive protein, mg/l	− 0.07	0.446

Spearman rank correlation coefficient

($r = -0.321$, $p < 0.001$), and plasma C-peptide level ($r = -0.23$, $p = 0.011$). Interestingly, the plasma levels of AGE, Nε-(carboxymethyl) lysine and an AGE-precursor, 3-doxyglucosone, showed no significant associations with skin AF. In addition, surrogate markers of oxidative stress, such as 8-OHdG, 8-isoprostane, and MDA-LDL, did not correlate with skin AF. Next, we performed multiple linear regression analyses to identify variables independently related to skin AF (**Table 3**). According to multivariate analysis, age and systolic blood pressure were strongly positively related to skin AF, whereas eGFR showed a negative correlation with skin AF values. Intriguingly, logCACS was identified as a variable independently associated with skin AF, whereas neither max IMT nor baPWV showed a significant association with AF. Certain classes of oral medications, such as metformin, dipeptidyl peptidase (DPP)-4 inhibitors, statins, and renin-angiotensin system (RAS) inhibitors, apparently have minor effects on skin AF values.

In our Japanese subjects with type 2 diabetes, CACS showed a strong association with tissue accumulation of AGE, as evaluated employing a skin AGE analyzer. Thus, we next performed further examinations to detect factors influencing CACS. When we stratified patients by tertile of skin AF, a significant increase in logCACS was observed across these tertiles (**Fig. 1**). This result confirmed the strong association of skin AF with coronary atherosclerosis. Next, we stratified the patients into groups according to the degree of CACS, i.e., CACS = 0 ($n = 43$), CACS ≥ 1 to 399 ($n = 59$), and CACS ≥ 400 ($n = 20$), and performed linear regression analysis. We observed significant increasing trends for age ($p < 0.001$), diabetes duration ($p = 0.01$), max IMT ($p < 0.001$), baPWV ($p < 0.001$), and skin AF ($p = 0.008$). In contrast, fasting plasma glucose ($p = 0.014$) and eGFR ($p = 0.001$) showed decreasing trends (**Table 4**). Multiple linear regression analysis, adjusted for age, gender, BMI, diabetes duration, HbA1c, history of smoking, systolic blood pressure, eGFR, skin AF, and max IMT, revealed age ($\beta = 0.366$, $p < 0.01$), max IMT ($\beta = 0.351$, $p < 0.01$) and skin AF ($\beta = 0.169$, $p = 0.026$) to be the only parameters showing independent statistically significant asso-

Table 3. Determinants of skin AF in multivariate regression analysis

Factors	β	*P* value
Age	0.269	0.041
Sex	0.060	n.s.
History of smoking	0.034	n.s.
BMI	−0.140	n.s.
Systolic blood pressure	0.218	0.017
HbA1c	0.077	n.s.
Fasting blood glucose	0.160	n.s.
eGFR	−0.256	0.007
max IMT	−0.135	n.s.
baPWV	−0.030	n.s.
logCACS	0.222	0.04
Metformin	−0.137	n.s.
DPP-4 inhibitors	−0.037	n.s.
Statins	−0.149	n.s.
RAS inhibitors	0.082	n.s.

β is the standard coefficient; the multiple coefficient of determination (R2) = 0.308

ciations with CACS (**Table 5**). In contrast, a similar multiple linear regression analysis revealed skin AF to not be independently associated with baPWV. Finally, to determine the significance of measuring skin AF as a predictor of subclinical atherosclerosis in Japanese population, the AUC was assessed using ROC analysis of surrogate markers of atherosclerosis. The AUC for skin AF to discriminate CACS >100, which was reportedly recognized as a cut-off value for predicting cardiovascular morbidity in Japanese people[27], was 0.698 (95% CI, 0.602−0795, $p<0.001$), a value much higher than those obtained for PWV and max IMT (**Fig. 2**).

Discussion

To our knowledge, this study is the first to demonstrate a close relationship between coronary artery calcification and skin AF, reflecting tissue accumulation of AGE in Japanese subjects with type 2 diabetes. Because the incidence of CVD in the Japanese population has been rising in recent decades, a rapid, cost-effective, and simple method of evaluating atherosclerosis in routine practice is urgently needed. Our present observations support the advantages of measuring skin AF, a useful marker for detecting subclinical atherosclerosis.

For the 10 years since the initial report introducing the AGE Reader[14], evidence has been mounting that skin AF may predict vascular complications in Caucasians[28]. Because AGE result from hyperglycemia, a number of studies have been conducted to assess the relationship between skin AF and diabetic microvascular complications, including nephropathy and neuropathy[19]. Moreover, the associations with surrogate markers of atherosclerosis[29], as well as cardiovascular mortality[17], have been documented in Caucasian populations. The relationship between coronary artery calcification and skin AF was also examined in Caucasian subjects with type 1 diabetes[18] and nondiabetes[30]. Several studies of Asian populations have identified associations of skin AF with markers of atherosclerosis, including coronary artery calcification in CKD subjects[31]. Furthermore, the associations of skin AF with the presence of CVD[32] and cardiovascular mortality[33] were also examined mainly in patients with CKD. Therefore, the present results obtained in Japanese subjects with type 2 diabetes may facilitate understanding the utility of measuring skin AF for predicting atherosclerosis development in Japanese populations.

Because AGE are eliminated by the kidneys, we excluded subjects with renal failure from this study, as decreased clearance would result in tissue AGE accumulation[34]. Patients with renal dysfunction are regarded as being extremely sensitive to tissue AGE accumulation. In fact, the associations of skin AF with various complications have been most extensively investigated in subjects with CKD[16]. Although it is still possible that the relationship between skin AF and CACS observed in this study is partially attributable to confounding by renal dysfunction, the statistical significance of this relationship was independent of eGFR in our diabetic subjects.

Skin AF was measured noninvasively, quickly, and conveniently employing a desktop instrument. In addition, the inter-observer variability of skin AF values was relatively small[14] as compared to other physiological test values requiring more complex techniques. Furthermore, CACS has been established as a reliable marker for detecting subclinical atherosclerosis and predicting CVD events. In the United States and European countries, CACS measurement is recommended in asymptomatic subjects with intermediate risk (10−20% CVD risk over 10 years) for assessing whether preventive therapy is needed[4]. However, MDCT examinations can be inconvenient and rather expensive for routine practice in subjects with type 2 diabetes, whereas radiation exposure with MDCT, up to 1.2 millisieverts, is not considered to pose a significant risk. The most important aspect of this study is that several surrogate markers, such as carotid IMT, PWV, and CACS, widely used in daily practice, were comparatively assessed to demonstrate the significance of skin AF for the evaluation of atherosclerosis in a

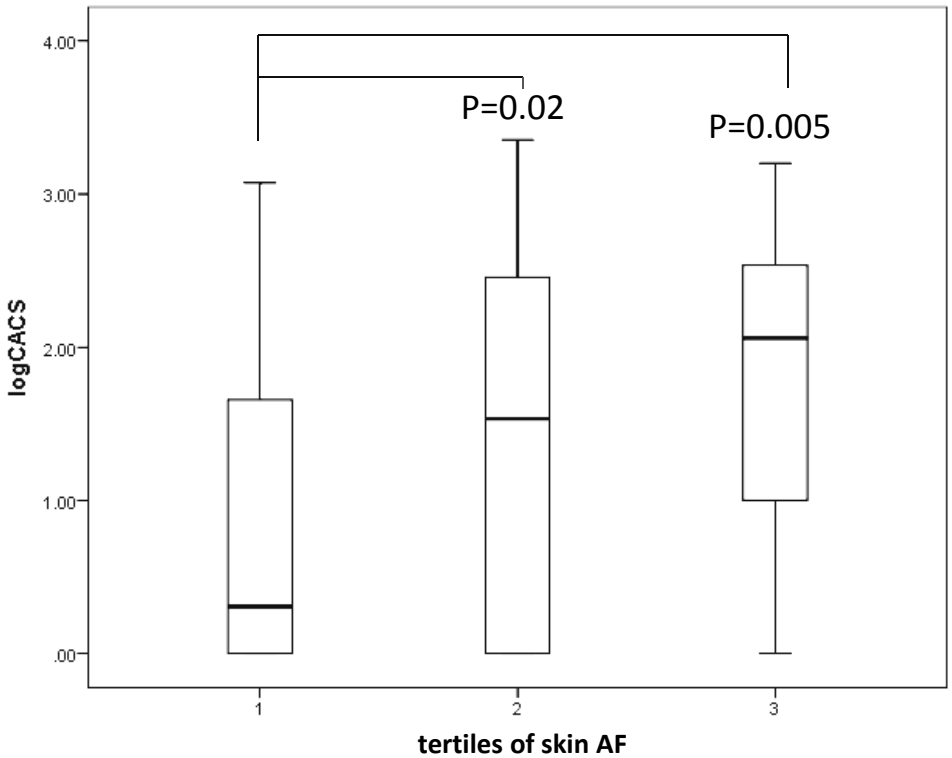

Fig. 1. Box plot of logarithmically converted CACS according to stratification of the subjects by tertiles of skin AF.

Japanese population. Previously, the relationships between skin AF and CACS were only reported in a European population with a small number of diabetic subjects[30], American subjects with type 1 diabetes[18] and Chinese subjects with CKD[31]. Furthermore, the relationships between skin AF and IMT were assessed in European nondiabetic subjects[29] and Japanese CKD subjects[35]. The association of skin AF with PWV has not yet been reported. Interestingly, Dekker *et al*. reported that skin AF correlated with carotid IMT but not with CACS in a study population with a small number of diabetic subjects[30]. As shown in **Fig. 2**, our results revealed skin AF to show a stronger association with CACS than either PWV or IMT, suggesting that prolonged hyperglycemia-induced AGE accumulation may be closely associated with the pathogenesis of aortic calcification. Therefore, skin AF measurement holds great promise as a screening tool for diabetic vascular complications.

Accumulating evidence obtained with the AGE reader has raised unexpected issues regarding the pathological roles of tissue AGE aggregation in various disorders, such as rheumatoid arthritis[36], foot ulceration[37], schizophrenia[38], and cognitive dysfunction[39]. Because the reasons for tissue AGE accumulation being related to these conditions are unclear, further research aimed at both elucidating the underlying mechanism and developing strategies for reducing AGE accumulation is required. Although statins and RAS inhibitors[40] reportedly reduce circulating levels of AGE, currently administered medications, such as statins, anti-diabetic, and anti-hypertensive agents, did not alter the skin AF values in our small-group investigation. Clinical trials of interventions with agents that decrease AGE accumulation, i.e., AGE breakers or inhibitors of AGE formation, are expected in the near future. We anticipate that skin AF measurement will ultimately come into widespread use for investigating the roles of AGE in various diseases.

In our subjects, the serum levels of nonfluorescent AGE, such as Nε-(carboxymethyl) lysine and 3-deoxyglucosone, showed no associations with CACS. Furthermore, biological markers of oxidative stress, such as plasma MDA-LDL and urinary 8-OHdG and 8-isoprostane, also showed no associations with either skin AF or CACS in this study. These results may suggest the development of macrovascular complications are affected by long-term hyperglycemia-induced tissue AGE accumulation rather than current increases in markers of AGE and oxidative stress in plasma.

The major limitation of this study is its cross-sectional design, raising the possibility that our results

Table 4. Characteristics of Patients According to Coronary Artery Calcium Scores

	CACS 0 ($n=43$)	CACS 1-399 ($n=59$)	CACS ≧400 ($n=20$)	P for Trend
Age, years	51.9 ± 13.7	64.6 ± 8.8	69.1 ± 8.9	$P<0.001$ *
Male sex, n (%)	26 (60)	33 (56)	13 (65)	$P=0.8$
History of smoking, n (%)	21 (49)	31 (53)	8 (40)	$P=0.6$
Body mass index, kg/m^2	27.4 ± 4.8	26.0 ± 5.5	25.7 ± 3.8	$P=0.2$
Diabetes duration, years	8.1 ± 7.3	11.0 ± 10.2	15.2 ± 9.0	$P=0.01$ *
Skin AF, AU	2.27 ± 0.40	2.48 ± 0.43	2.57 ± 0.32	$P=0.008$ *
Systolic blood pressure, mmHg	131.0 ± 17.7	132.0 ± 18.8	132.3 ± 15.1	$P=0.9$
Diastolic blood pressure, mmHg	75.4 ± 10.3	76.1 ± 13.4	71.3 ± 11.1	$P=0.4$
HbA1c, %	9.66 ± 2.44	7.85 ± 2.10	8.33 ± 2.02	$P<0.001$ *
Fasting blood glucose, mg/dl	172.8 ± 60.4	145.0 ± 41.0	137.7 ± 45.6	$P=0.014$ *
eGFR (ml/min per 1.73 m^2)	85.1 ± 20.7	76.2 ± 25.3	64.4 ± 17.8	$P=0.001$ *
LDL cholesterol, mg/dl	107.2 ± 26.5	113.3 ± 33.0	106.0 ± 27.7	$P=0.6$
HDL cholesterol, mg/dl	49.4 ± 11.8	53.8 ± 16.3	53.3 ± 23.3	$P=0.6$
TG, mg/dl	143.6 ± 67.5	141.8 ± 66.7	117.0 ± 84.3	$P=0.4$
Urinary 8-isoprostane, pg/mgCr	260.8 ± 140	248 ± 207	321 ± 507	$P=0.2$
Urinary 8-OHdG, ng/mgCr	11.3 ± 7.9	11.2 ± 7.4	12.3 ± 9.2	$P=1.0$
MDA-LDL-C, U/dl	125.5 ± 31.9	139.6 ± 45.2	130.0 ± 51.4	$P=0.3$
High-sensitivity C-reactive protein, mg/l	0.57 ± 2.44	0.19 ± 0.33	0.064 ± 0.046	$P=0.1$
Nε-(carboxymethyl) lysine, μg/ml	3.55 ± 0.99	3.42 ± 1.03	3.72 ± 1.08	$P=0.4$
3-deoxyglucosone, ng/ml	26.6 ± 18.1	23.9 ± 13.2	23.6 ± 14.2	$P=0.9$
max IMT, mm	1.32 ± 0.6	1.66 ± 0.61	2.3 ± 0.70	$P<0.001$ *
mean baPWV	1425 ± 235	1579 ± 284	1851 ± 344	$P<0.001$ *

Values are means ± SD.
# $p<0.05$ (Kruskal Wallis test and the Chi-Square test)

show only associations. However, coronary artery calcification as assessed by MDCT is regarded as an excellent surrogate marker of cardiovascular mortality in Japanese subjects[5], such that the observed cross-sectional associations in our living study participants still have important clinical implications. Second, the instrument used, the AGE reader, cannot be applied to subjects with dark skin because of the high absorption grade of the excited light. To address this problem, we excluded patients with skin reflectance below 6% in accordance with a recent report on a non-Caucasian population[31]. Although Meerwaldt *et al.* demonstrated significant correlations of skin AF with tissue levels of Nε-(carboxymethyl) lysine, Nε-(carboxyethyl) lysine, and pentosidine[14, 41], we did not directly perform histological examinations of skin accumulation of AGE in our subjects. Moreover, the detection capability of skin AGE accumulation by the AGE reader is limited because some types of AGE are not fluorescent. A critical issue of our study is the lack of measurement for serum concentrations of fluorescent AGE, such as pyrraline and pentosidine. In particular, pentosidine is considered as one of the major components of AGEs, leading to vascular complication. In a previous report with Japanese subjects, high serum concentration of pentosidine was closely associated with both increasing arterial stiffness and thickening carotid IMT[42]. We will try to clarify the relationships among diabetic complications, skin AF values, and serum concentration of fluorescent AGE in a further study. Third, our sample size was too small to allow sufficiently powered statistical analyses to be performed. Furthermore, a recent study showed that CAC scoring employing a combination that includes CAC density would increase the predictive values for CHD[43]. This possibility merits further examination.

Table 5. Determinants of logCACS in multiple regression analysis

Factors	β	P value
Age	0.366	<0.01
max IMT	0.351	<0.01
skin AF	0.169	0.026

β is the standard coefficient; the multiple coefficient of determination (R2) = 0.439

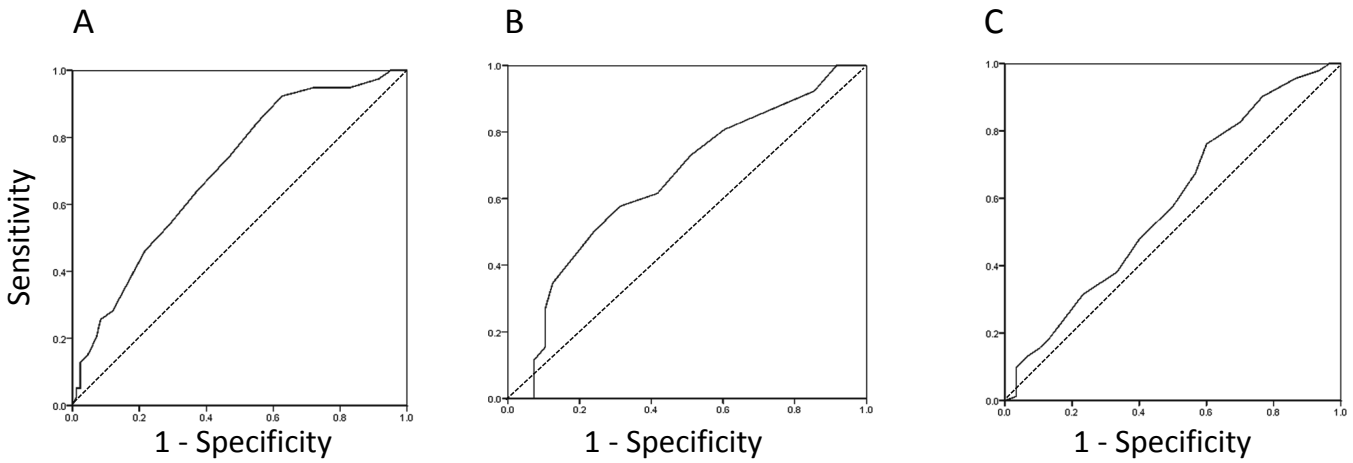

Fig. 2. ROC curve analysis of AF for predicting subclinical atherosclerosis.

A. CACS >100, area under the curve (AUC)=0.698 (95% CI, 0.602−0795, $p<0.001$). B. PWV >1800 cm/s, AUC=0.655 (95% CI, 0.538−0773, $p<0.05$). C. max IMT >1.1 mm, AUC=0.582 (95% CI, 0.460−0704, n.s.).

Conclusion

Skin AF results positively correlated with CACS in Japanese subjects with type 2 diabetes. This observation indicates tissue AGE accumulation plays a role in the pathogenesis of diabetic macrovascular disease. Skin AF measurements may be useful for assessing the severity of diabetic complications in Japanese patients.

Acknowledgments

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Conflict of Interest

Dr. Yasushi Ishigaki has received lecture fees from Astellas Pharma Inc., Novartis Pharma K.K., Novo Nordisk Pharma Ltd., Kowa Pharmaceutical Co., Ltd., Sanofi Co., Ltd., Mitsubishi Tanabe Pharma Co., Ltd., Ono Pharmaceutical Co., Ltd., and MSD Co., Ltd. and scholarship grants from Mitsubishi Tanabe Pharma Co., Ltd., Ono Pharmaceutical Co., Ltd. and MSD Co., Ltd. Dr. Jo Satoh has received lecture fees from Astellas Pharma Inc., Astra Zeneca Co., Ltd., Dainippon-Sumitomo Pharma Co., Ltd., Sanofi Co., Ltd., Mitsubishi Tanabe Pharma Co., Ltd., Ono Pharmaceutical Co., Ltd., and MSD Co., Ltd.

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