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Original Article

Skin Autofluorescence is Associated with Early-stage Atherosclerosis in Patients with Type 1 Diabetes

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***Aim*: Accumulation level of fluorescent advanced glycation end products (AGEs) in the skin can be measured non-invasively as skin autofluorescence (skin AF) by autofluorescence reader. The aim of this study was to assess possible associations between skin AF and diabetic complications, especially early-stage atherosclerosis, in Japanese type 1 diabetic patients.**

***Methods*: Skin AF was measured by AGE reader® in 105 Japanese type 1 diabetic patients (34 men and 71 women, aged 37.4 ± 12.4 years (± SD)) and 23 age-matched healthy non-diabetic subjects. Ultrasonic carotid intima-media thickness (IMT), ankle-brachial index (ABI), and brachial ankle pulse wave velocity (baPWV) were evaluated as indices of early-stage diabetic macroangiopathy. Urinary albumin-to-creatinine ratio (UACR), the coefficient of variation of R-R intervals (CVR-R), and presence of retinopathy were also evaluated.**

***Results*: Skin AF values were significantly higher in type 1 diabetic patients than in healthy controls (2.07 ± 0.50 (mean ± SD) and 1.90 ± 0.26, respectively, $p = 0.024$). Skin AF was associated with carotid IMT ($r = 0.446$, $p < 0.001$) and baPWV ($r = 0.450$, $p < 0.001$), but not with ABI ($r = -0.019$, $p = 0.8488$). Notably, skin AF was an independent risk factor for IMT thickening. Similarly, skin AF was associated with log (UACR) ($r = 0.194$, $p = 0.049$) and was an independent risk factor for UACR. Furthermore, skin AF values were significantly higher in patients with diabetic retinopathy than in those without (2.21 ± 0.08 and 1.97 ± 0.06, respectively, $p = 0.020$).**

***Conclusions*: Skin AF was significantly associated with the presence and/or severity of diabetic complications and was an independent risk factor for carotid atherosclerosis.**

Key words: Advanced glycation end products (AGEs), Skin autofluorescence, Carotid intima-media thickness, Type 1 diabetes

Since diabetic micro- and macrovascular complications lead to impairment of quality of life and are major causes of mortality in patients with type 1 diabetes mellitus, risk stratification and subsequent rapid intervention against them are important in the management of type 1 diabetes mellitus[1, 2]. Although poor glycemic control is one of the most important risk factors for diabetic complications[3, 4], indices of current or recent glycemic control status such as blood glucose levels, glycoalbumin (GA), and glycated hemoglobin A1c (HbA1c) do not fully reflect the risk of diabetic complications and are insufficient for risk stratification[1].

Advanced glycation end products (AGEs), a complex group of compounds produced from slowly occurring nonenzymatic glycation of proteins, represent the integration of various vascular risk factors, such as chronic hyperglycemia, insulin resistance, oxidative stress, aging, and smoking[5, 6], and play important roles in the development of vascular disease[7, 8].

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Furthermore, since relatively long-term metabolic status affects the formation of AGEs, AGEs are regarded as a marker of so-called "metabolic memory." The Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications (DCCT/EDIC) study revealed that early intensive glycemic control retards the development and progression of diabetic complications in the long term, despite worsening glycemic control[4, 9]. Interestingly, in that study, long-term intensive treatment of hyperglycemia, as compared to conventional treatment, was associated with lower levels of AGEs in skin collagen[10]. It was also demonstrated that level of skin AGEs predicts the risk of future 10-year progression of diabetic retinopathy and nephropathy independently of past HbA1c[11]. Thus, assessment of AGEs accumulated in the skin can improve the predictability of diabetic complications. However, skin biopsy cannot be applied in routine clinical practice, because of its invasive and costly characteristics and technical limitations.

Recently, accumulation level of fluorescent AGEs in the skin has become measurable non-invasively with skin autofluorescence (skin AF) using an autofluorescence reader. Skin AF correlated with accumulation of skin AGEs assessed by skin biopsy[12]. Several reports described elevation of skin AF in both type 1 and type 2 diabetes compared with healthy controls[13-16]. Furthermore, in type 1 diabetes, it was reported that skin AF correlated with nephropathy[13, 17, 18], neuropathy[17, 19], and retinopathy[13], suggesting that skin AF may become a surrogate marker candidate for diabetic microangiopathy. However, still few studies have assessed the association between skin AF and macroangiopathy, especially early-stage atherosclerosis, in type 1 diabetes. In addition, it is of note that most of the previous studies dealt with Caucasian subjects, although there seems to be ethnic differences in skin AF values[20].

The aims of this study were: (1) to evaluate skin AF in Japanese patients with type 1 diabetes in comparison with healthy controls, and (2) to assess of possible associations between skin AF and diabetic complications, especially early-stage atherosclerosis, in this population.

Research Design and Methods

Study Population

Study subjects were recruited from patients at the Diabetes Clinic of Osaka University Hospital and the Osaka Police Hospital during the period from June 2014 to August 2015. Patients with type 1 diabetes diagnosed by diabetologists were considered eligible as subjects. All patients were diagnosed as type 1 diabetes at the time of presenting with hyperglycemia and/or ketosis in their clinical history, and since that time, all had been under insulin therapy. The patients on dialysis were excluded. Finally,, 105 type 1 diabetes patients were enrolled. As control subjects, 23 age-matched healthy volunteers were also enrolled. The study protocol was approved by the local ethics committee and the study was conducted in accordance with the principles of the Helsinki Declaration. All participants provided written informed consent.

Clinical and Biochemical Assessment

The clinical assessments included a medical history interview, physical examination (height, weight, and resting blood pressure), electrocardiography, and laboratory testing. Smoking status was classified as having a current smoking habit or not. Venous blood samples were taken, and serum total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, serum triglyceride, and HbA1c levels were measured by SRL Inc. (Tokyo, Japan) using standard laboratory protocols. Plasma pentosidine levels were also measured by SRL Inc. (Tokyo, Japan) using enzyme-linked immunosorbent assay (ELISA) (CV=6.4%).

Assessment of Diabetic Macroangiopathy

Ultrasonic carotid intima-media thickness (IMT), ankle-brachial index (ABI), and brachial ankle pulse wave velocity (baPWV) were evaluated as indices of early-stage diabetic macroangiopathy. B-mode ultrasonography of the carotid artery was performed using an ultrasound machine with a 7.5-MHz linear transducer. In accordance with the guidelines of the Japan Society of Ultrasonics[21], all scanning was conducted by experienced physicians using the same ultrasound system and the same measuring method. Scanning of the extracranial common carotid artery, the carotid bulb, and the internal carotid artery in the neck was performed bilaterally from three different longitudinal projections (i.e., anterior-oblique, lateral, and posterior-oblique). The carotid IMT was measured as the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line. The thickest points of IMT (including plaque lesions) were measured, and the highest value among them was defined as maxIMT and used as the representative value for each individual.

Measurement of ABI and baPWV was performed using the same volume-plethysmographic apparatus as for ABI (BP-203RPE II form PWV/ABI), with subjects in the supine position after at least 5 min of rest. Oscillometric cuffs, each connected to a plethysmographic sensor that determined volume pulse form

Table 1. The clinical characteristics of the study subjects.

	controls ($n=23$)	type 1 diabetes ($n=105$)	p
Age (years)	34.7 ± 6.2	37.4 ± 12.4	NS
female n (%)	8 (34.8)	71 (65.7)	0.006
duration of diabetes (years)	−	21.9 ± 9.2	−
Smoking n (%)	2 (8.7)	35 (34.0)	0.016
BMI (kg/m^2)	20.6 ± 2.6	23.0 ± 3.0	< 0.001
Systolic BP (mmHg)	114 ± 7	117 ± 14	NS
Diastolic BP (mmHg)	68.9 ± 5.9	69.7 ± 9.2	NS
AST (U/L)	18.5 ± 4.0	18.4 ± 5.5	NS
ALT (U/L)	14.9 ± 6.2	15.4 ± 9.3	NS
GGT (U/L)	20.1 ± 9.6	24.4 ± 36.7	NS
Glucose (mg/dL)	92.8 ± 8.3	156.7 ± 75.0	< 0.001
HbA1c (%)	5.1 ± 0.2	7.7 ± 1.4	< 0.001
GA (%)	13.8 ± 1.1	23.5 ± 5.0	< 0.001
TC (mg/dL)	181.8 ± 23.9	186.6 ± 32.2	NS
TG (mg/dL)	59 (30-623)	66 (21-757)	NS*
HDL-C (mg/dL)	62.8 ± 13.1	67.3 ± 13.2	NS
LDL-C (mg/dL)	104.1 ± 22.1	102.6 ± 26.8	NS
UA (mg/dL)	5.2 ± 1.5	4.4 ± 1.3	0.012
Creatinine (mg/dL)	0.82 ± 0.16	0.71 ± 0.17	0.005
eGFR (ml/min/1.73 m^2)	81.4 ± 11.3	87.5 ± 17.2	NS
UACR (mg/g/Cr)	−	45.3 ± 320	−
CVR-R (%)	5.47 ± 2.71	3.94 ± 1.84	0.001
Nephropathy (no n (%))	−	95 (91.3)	−
Retinopathy (no n (%))	−	58 (59.2)	−
maxIMT (mm)	0.76 ± 0.21	1.09 ± 0.48	0.002
ABI	1.07 ± 0.08	1.07 ± 0.08	NS
ba-PWV (cm/sec)	1225 ± 156	1318 ± 248	NS
Skin AF (AU)	1.90 ± 0.26	2.07 ± 0.50	0.024

Data are expressed as mean ± SD, median (range), or number and percentage. Unpaired t-test, Welch's t-test or the *Mann-Whitney U test was performed. P-value < 0.05 was considered statistically significant.
Abbreviations: BMI, body mass index; BP, blood pressure; AST, aspartate transaminase; ALT, alanine transaminase; GGT, γ-glutamyltransferase; HbA1c, glycated hemoglobin A1c; GA, glycoalbumin; TC, total cholesterol; TG, serum triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; eGFR, estimated glomerular filtration rate; UACR, urinary albumin-to-creatinine ratio; CVR-R, coefficient of variation of R-R intervals; IMT, intima media thickness; ABI, ankle-brachial index; PWV, pulse wave verbosity.

and to an oscillometric pressure sensor that measured blood pressure, were wrapped on both ankles and upper arms of each subject, and an electrocardiogram electrode was placed on each wrist. The cuffs were simultaneously pressurized to the approximate value of the subject's diastolic pressure so that the pulse volume waveforms could be recorded using semiconductor pressure sensors. The distance between sampling points of baPWV was calculated based on the height of the subject. The path length from the suprasternal notch to the ankle (La) was calculated as: La = 0.8129* height (in cm) + 12.328. The path length from the suprasternal notch to the brachium (Lb) was calculated as: Lb = 0.2195*height − 2.0734. The baPWV was calculated according to the following formula: baPWV = (La − Lb)/Tba, where Tba was the time interval between the wave front of the brachial waveform and that of the ankle waveform[22]. Two simultaneous measurements of baPWV were recorded, on the right side and left side, respectively, and the higher of these readings was used as the representative value for each individual.

Assessment of Diabetic Microangiopathy

Presence of retinopathy was diagnosed by a diabetologist based on the findings of single-field fundus photography under regular check up by an ophthalmologist, and classified as no diabetic retinopathy

Table 2. Relative risk factors of skin AF in type 1 diabetes

	Univariate		Multivariate	
	r	*p*	*β*	*p*
Age (year)	0.470	<0.001	0.412	<0.001
Gender (male)	0.213	0.029	NI	
Duration of diabetes (years)	0.248	0.003	NI	
Smoking	0.332	<0.001	0.225	0.012
BMI (kg/m^2)	0.170	NS		
Systolic BP (mmHg)	0.173	NS		
Diastolic BP (mmHg)	0.153	NS		
GGT (U/L)	0.320	<0.001	NI	
Glucose (mg/dL)	0.118	NS		
HbA1c (%)	0.217	0.026	NI	
TG (mg/dL)	0.130	NS		
LDL-C (mg/dL)	0.106	NS		
HDL-C (mg/dL)	−0.072	NS		
UA (mg/dL)	0.246	0.011	NI	
eGFR (ml/min/1.73 m^2)	−0.246	0.011	NI	
R^2			0.269	

The threshold of statistical significance was defined as $p<0.05$.
Gender: male=1, female=0, Smoking: yes=1, no=0.
Stepwise multivariate regression analysis was performed to determine the predictors of skin AF. The significant ($p<0.05$) variables in Pearson's univariate analysis were selected for multivariate regression analysis.
Abbreviations: β, partial regression coefficient; NS, not significant; NI, not included in the model; Gender: male=1, female=0, Smoking: yes=1, no=0; BMI, body mass index; BP, blood pressure; GGT, γ-glutamyltransferase; HbA1c, glycated hemoglobin A1c; GA, glycoalbumin; TG, serum triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; eGFR, estimated glomerular filtration rate.

(NDR) or simple or more advanced diabetic retinopathy (≥SDR). The coefficient of variation of R-R intervals (CVR-R) was measured as an index of autonomic nerve function. Presence and severity of diabetic nephropathy were diagnosed based on glomerular filtration rate (GFR) and urine albumin excretion. GFR was estimated using the abbreviated Modification of Diet in Renal Disease (MDRD) for Japanese. Urine albumin excretion was evaluated by turbidimetric immunoassay and expressed as urinary albumin-to-creatinine ratio (UACR).

Measurement of Skin Autofluorescence

Skin autofluorescence was measured by AGE reader (DiagnOptics BV, Groningen, the Netherlands). The detailed measurement protocol was reported previously[12]. Briefly, the apparatus illuminates the skin with an excitation light source of 300 to 420 nm. Subsequently, emitted light and light reflected from skin are assessed with a spectrometer in the range 300-600 nm. Skin autofluorescence is calculated in arbitrary units (AU) by dividing the area under the curve between 420 and 600 nm (emission spectrum) by the area under the curve between 300 and 420 nm (excitation spectrum). For each subject, skin AF was measured three times in series and the arithmetical mean of those assessments was used. AF was measured on ventral site of the forearm.

Statistical Analysis

All values are reported as mean±SD, or median for continuous variables, or number with percentage in parentheses for categorical variables. Comparisons between the groups were assessed using an unpaired Student's *t*-test (or Welch's *t*-test when two groups were assumed to have unequal population variance) for parametric data, or the Mann−Whitney *U*-test for nonparametric data. Differences in proportions were tested using the χ^2-test or Fisher's exact test. Associations between variables were tested with Pearson's univariate test. Multivariate regression analysis was performed to assess variables significantly associated with an objective variable. In case of multivariate regression analysis for categorical data, we performed logistic regression analysis to derive the odds ratio (OR) and 95% confidence intervals (CI). A two-sided *p* value <0.05 was considered statistically significant. All analyses were performed with the use of JMP® pro 11.1.1

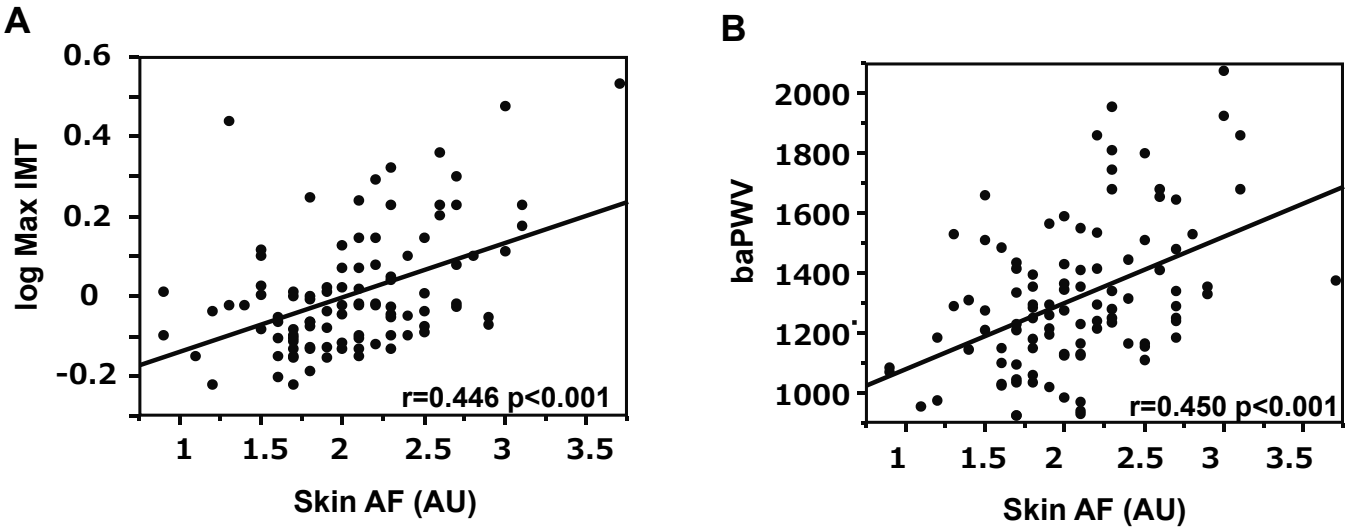

Fig. 1. Association between skin AF and (A) maxIMT and (B) baPWV. Statistical analysis for association was performed using Pearson's univariate test.

(SAS Institute Inc., Cary, NC, USA).

Results

Skin AF Values in Type 1 Diabetic Subjects in Comparison with Healthy Controls

The clinical characteristics of the study subjects are presented in **Table 1**. Smoking rates, plasma glucose, HbA1c, GA, and BMI levels were significantly higher, and uric acid (UA) levels were significantly lower in subjects with type 1 diabetes as compared to non-diabetic subjects ($p<0.05$). There was no significant difference between the two groups regarding the other clinical parameters such as age, systolic and diastolic BP, eGFR, total, LDL, and HDL cholesterol, and triglyceride levels. Skin AF values were significantly higher in type 1 diabetic subjects than in healthy controls (2.07 ± 0.50 (mean ± SD) and 1.90 ± 0.26, respectively, $p=0.024$).

Next, to assess what factors affected skin AF values in the type 1 diabetic subjects, we analyzed associations between skin AF value and clinical parameters. Univariate regression analysis showed that skin AF was significantly associated with age, gender, smoking status, duration of diabetes, HbA1c, GA, aspartate transaminase (AST), γ-glutamyltransferase (GGT), UA, serum Creatinine, and eGFR in the type 1 diabetic subjects (**Table 2**, **Supplementary Fig. 1A**). A stepwise multivariate regression analysis including variables that were significantly associated with skin AF in univariate analysis as independent variables demonstrated that age and smoking status were independent determinants of skin AF, while HbA1c and GA were not (**Table 2**). Interestingly, there was also a statistically significant association between skin AF and the average HbA1c value for a long term (utmost past 10 years) in 67 subjects whose past HbA1c levels were available (**Supplementary Fig. 1B**). Using this average HbA1c value as an index of glycemic control, instead of the single-point HbA1c value measured at the time of enrollment, showed the average HbA1c to be an independent determinant of skin AF ($\beta=0.350$, $p=0.002$), even after adjustment for the other variables.

Associations between Skin AF and Diabetic Complications

Skin AF was associated with maxIMT ($r=0.446$, $p<0.001$, **Fig. 1A**) and baPWV ($r=0.450$, $p<0.001$, **Fig. 1B**) but not with ABI ($r=-0.019$, $p=0.8488$). Since maxIMT was also associated with other variables such as age, duration of diabetes, systolic BP, GGT, LDL-C, UA, serum Cr, and eGFR, a stepwise multivariate regression analysis was performed to evaluate whether skin AF value was an independent determinant for maxIMT ($\beta=0.247$, $p=0.008$), even after adjustment for these variables. This analysis revealed that skin AF value and age were significant independent determinants for maxIMT (**Table 3**). Furthermore, another multivariate regression analysis revealed that skin AF value was a significant independent determinant for maxIMT ($\beta=0.201$, $p=0.043$) even after mandatory adjustment for these variables. On the other hand, skin AF was no longer an independent determinant for baPWV after adjustment for age, duration of diabetes, systolic BP, and eGFR, all of which were significantly associated with baPWV

Table 3. Relative risk factors of maxIMT in type 1 diabetes

	Univariate		Multivariate	
	r	*p*	*β*	*p*
Age (year)	0.538	< 0.001	0.422	< 0.001
Gender (male)	0.165	NS		
Duration of diabetes (years)	0.286	0.003	NI	
Smoking	0.197	0.045	NI	
BMI (kg/m^2)	0.102	NS		
Systolic BP (mmHg)	0.210	0.033	NI	
GGT (U/L)	0.277	0.004	NI	
HbA1c (%)	0.116	NS		
TG (mg/dL)	0.108	NS		
LDL-C (mg/dL)	0.196	0.048	NI	
HDL-C (mg/dL)	−0.110	NS		
UA (mg/dL)	0.208	0.034	NI	
eGFR (ml/min/1.73 m^2)	−0.357	< 0.001	NI	
Skin AF (AU)	0.446	< 0.001	0.247	0.008
R^2			0.337	

The threshold of statistical significance was defined as $p < 0.05$.
Gender: male = 1, female = 0, Smoking: yes = 1, no = 0.
Stepwise multivariate regression analysis was performed to determine the predictors of maxIMT. The significant ($p < 0.05$) variables in Pearson's univariate analysis were selected for multivariate regression analysis.
Abbreviations: β, partial regression coefficient; NS, not significant; NI, not included in the model; BMI, body mass index; BP, blood pressure; GGT, γ-glutamyltransferase; HbA1c, glycated hemoglobin A1c; GA, glycoalbumin; TG, serum triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; eGFR, estimated glomerular filtration rate.

(**Supplementary Table 1**).

Although skin AF values were significantly higher in subjects with diabetic retinopathy than in those without it (2.21 ± 0.08 and 1.97 ± 0.06, respectively, $p = 0.02$), a multiple logistic regression analysis revealed that skin AF was no longer an independent determinant for the presence of diabetic retinopathy after adjustment for duration of diabetes and plasma glucose level (odds ratio; 1.94, 95% confidence interval; 0.70-5.85) (**Supplementary Table 2**). Similarly, univariate analysis revealed that skin AF was significantly associated with CVR-R ($r = -0.342$, $p < 0.001$), a multiple regression analysis revealed that skin AF was no longer an independent determinant for CVR-R after adjustment for age and HbA1c (**Supplementary Table 3**).

Univariate analysis revealed that log (UACR) was associated with gender, HbA1c, and skin AF value. Skin AF was a significant independent determinant for UACR even after adjustment for gender and HbA1c (**Supplementary Table 4**). Similarly, univariate analysis revealed that there were significant associations between skin AF value and eGFR ($r = -0.246$, $p = 0.0113$), while a multiple regression analysis revealed that skin AF is no longer an independent determinant for eGFR after adjustment for age, duration of diabetes, and serum UA levels.

Associations between Plasma Pentosidine and Diabetic Complications

We also assessed associations between diabetic complications and plasma pentosidine, which is a well-known circulating AGE. Although plasma pentosidine levels were not associated with skin AF ($r = 0.075$, $p = 0.414$), plasma pentosidine levels were significantly higher in type 1 diabetes than in healthy controls (0.043 ± 0.013 (mean ± SD) and 0.036 ± 0.001 respectively, $p < 0.001$). However, plasma pentosidine levels were not associated with either single-point or average HbA1c levels. In addition, there were no associations between plasma pentosidine levels and any markers of diabetic macroangiopathy (maxIMT, baPWV, and ABI) or microangiopathy (presence of retinopathy, CVR-R, UACR, and, eGFR) in this population (all $p > 0.05$).

Discussion

In the present study, we confirmed that skin AF was higher in participants with type 1 diabetes as

compared to non-diabetic control subjects and that average past HbA1c level was a determinant of skin AF independent of age, gender, duration of diabetes, and smoking status, although single-point HbA1c was not associated with skin AF after adjustment for confounding factors. These findings are consistent with previous reports[13, 16] and support the idea that skin AF as an index of relatively long-term glycemic control (historical glycemic exposure) can be a better marker of diabetic complications than single-point HbA1c.

Notably, the present study revealed that skin AF values are associated with markers of early-stage atherosclerosis such as maxIMT and baPWV. This finding supports the idea that AGEs are accumulated before development of atherosclerosis. On the other hand, there was no significant association between skin AF and ABI in the present study. One possible explanation for this phenomenon is that the ABI values in the patients with type 1 diabetes were not increased in the present study, and thus, cannot be used as an index of atherosclerotic change. Indeed, in this study, mean and SD values of ABI in the patients with type 1 diabetes were similar to those of healthy controls and in almost normal range. Although there have been several studies that evaluated the association between skin AF and early-stage subclinical atherosclerosis in diabetic patients[23-27], very few of them were concerned with non-Caucasian patients. Very recently, the associations between skin AF and coronary artery calcification score[28] or IMT[24] have been shown in Japanese type 2 diabetes mellitus. Our study confirmed the findings of these previous studies and extended them to Japanese patients with type 1 diabetes mellitus. Furthermore, to the best of our knowledge, this is the first study to find that skin AF was an independent risk factor for IMT even after adjustment for the other established risk factors for atherosclerosis. It would be important to demonstrate whether skin AF reflects the risk of atherosclerosis even in its subclinical stage, while several clinical studies have already shown positive association between skin AF and cardiovascular disease[15, 29-32]. Increased skin AF may reflect early abnormalities in processes involved in atherosclerosis development.

This study also confirmed positive associations between skin AF and presence and/or severity of diabetic microangiopathies. Skin AF values were significantly higher in subjects with diabetic retinopathy and significantly associated with CVR-R, a marker of diabetic neuropathy. However, these associations were no longer statistically significant after adjustment for the established risk factors (e.g., age, duration of diabetes, HbA1c.) These results suggest that much of this association appears to be related to historical glycemic exposure, as shown in the DCCT/EDIC study[3].

On the other hand, skin AF was an independent risk factor for UACR even after adjustment for the other established risk factors for nephropathy. This finding is compatible with the experimental studies indicating that AGEs play important roles for development and progression of chronic renal disease, especially diabetic nephropathy[33]. Although these results were already reported in previous studies based on Caucasian patients[13, 17, 18], there are still few reports concerning Asian patients[13].

Experimental studies have shown that AGEs and their receptor system (RAGE) play an important role in the development of diabetic vascular complications. AGEs are engaged in vascular complications through the changing of three-dimensional structure of proteins by cross-linking. The binding of AGEs to RAGE is also known to cause phenotypic changes in various cells such as endothelial cells, smooth muscle cells, pericytes, and renal mesangial cells, leading to the pathogenesis of diabetic retinopathy, nephropathy, and macroangiopathies[8, 34-43]. Thus, AGEs not only represent various metabolic disorders as a marker of "metabolic memory", but also directly play critical pathogenic roles, which would be reason why skin AF can be a marker of diabetic complications independent of the other established risk factors.

We also assessed association between diabetic complications and pentosidine, a well-known fluorescent AGE for which causal relationship with renal disease has been reported[44, 45]. However, the present study found no significant association between serum pentosidine levels and diabetic complications in Japanese patients with type 1 diabetes mellitus. Similar to our result, several studies showed that tissue AGEs are better predictor for vascular disease as compared to circulating AGEs[25, 46, 47]. One of the reasons might be that tissue AGEs have slower turnover than circulating AGEs[48]. These findings suggest that skin AF may more useful marker for diabetic complications than circulating AGEs.

There were several limitations in this study. First, the number of study subjects was small. Therefore, to establish the utility of the measurement of skin AF in daily clinical practice, another study with larger sample size should be undertaken. Second, all participants were Japanese, and this may limit the generalizability of the results, since it is reported that there is an ethnic heterogeneity in skin AF[20]. However, from a different perspective, this report is valuable in that our showed the utility of skin AF in Japanese, since most of the previous studies were concerned with Caucasians. Third, skin AF cannot be measured by non-flu-

orescent AGEs, such as CML and CEL, because of the characteristics of the measurement principle of the AGE Reader. However, it was already demonstrated that non-fluorescent AGEs accumulated in skin are also strongly correlated with skin AF[12]. Fourth, although we tried to exclude the influence of the known interfering factors that can affect skin AF value, such as body creams and sunscreen creams[49], we could not eliminate the influence of unknown interfering factors. Finally, because of the cross-sectional nature of this study, the associations do not necessarily indicate causality. Further longitudinal studies are necessary to confirm the significance of skin AF in type 1 diabetes.

In conclusion, our study confirmed that skin AF represents historical glycemic exposure and that there were moderately strong associations between skin AF and a number of markers of diabetic complications even in Japanese patients with type 1 diabetes mellitus. Especially, skin AF was an independent risk factor for IMT even after adjustment for the other established risk factors for atherosclerosis. These findings suggest that increased skin AF may reflect early atherosclerotic changes and may be a good predictor of development and progression of diabetic macroangiopathy.

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Author Contributions S.O., N.K., and M.M. analyzed data and wrote the manuscript. M.T., F.S., D.K., A.K., and T.M. analyzed the data. I.S. contributed to the interpretation of the results and the discussion. All authors reviewed and approved the report. N.K. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.

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Conflict of Interest Disclosures

None.

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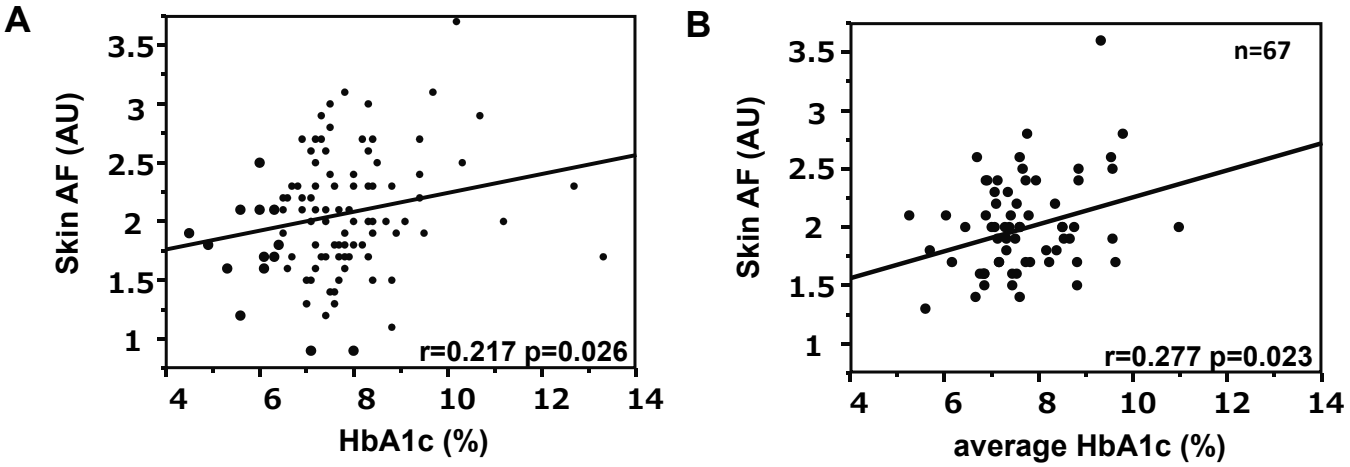

Supplementary Fig. 1. Association between skin AF and (A) single-point HbA1c, (B) average of past HbA1c values. Statistical analysis for association was performed using Pearson's univariate test.

Supplementary Table 1. Relative risk factors of baPWV in type 1 diabetes

	Univariate		Multivariate	
	r	*p*	*β*	*p*
Age (year)	0.691	< 0.001	0.529	< 0.001
Gender (male)	0.330	< 0.001	NI	
Duration of diabetes (years)	0.480	< 0.001	0.117	0.011
Smoking	0.196	0.047	NI	
BMI (kg/m^2)	0.247	0.011	NI	
Systolic BP (mmHg)	0.519	< 0.001	0.370	< 0.001
GGT (U/L)	0.218	0.026	NI	
HbA1c (%)	0.068	NS		
TG (mg/dL)	0.144	NS		
LDL-C (mg/dL)	0.111	NS		
HDL-C (mg/dL)	− 0.154	NS		
UA (mg/dL)	0.298	0.002	NI	
eGFR (ml/min/1.73 m^2)	− 0.417	< 0.001	− 0.142	0.036
Skin AF (AU)	0.450	< 0.001	NI	
R^2			0.700	

The threshold of statistical significance was defined as $p < 0.05$.
Gender: male = 1, female = 0, Smoking: yes = 1, no = 0.
Stepwise multivariate regression analysis was performed to determine the predictors of baPWV. The significant ($p < 0.05$) variables in Pearson's univariate analysis were selected for multivariate regression analysis.
Abbreviations: β, partial regression coefficient; NS, not significant; NI, not included in the model; BMI, body mass index; BP, blood pressure; GGT, γ-glutamyltransferase; HbA1c, glycated hemoglobin A1c; GA, glycoalbumin; TG, serum triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; eGFR, estimated glomerular filtration rate.

Supplementary Table 2. Relative risk factors of presence of retinopathy in type 1 diabetes

	Univariate		Multivariate	
	r	*p*	OR	95%CI
Age (year)	0.051	NS		
Gender (male)	−0.108	NS		
Duration of diabetes (years)	0.433	<0.001	1.19	1.10-1.30
Smoking	−0.046	NS		
BMI (kg/m^2)	0.034	NS		
Systolic BP (mmHg)	0.091	NS		
GGT (U/L)	−0.033	NS		
PG (mg/dl)	0.227	0.022	1.01	1.00-1.02
HbA1c (%)	0.140	NS		
TG (mg/dL)	−0.099	NS		
LDL-C (mg/dL)	−0.049	NS		
HDL-C (mg/dL)	0.128	NS		
UA (mg/dL)	−0.147	NS		
eGFR (ml/min/1.73 m^2)	−0.146	NS		
Skin AF (AU)	0.221	0.026	2.43	0.87-7.44
R^2			0.337	

The threshold of statistical significance was defined as $p<0.05$.
Gender: male=1, female=0, Smoking: yes=1, no=0.
Multiple logistic regression analysis was performed to determine the predictors of presence of retinopathy (yes=1, no=0). The significant ($p<0.05$) variables in Pearson's univariate analysis were selected for multiple logistic regression analysis.
Abbreviations: OR, odds ratio; NS, not significant; NI, not included in the model; BMI, body mass index; BP, blood pressure; GGT, γ-glutamyltransferase; HbA1c, glycated hemoglobin A1c; GA, glycoalbumin; TG, serum triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; eGFR, estimated glomerular filtration rate.

Supplementary Table 3. Relative risk factors of CVR-R in type 1 diabetes

	Univariate		Multivariate	
	r	*p*	*β*	*p*
Age (year)	−0.468	<0.001	−0.422	<0.001
Gender (male)	−0.124	NS		
Duration of diabetes (years)	−0.052	NS		
Smoking	−0.200	0.042	NI	
BMI (kg/m^2)	−0.094	NS		
Systolic BP (mmHg)	−0.144	NS		
GGT (U/L)	−0.119	NS		
HbA1c (%)	−0.356	<0.001	−0.290	<0.001
TG (mg/dL)	−0.099	NS		
LDL-C (mg/dL)	−0.112	NS		
HDL-C (mg/dL)	0.151	NS		
UA (mg/dL)	−0.163	NS		
eGFR (ml/min/1.73 m^2)	0.170	NS		
Skin AF (AU)	−0.342	<0.001	NI	
R^2			0.301	

The threshold of statistical significance was defined as $p<0.05$.
Gender: male=1, female=0, Smoking: yes=1, no=0.
Stepwise multivariate regression analysis was performed to determine the predictors of CVR-R. The significant ($p<0.05$) variables in Pearson's univariate analysis were selected for multivariate regression analysis.
Abbreviations: β, partial regression coefficient; NS, not significant; NI, not included in the model; BMI, body mass index; BP, blood pressure; GGT, γ-glutamyltransferase; HbA1c, glycated hemoglobin A1c; GA, glycoalbumin; TG, serum triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; eGFR, estimated glomerular filtration rate.

Supplementary Table 4. Relative risk factors of log(UACR) in type 1 diabetes

	Univariate		Multivariate	
	r	*p*	*β*	*p*
Age (year)	0.016	NS		
Gender (male)	−0.260	0.008	−0.318	0.001
Duration of diabetes (years)	−0.094	NS		
Smoking	0.044	NS		
BMI (kg/m^2)	−0.069	NS		
Systolic BP (mmHg)	−0.028	NS		
GGT (U/L)	0.084	NS		
HbA1c (%)	0.247	0.012	NI	
TG (mg/dL)	−0.001	NS		
LDL-C (mg/dL)	−0.034	NS		
HDL-C (mg/dL)	0.041	NS		
UA (mg/dL)	0.128	NS		
eGFR (ml/min/1.73 m^2)	−0.142	NS		
Skin AF (AU)	0.194	0.049	0.264	0.007
R^2			0.134	

The threshold of statistical significance was defined as $p < 0.05$.
Gender: male = 1, female = 0, Smoking: yes = 1, no = 0.
Stepwise multivariate regression analysis was performed to determine the predictors of log(UACR). The significant ($p < 0.05$) variables in Pearson's univariate analysis were selected for multivariate regression analysis.
Abbreviations: β, partial regression coefficient; NS, not significant; NI, not included in the model; BMI, body mass index; BP, blood pressure; GGT, γ-glutamyltransferase; HbA1c, glycated hemoglobin A1c; GA, glycoalbumin; TG, serum triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; eGFR, estimated glomerular filtration rate.