Dokkyo Medical Journal
2(1): 31-42, 2023

Original

Association of Skin Autofluorescence with Serum Lipids, Insulin Secretion Ability and Diabetic Complications in Patients with Poorly Controlled Type 2 Diabetes

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Summary

Aims: The current study was performed to evaluate the association between the accumulation of advanced glycation end-products (AGEs) in skin and diabetes-related metabolic markers, atherosclerosis and diabetic complications in patients with poorly-controlled type 2 diabetes.

Methods: The skin AGE levels were assessed non-invasively by measuring skin autofluorescence (SAF). The modified low-density lipoprotein (LDL) was assessed by measuring lectin-like oxidized LDL receptor-1 ligand-containing apolipoprotein B (LAB).

Results: SAF was significantly higher in patients with type 2 diabetes than in the healthy control subjects. In patients with type 2 diabetes, SAF correlated positively with age and duration of diabetes. Furthermore, SAF exhibited significant negative correlations with LDL-cholesterol (C), triglyceride (TG) and body mass index. SAF was also negatively correlated with urinary C peptide immunoreactivity (U-CPR). In addition, SAF also exhibited significant positive correlations with cardio-ankle vascular index (CAVI) and intimal medial complex thickness of the carotid artery (IMT). Furthermore, there were significant correlations between SAF and estimated glomerular filtration rate (eGFR)(negative), urinary albumin excretion (positive).

Conclusion: SAF may be negatively associated with serum lipids such as LDL-C and TG, insulin sensitivity ability and renal dysfunction evaluated by eGFR, and positively associated with aging, duration of diabetes, markers of atherosclerosis (CAVI and IMT) in patients with poorly-controlled type 2 diabetes.

Key Words: SAF, type 2 diabetes, LDL-C, U-CPR

Received October 3, 2022; accepted October 9, 2022; advance publication by J-STAGE June 22, 2023
https://doi.org/10.51040/dkmj.2022-041
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Introduction

Advanced glycation end-products (AGEs) are generally defined as a heterogeneous class of final compounds that are mainly produced due to glycation of amino groups on proteins, lipids, and nucleic acids by non-enzymatic mechanisms, which are known as Maillard reactions, and can accumulate in most tissues (especially in long-lived tissues) including the vasculature, muscle, skin, liver, kidney, and crystalline lens[1,2]. The accumulation of these AGEs in various tissues can be derived both from endogenous production, and from exogenous diet, especially in which modern western diet might accelerate the accumulation[2-4]. Although the accumulation of AGEs in tissues physiologically increases with age at a relatively slow rate[5,6], its production and accumulation is accelerated by hyperglycemia and/or oxidative stress[7-9]. Therefore, diabetes can be an important risk factor for the increased accumulation of AGEs.

Importantly, the AGEs per se can, in turn, increase oxidative stress directly, or via the AGEs-receptor for AGEs (RAGE) axis[1,2,10], which plays an important role in the progression of diabetic cardiovascular diseases[1]. In the clinical studies, it has been reported that the baseline circulating AGE levels were associated with the incidence of cardiovascular diseases in type 1 diabetes[11] or the cardiovascular and coronary mortality in women with type 2 diabetes[12]. However, the circulating AGE levels do not necessarily correlate with the AGE accumulation in tissues[13]. Given the fact that the AGEs accumulate systemically and remain relatively stable over time, while the circulating AGEs have relatively short half-lives[2,14], the AGE levels in tissues may be more appropriate as a marker to predict the progression of diabetic complications than the circulating AGE levels. Recently, it has become possible to assess skin AGE levels very easily and non-invasively by measuring autofluorescence (AF)[15,16]. A previous study showed that skin autofluorescence of AGEs (SAF), but not the circulating N-(carboxymethyl) lysine levels (CML: one of the AGEs), exhibited an independent association with arterial stiffness, as measured by the aortic pulse wave velocity, in type 1 diabetes without clinical cardiovascular events[17]. Furthermore, it has been reported that SAF was associated with cardiac mortality in patients with diabetes[18], or future cardiovascular events and/or mortality in patients with type 2 diabetes[19]. These findings suggest that SAF may reflect the degree of cardiovascular damage and may be a useful marker to predict future cardiovascular events and the mortality in patients with diabetes. However, the associations between SAF and various metabolic markers related to diabetes, degrees of atherosclerosis, or diabetic complications are not yet fully evident, especially in patients with poorly controlled type 2 diabetes. Therefore in the current study, we performed a cross-sectional analysis on these associations in patients with poorly controlled type 2 diabetes who required hospitalization for glycemic control.

Patients and Methods

Patients

In the patients (n = 70) who had taken part in our previous study [registered as UMIN000025767[20]], the 69 patients in whom measurement of SAF was performed were included for analyses in this study. In brief, in the previous study[21], patients with type 2 diabetes who were hospitalized for treatment of poor glycemic control were prospectively and consecutively enrolled from February 2017 to August 2017.

The detailed key inclusion and exclusion criteria of type 2 diabetic patients in the study has been previously described at the UMIN clinical trials registry[20]. Twenty healthy control subjects were newly enrolled for this study. The control subjects were not hospitalized. The characteristics and laboratory data of the 69 patients and 20 healthy subjects at the time of enrollment are shown in Table 1.

Methods

Blood tests on the type 2 diabetic patients were basically performed at 9:00 a.m., after an overnight fast for at least 10 hrs, on the day after hospitalization. Until analysis for soluble lectin-like oxidized low density lipoprotein receptor-1 ligands containing apolipoprotein B (LAB), samples were preserved frozen at -80℃. Body weight (BW) and blood pressure were also measured during admission. The measurement-methods for LAB, high sensitivity C-reactive protein (hsCRP), fibrinogen, fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), insulin, estimated glomerular filtration rate (eGFR), and

Table 1 Clinical features and laboratory data at baseline in patients with type 2 diabetes and control subjects

	Control subjects	Patients with type 2 diabetes	P (95%CI)
No. (male/female)	20 (5/15)	69 (44/25)	
Age (year)	64 (56.3, 67.5)	62 (48, 72)	0.8402
Duration of diabetes (year)		7 (2, 12)	
BMI (kg/m^2)	22.0 ± 2.9	24.5 ± 4.5	0.0043 (0.8277-4.2175) *
FPG (mg/dL)	91 (87, 97.8)	176 (142, 258)	< 0.001*
HbA1c (%)	5.5 (5.5, 5.8)	9.8 (8.8, 11.7)	< 0.001*
eGFR (ml/min/1.73 m^2)	78.8 (64.4, 81.7)	79.6 (63.8, 94.3)	0.8110
TG (mg/dL)	66 (55.3, 85.3)	129 (99.8, 177.8)	< 0.001*
HDL-C (mg/dL)	71.5 (66.5, 90)	47 (40.8, 61.5)	< 0.001*
LDL-C (mg/dL)	114.7 ± 28.0	123.5 ± 45.3	0.2941
LAB (ng cs/mL)		3.1 (2.6, 3.8)	
Insulin (uU/mL)		5.4 (4, 9.5)	
hsCRP (mg/dL)		0.144 (0.062, 0.358)	
Fibrinogen (mg/dL)		349.9 ± 98.9	
AST (U/L)		18 (15, 23)	
ALT (U/L)		18 (13, 27)	
GGT (U/L)		30 (20, 39)	
CAVI			
IMT (mm)		1 (0.8, 1.2)	
UAE (mg/g.Cr)		16.7 (6.4, 72.6)	
U-CPR (ug/day)		56.8 (28.4, 94.0)	
CV_{RR} (%)		1.75 (1.31, 2.55)	
SAF (AU)	2.4 ± 0.4	2.8 ± 0.6	0.0023 (0.1409-0.6085) *
Diabetic therapy			
Insulin (-)		34	
Metformin (+)		24	
MD/MDA/MDP/MDSg/MDGn		7/3/2/2/1	
MDPSg/MDPSgGp/MDASg/		1/1/2	
MDASu/MGp/MGpPSg/		1/2/2	
Metformin (-)		10	
D/DA/DASu/DSu/PAD/PGp/N		2/1/1/1/1/1/3	
Insulin (+)		35	
Metformin (+)		16	
MD/ MDA/MDP/MDSg/MDGn		4/1/2/1/1	
MDPSg/MDPSgGp/MDASg/		1/0/0	
MDASu/MGp/MGpPSg/		0/0/0	
MDSu/MDPA/M/MA/MASu		1/1/2/1/1	
Metformin (-)		19	
D/DA/DASu/DSu/PAD/PGp/GpGn/N		5/2/0/0/0/0/3	
P/DP/DGn/A		3/4/1/1	
Anti-hypertensive drugs		31	
Ar/C/ArC/ArCB/ArCAb/ArCT/CT/CBAb		4/6/16/1/1/1/1/1	
Anti-hyperlipidemic drugs			
Statins (+)		20	
S/SE/SCo		18/2/0	
Statins (-)		49	
E/Co/N		2/1/46	

Data are expressed as median with 25th and 75th interquartile range because of the skewed distribution, excluding BMI, LDL-C and SAF. These are expressed mean ± standard deviation (SD) because of its normal distribution. Comparisons in variables between diabetic patients and healthy subjects were performed by a Mann-Whitney U test excluding BMI LDL-C and SAF. For these variables, the comparison was confirmed by an unpaired *t* test (Welch's *t* test). P: P value, $P < 0.05$ is defined as statistical significance (*).

Abbreviations: CI: confidence intervals, BMI: body mass index, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, eGFR: estimated glomerular filtration rate, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, SAF: skin autofluorescence, AU: autoflurorescence in arbitrary, Diabetic therapy: the number of the patients with respective diabetic therapies, M: metformin, D: dipeptidyl peptidase 4 (DPP4) inhibitors, A: α glucosidase inhibitor, P: pioglitazone, Sg: Sodium glucose cotransporter 2 (SGLT2) inhibitors, Gn: glinides, Gp: glucagon-like peptide (GLP) -1 receptor agonists, Su: sulfonylureas, N: no antidiabetic drugs, Antihypertensive drugs: the number of the patients with respective antihypertensive drugs, Ar: angiotensin-II receptor blockers (ARB), C: calcium channel blockers, T: thiazides, B: beta adrenalin receptor blockers, Ab: alpha adrenalin receptor blockers, Anti-lipids drugs: S: statins, E: ezetimibe, Co: colestimide

In therapies, for example, D and DA mean respectively DPP4 inhibitors alone and DPP4 inhibitors + α glucosidase inhibitors

the intimal medial complex thickness of the carotid artery (IMT) have been described in our previous study[21]. The mean HbA1c was calculated as the mean value for the past 1 year in those patients who visited the hospital at least 5 times within 1 year before the baseline time point.

Measurement of the SAF

Non-invasive measurement of SAF was performed in the right upper arm using the AGE reader mu (Diagnoptics Technologies B.V., Groningen, Netherlands), which can assess AF of AGEs accumulated in the region approximately 1 mm beneath the epidermis or dermis.

Measurement of the cardio-ankle vascular index

For the index of arterial stiffness, the cardio-ankle vascular index (CAVI) was used. The CAVI was assessed on the right side using a specific apparatus, VaSera VS3000 (Fukuda Denshi, Tokyo, Japan).

Measurement of serum lipids

Serum low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and serum triglycerides (TG) were measured using enzymatic assays. The Determiner L TG® reagents (Kyowa Medics, Tokyo, Japan) were used to measure TG levels. HDL-C was measured directly by a method based on selective solubilization of different lipoproteins by proprietary detergents using Cholestest N HDL-C® (Daiichi Pure Chemicals, Tokyo, Japan). LDL-C was also measured directly using Cholestest LDL® (Daiichi Pure Chemicals).

Measurement of coefficient of variation of RR intervals

The coefficient of variation of RR intervals (CV_{RR}) was evaluated on electrocardiogram by using recordings of consecutive cardiac cycles, measured in the morning, based on the following formula:

CV_{RR} = standard deviation of RR/mean RR × 100.

Any patient with arrhythmia was excluded.

Measurement of urinary albumin excretion

Urinary albumin excretion was measured once in a 24-hour urine specimen during admission by an immunoturbidimetric methods using the reagent kit, TAC-2 test albumin U (Medical & biological laboratories Co., LTd, Nagoya, Japan). Albumin values were corrected for urinary creatinine concentration.

Measurement of urinary C-peptide

Urinary C peptide (U-CPR) was measured once in a 24-hour urine specimen during admission by a chemiluminescent enzyme assay, based on a 2 step sandwich method using the reagent kit, Lumipulse-presto C-peptide (Fujirebio Co., Ltd, Tokyo, Japan).

Evaluation of diabetic retinopathy

Diabetic retinopathy, i.e., no diabetic retinopathy (NDR), simple diabetic retinopathy (SDR), or proliferative diabetic retinopathy (PDR), was evaluated based on Davis' criteria by ophthalmologists in our hospital.

Ethics Policy

All subjects gave written informed consent for inclusion in the study, registered as UMIN000025767. The previous and the current studies were approved by the Local Ethics Committee at Dokkyo Medical University Saitama Medical Center (study numbers and approval days: 1654, 1/20/2017 and 2072, 10/16/2020 respectively). This study was performed according to the guidelines of the Declaration of Helsinki.

Statistical methods

The normality of the data for each variable was confirmed by Kolmogorov-Smirnov test and/or Shapiro-Wilk test. The SAF exhibited a normal distribution. Among the variables included in Table 2, body mass index (BMI), diastolic blood pressure (DBP), LDL-C, fibrinogen, and CAVI exhibited a normal distribution. Therefore, the correlations between SAF and these variables were examined using Pearson's correlation. The remaining variables exhibited skewed distributions. Among the variables with a skewed distribution, FPG, HbA1c, TG, HDL-C, LAB, insulin, hsCRP, U-CPR and CV_{RR} exhibited normal distributions after $\log_{10}$-transformation. Therefore, the associations between SAF and these variables were examined using the data after $\log_{10}$-transformation by Pearson's correlation. None of the remaining variables exhibited a normal distribution, even after $\log_{10}$-transformation. Thus, the correlations between SAF and these variables were examined using Spearman's correlation as a non-parametric coefficient. In multiple regression analysis, we established 4 models, in which the dependent variables that we considered as important atherosclerotic or metabolic markers were selected. In these models, the HbA1c, HDL-C, LAB andU-CPR were $\log_{10}$-transformed due to above-described reason. In model 4, we decided to add $\log_{10}$-transformed age, duration of diabetes, and eGFR, although these markers did not

Table 2 The correlation of SAF with multiple variables

	r or ρ	P
Age (69)	0.4866	< 0.001*
Duration of diabetes (69)	0.3112	0.0093*
BMI (69)	-0.4002	< 0.001*
FPG (69)	-0.1478	0.2254
HbA1c (69)	-0.1711	0.1599
Mean HbA1c in an year (16)	0.0586	0.8294
SBP (69)	-0.0068	0.9559
DBP (69)	-0.0298	0.8081
TG (68)	-0.2436	0.0453*
HDL-C (68)	0.1014	0.4108
LDL-C (68)	-0.4192	< 0.001*
LAB (69)	0.1377	0.2591
Insulin (29)	-0.1734	0.3682
hsCRP (69)	0.0505	0.6804
Fibrinogen (69)	0.1029	0.4001
AST (69)	0.00215	0.8607
ALT (69)	-0.0936	0.4442
GGT (69)	-0.0435	0.7288
eGFR (69)	-0.4453	< 0.001*
CAVI (65)	0.3160	0.0103*
IMT (56)	0.2991	0.0238*
UAE (69)	0.4133	< 0.001*
U-CPR (68)	-0.3038	0.0118*
CV_{RR} (62)	-0.3761	0.0026*

SAF, BMI, DBP, LDL-C, Fibrinogen, CAVI-index followed a normal distribution as confirmed by a Kolmogorov-Smirnov test and/or Shapiro-Wilk test. Because of the skewed distribution for FPG, HbA1c, TG, HDL-C, insulin, hsCRP, U-CPR and CV_{RR}, these variables are log_{10}-transformed. After log_{10}-transforming, these variables followed a normal distribution. These correlations were evaluated using Pearson's correlation coefficient. For age, duration, SBP, AST, ALT, GGT, IMT, eGFR, UAE because these variables had skewed distribution even after log_{10}-transforming, these correlations were evaluated using Spearmann's correlation coefficient. For insulin, only the patients without insulin-treatment were included. r and ρ means respectively Pearson's and Spearmann's correlation. P: P value, $P < 0.05$ is defined as statistical significance (*). Parentheses mean the number of patients.

Abbreviations: SAF: skin autofluorescence, BMI: body mass index, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, SBP: systolic blood pressure, DBP: diastolic blood pressure, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, LAB: soluble lectin-like oxidized LDL receptor 1 ligands containing apolipoprotein B, hsCRP: high-sensitivity C reactive protein, AST: aspartate transaminase, ALT: alanine transaminase, GGT: gamma-glutamyl transpeptidase, eGFR: estimated glomerular filtration rate, CAVI: cardio-ankle vascular index, IMT: intimal medial complex thickness, UAE: urinary albumin excretion, U-CPR: Urinary C-peptide immunoreactivity, CV_{RR}: Coefficient of variation of R-R interval on electronic cardiogram

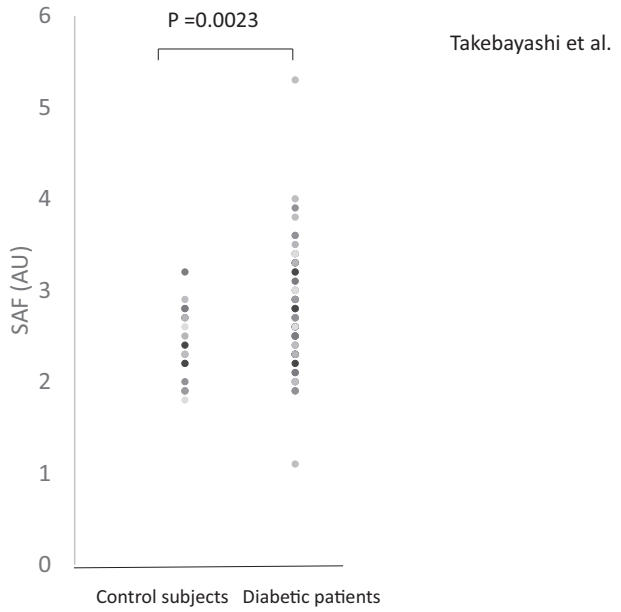

Figure 1 SAF levels in healthy subjects without diabetes (n = 20) and in patients with type 2 diabetes (n = 69). SAF: skin autofluorescence

exhibit normal distributions even after $\log_{10}$-transformation as described above, because we considered that these markers may demonstrate important associations with SAF. Comparisons of BMI, LDL-C, and SAF between type 2 diabetic patients and healthy subjects were performed using an unpaired *t* test; Welch's *t* test was chosen based on the significant difference in the variance by F test. Comparisons between these groups for age, FPG, HbA1c, eGFR, TG, and HDL-C were performed using the Mann-Whitney U test because of the skewed distribution of these variables. Comparisons of SAF between the two subgroups in patients with type 2 diabetes were performed using an unpaired *t*-test; Student's *t* test was chosen. For comparison for SAF among the 3 groups, homogeneity was confirmed by using the Bartlett test. A parametric comparison was conducted using one-way analysis of variance (ANOVA) after confirmation of equal variance. After confirmation of the significance by ANOVA, a *post hoc* Holm test was conducted.

All statistical analyses were performed using Bell-Curve for Excel software (Social Survey Research Information Co., Ltd, Tokyo, Japan). A *P*-value of less than 0.05 was estimated as indicating statistical significance (two-sided).

Results

SAF was significantly higher in diabetic patients than the healthy control subjects (Table 1 and Fig. 1). SAF correlated significantly and positively with age, duration of diabetes, CAVI, IMT and UAE. There were negative correlations between SAF and BMI, TG, LDL-C, eGFR, U-CPR, and CV_{RR}. On the other hand, no correlations were significant between SAF and FPG, HbA1c and mean HbA1c over one year. These results are summarized in Table 2. The correlations between SAF and LDL-C, TG, LAB, BMI, U-CPR and CAVI are also presented in Fig. 2A-F.

There was a significant positive correlation between LDL-C and $\log_{10}$-transformed LAB ($r = 0.4601$, $P < 0.001$: Fig. 2G). When the patients were divided into two groups based on the presence or absence of statin-treatment, a significant negative correlation was only observed between SAF and LDL-C in the patients without statins ($r = -0.4773$, $P < 0.001$ in the no-statin group respectively; $r = -0.2366$, $P = 0.3152$ in the statin group, respectively).

In multiple regression analysis with the dependent variables, significant negative associations between SAF and LDL-C were evident in models 1-4. SAF also exhibited a significant negative association with $\log_{10}$-transformed U-CPR in one model (model 3) including this variable. SAF was significantly and positively associated or had a tendency towards an association with CAVI in models 1, 2 and 3, respectively. Significant negative associations between SAF and BMI were found in models 2 and 4. There were no associations between SAF and $\log_{10}$-transformed HbA1c in any of the models. Tendencies towards associations with $\log_{10}$-transformed age or eGFR were observed in model 4. These results are presented in Table 3.

There were no significant differences in SAF between the patients treated with or without insulin, metformin, dipeptidyl peptidase 4 (DPP4) inhibitors or statins. There were no gender-related differences in SAF. No difference in SAF was found between the patients with or without a smoking history. As for diabetic retinopathy, there were no significant differences in SAF among the patients with NDR, SDR, and PDR.

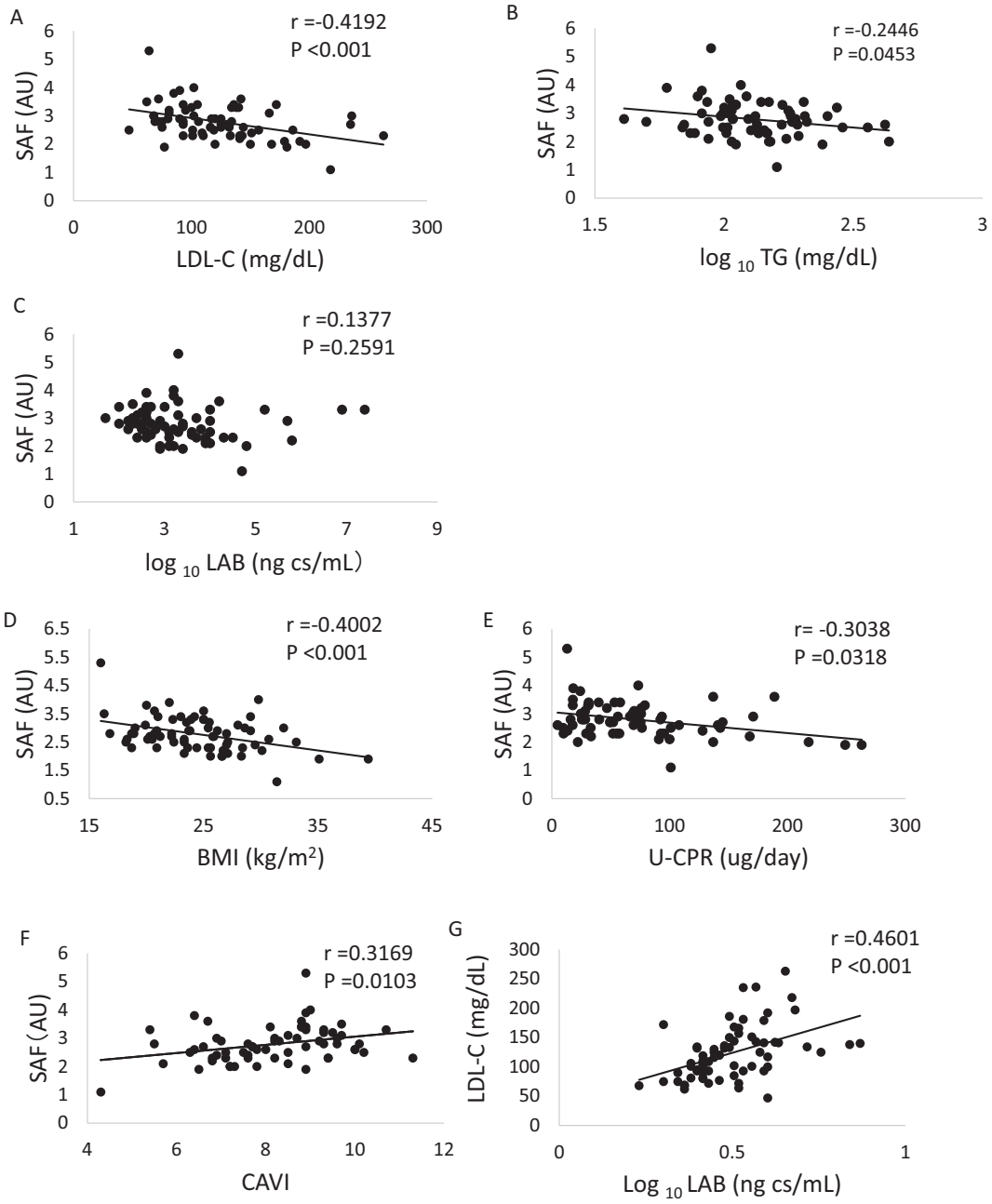

Figure 2 **A-F**. Correlations between SAF and LDL-C, TG, LAB, BMI, U-CPR. **G**. Correlation between LDL-C and LAB. TG, LAB, and U-CPR were log_{10}-transformed due to the skewed distribution. SAF: skin autofluorescence, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, LAB: soluble lectin-like oxidized LDL receptor 1 ligands containing apolipoprotein B, BMI: body mass index, U-CPR: Urinary C-peptide immunoreactivity

Discussion

In the current study, we examined patients with type 2 diabetes who were under poorer glycemic control than those in most previous studies. First, we compared SAF in these patients with those in healthy subjects, and then investigated the association between SAF and various metabolic or atherosclerosis-related markers in these patients. SAF was significantly elevated in patients with type 2 diabetes compared with control subjects, which was consistent with previous reports in patients with diabetes or type 2 diabe-

Table 3 Multiple regression analysis with SAF as the dependent variable

Model 1	β	P	(R^2: 0.2555)
CAVI	0.2853	0.0167*	
HbA1c (%)	0.0294	0.8058	
LDL-C (mg/dL)	-0.4021	< 0.001*	
Model 2	β	P	(R^2: 0.3234)
CAVI	0.1931	0.1156	
HbA1c (%)	-0.0014	0.9903	
HDL-C (mg/dL)	-0.0768	0.5175	
BMI (kg/m^2)	-0.3022	0.0184*	
LDL-C (mg/dL)	-0.3513	0.0028*	
Model 3	β	P	(R^2: 0.3071)
CAVI	0.2107	0.0790	
HbA1c (%)	-0.0588	0.6302	
LDL-C (mg/dL)	-0.3579	0.0015*	
U-CPR (ug/day)	-0.2540	0.0280*	
Model 4	β	P	(R^2: 0.4007)
Age (year)	0.1985	0.1890	
Duration of diabetes (year)	-0.0293	0.8117	
HbA1c (%)	0.0349	0.7471	
eGFR (ml/min/1.73 m^2)	-0.2336	0.0861	
BMI (kg/m^2)	-0.2516	0.0301*	
LDL-C (mg/dL)	-0.2833	0.0124*	

SAF, CAVI, LDL-C, and BMI followed the normal distribution confirmed by a Kolmogorov-Smirnov test and/or Shapiro-Wilk test. All variables except for CAVI, LDL-C, and BMI were $\log_{10}$-transformed because of the skewed distribution.

β: standard partial regression, R^2: coefficient of determination, P: P value, P < 0.05 is defined as statistical significance (*).

Abbreviations: CAVI: cardio-ankle vascular index, HbA1c: hemoglobin A1c, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, BMI: body mass index, LAB: soluble lectin-like oxidized LDL receptor 1 ligands containing apolipoprotein B, duration: duration of diabetes, eGFR: estimated glomerular filtration rate

tes[18,22,23]. Because diabetes and hyperglycemia accelerate the formation and the accumulation of AGEs[5,9,24], the result is plausible.

SAF correlated positively with age and the duration of diabetes, which is also consistent with previous studies[15,25]. Because the accumulation of AGEs is largely influenced by aging[5,6] and diabetes, and because the AGEs are hardly removed and remain in tissues in the long-term, even after glycemic control is ameliorated[14], these associations also appears to be compatible. On the other hand, SAF did not correlate with HbA1c or FPG, as markers of glycemic control, in this study. The negative findings for an association between SAF and HbA1c are essentially consistent with previous reports[15,18,22,25-27]. No correlation was observed, even between SAF and mean HbA1c over one year, unlike the previous reports in patients with diabetes[15,18]. However, the number of patients in whom the mean HbA1c could be assessed was very small in this study, which may have affected the negative result. In addition, a mean HbA1c longer than 1 year may appropriately determine an association.

In the current study, significant negative correlations were found between SAF and LDL-C or TG. One report showed a negative correlation between SAF and TC in patients who have chronic kidney disease (CKD) stage 3 with or without diabetes[23]. In contrast, Meerwaldt et al. showed the significant positive corre-

lation between SAF and LDL-C and TG in either patients with type 1 or type 2 diabetes[18]. On the other hand, some studies showed no correlation between SAF and LDL-C, HDL-C, TG[17,22,26,27]. We cannot fully explain the reason for the discrepancy in results, particularly between this study and that in Meerwaldt et al. However, the differences in patients' backgrounds including race (Japanese in this study vs. Caucasians more than 90% in Meerwaldt et al.) and glycemic control (higher in this study), ratio of the patients receiving statins (lower in this study) might have affected the results. The results in this study is interesting because both LDL-C and AGEs are known as risk factors of cardiovascular disease[18,19,28]. Notably, the significant correlation between SAF and LDL-C was only observed in patients that were not treated with statins when the patients were divided into 2 groups with or without statins. It might be interesting to speculate that the complicated effect of statins including LDL-C lowing and anti-oxidative effects modifies the real association between AGEs and lipids at least in part.

Interestingly, a significant negative correlation between SAF and U-CPR, which reflects endogenous insulin secretion ability, was also noted. Although we were not aware of any previous studies investing this relationship, the finding appears to be plausible, since it is likely that the reduction of endogenous insulin secretion ability likely leads to an intermittent or a continuous hyperglycemia in relatively long-term, despite under the administration of anti-hyperglycemic drugs, including insulin injection.

We found a significant negative correlation between SAF and BMI in this study. The previous reports of associations between SAF and BMI are also discrepant. While some reports have demonstrated significant positive associations between SAF and BMI[18] or no association[23] in patients with type 2 diabetes, a significant negative correlation or a tendency towards a negative correlation have been observed in Japanese type 2 diabetic patients[22,27], which are consistent with our findings. It is difficult to explain these discrepancies properly. However, the differences in the patients' backgrounds, including race, might at least in part explain the discordant results. As expected, SAF positively correlated with CAVI, reflecting arterial stiffness, and IMT (a surrogate marker of atherosclerosis). These results are consistent with the previous studies showing significant associations or a tendency towards associations between SAF and CAVI or brachial-ankle pulse wave velocity (baPWV), or IMT in patients with type 2 diabetes[25,27,29]. The results are considered relevant because the AGEs can promote the modification of matrix proteins, such as collagen and elastin, in the arteries, leading to an increase in the risk of cardiovascular diseases[7,24].

There were significant correlations between SAF and eGFR (negatively) or UAE (positively), both of which reflect the degree of renal dysfunction. In previous study, the AGEs could accumulate at high levels in the glomerular basement[2], which likely influences renal function. In addition, because it is known that the AGEs are excreted through the kidneys[30], renal dysfunction could increase the circulating AGEs levels[31], which would result in the accumulation of AGEs in systemic tissues. However, in the current study, it is unclear whether the associations observed in diabetic patients between AGEs and renal dysfunction as reflected by eGFR or UAE was direct, because there was no difference in eGFRs between control subjects and diabetic patients despite the fact that AGEs was elevated in the latter compared with the former. In fact, in multiple regression analysis, the association of eGFR with SAF as dependent variable was weak and no significant. SAF also exhibited a significant negative correlation with CV_{RR}, which may reflect parasympathetic function possibly due to diabetic neuropathy. On the other hand, there were no differences on SAF among patients classified by the degree of diabetic retinopathy (i.e., NDR, SDR or PDR). These findings are consistent with a previous report showing that SAF in patients with type 2 diabetes[32] was associated with diabetic neuropathy, but not retinopathy. However, it should be noted that a recent report suggested a close association between SAF and the retinopathy stages[33].

There are some limitations of this study. Firstly, the number of patients was relatively small. Secondary, the study was based on cross-sectional observation. On the other hand, few studies have investigated SAF in the patients with very poor glycemic control, as reflected by HbA1c of approximately 10%. Our study demonstrated a possible association between SAF and various markers related to diabetes in such patients. In

a previous study in type 2 diabetic patients with relatively good control (median HbA1c 6.6%)[26], although the correlations between SAF and parameters such as age (positive), TG (negative) and HbA1c (no correlation) were similar with the results in this study, the results with the correlation of SAF with duration of diabetes (no correlation) or hsCRP (negative correlation) were different compared with those in this study. Therefore, because the situation of glycemic control may influence the associations between SAF and some parameters, it may be interesting to investigate the differences on these associations between patients with better or poorer glycemic control in a future study. Finally, we could not evaluate the blood-flow-dependent vasodilator response (FMD) of brachial artery using Endo-PAT® or the dedicated ultrasound diagnostic equipment for the assessment of early cardiovascular damages, mainly because of technical problem. This is also one of the limitations in this study.

In conclusion, in patients with poorly controlled type 2 diabetes, SAF correlated positively with both age and duration of diabetes. Furthermore, SAF exhibit a significant negative correlation with LDL-C and TG, and U-CPR. SAF also exhibited significant positive associations with CAVI and IMT. In addition, there were significant correlations between SAF and eGFR (negatively) and UAE (positively), and BMI (negatively). Thus, more detailed analysis is warranted here in a future prospective study in particular, with respect to serum lipids, especially LDL-C, and U-CPR as a marker of insulin secretion ability.

Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science (JSPS) [grant Numbers 19K09018 (Koshi Hashimoto.) and 20K11394 (Kenji Hara)].

Author contributions

K.T. and T.S. investigated the patients. K.T. examined the data and wrote this manuscript. T.T., H.S., M. Y., K.H., and K. H., reviewed this manuscript.

Institutional Review Board Approval

Approval number: 1654 and 2072 at the Local Ethics Committee at Dokkyo Medical University Saitama Medical Center

Conflict of interest

All authors have no conflict of interest to declare.

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