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Research article

Skin accumulation of advanced glycation end products and cardiovascular risk in Korean patients with type 2 diabetes mellitus

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HIGHLIGHTS

- Unclear association between skin accumulation of AGEs and T2DM complication risk.
- Study Population: Korean patients with T2DM.
- Strong correlation between skin levels of AGEs and diabetes duration.
- Independent association between AGEs and arterial pulse wave conduction velocity.
- Association between increased SAF levels in with PWV, vein age, arterial stiffness.

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ABSTRACT

Background: The formation of advanced glycation end products (AGEs) takes place during normal aging; however, their production is faster in people having diabetes. The accumulated AGEs reportedly play a role in the occurrence of various age-related disorders. Furthermore, the skin autofluorescence (SAF) technique can be used to detect accumulated AGEs levels. There are few reports on the association between skin accumulation of AGEs and risk of complications in type 2 diabetes mellitus.

Methods: In this study, we aimed to describe the association between the skin accumulation of AGEs and cardiovascular risk factors in Korean patients with type 2 diabetes. A total of 310 Korean patients with diabetes were enrolled, and the levels of AGEs were measured using SAP. Levels of fasting blood glucose (FBS), triglycerides, total cholesterol, low- and high-density lipoprotein cholesterol, proteinuria, arterial pulse wave velocity (PWV), and blood vessel age were measured using an automatic waveform analyzer. General linear models were used to identify the independent effect of AGEs after adjusting for covariates (age, weight, and duration of diabetes).

Results: The skin levels of AGEs were strongly correlated with the diabetes duration. Significant independent associations were observed for AGEs with FBS ($P < 0.01$), proteinuria ($P < 0.001$), and PWV ($P < 0.001$). The advanced glycated product was independently associated to the arterial pulse wave conduction velocity that is used as a representative method for measuring arteriosclerosis by analysis early cardiovascular risk factors.

Conclusion: Our results show that an increase in SAF levels in Korean patients with type 2 diabetes is associated with PWV and vein age, and thereby with arterial stiffness. Therefore, our results suggest that AGEs are associated with cardiovascular risk factors. The level of AGEs can thus be used as an indicator of cardiovascular diseases in the clinical diagnosis of patients with type 2 diabetes.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder caused by dysfunction of beta cells, which causes insulin resistance. It is one of the diseases with the highest prevalence in the 21st century [1]. In 2011, approximately 366 million cases of diabetes mellitus were registered globally; there is an expected increase to 552 million in 2030 [2]. According to the 2018 National Statistical office survey,

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T2DM was the 6th cause of death in Korea [3]. Moreover, one in three adults aged above 65 years were diagnosed with T2DM [4]. An increase in the 10-year prevalence rate of T2DM in Korea from 9% in 2012 to 11% in 2013 was observed [5]. Several studies have shown that patients with T2DM have an increased risk of short- and long-term complications, which lead to increased mortality and morbidity, and thereby early death [6, 7, 8].

Hyperglycemia, in addition to the formation of glycated hemoglobin (HbA1c), also leads to the formation of glycated low-density lipoprotein (LDL), which accelerates atherosclerosis of blood vessels and glycation of collagen, thus promoting aging [9, 10, 11]. Furthermore, hyperglycemic environments cause nerve cell damage through glycation of myelin compounds [12], and form advanced glycation end products (AGEs) through glycation of protein compounds [13, 14].

AGEs accumulate with age due to the constant formation of glycated products and the slow rate of their degradation. The Maillard reaction is a non-enzymatic glycation process comprising a series of complex biochemical reactions. The first reaction is an interaction between the amino group of proteins or nucleic acids with the aldehyde group of glucose, resulting in Schiff's base. This is followed by the formation of Amadori products, which comprises the rearrangement of Schiff's base, ensued by the formation of a stable ketoamine, and resulting in a reactive carbonyl compounds. The end product of this reaction is AGEs [15, 16]. AGEs are formed from the oxidation and non-enzymatic glycation of proteins, lipids, and nucleic acids. AGEs are produced during all stages of life; however, elevated levels of AGEs in patients with diabetes are due to biochemical, molecular, and functional changes [17]. Therefore, elevated levels of AGEs can be used as a biomarker for predicting diabetes.

Accumulated AGEs lead to tissue damage by worsening oxidative stress and inflammation, and causing micro- and macrovascular dysfunction, including retinopathy, nephropathy, neuropathy, myocardial infraction, and stroke [18, 19]. Elevated levels of AGEs have been reported in chronic conditions such as diabetes, aging, renal complications, and inflammation [20, 21, 22].

AGEs form both intracellular and extracellular cross links with protein components, thus altering their functional and mechanical properties [23]. The accumulated advanced glycated products induce the production of various cytokines, hormones, and free radicals. When these substances bind to the final advanced glycated product receptors (receptors for AGEs, RAGEs) in blood vessels, intracellular oxidative stress occurs [24, 25, 26]. It has been reported that oxidative stress contributes to the occurrence of complications in diabetes by damaging cells [27, 28]. Binding of AGEs to the cell surface receptors (RAGEs) leads to altered cell functioning.

Blood tests are an invasive, expensive, and time consuming technique for measuring HbA1c levels in the early or late stages of diabetes [29]. Blood tests also require a clinical setting and an adequate laboratory environment. However, with the development of spectrometry fluorescence techniques, glycated proteins can be assessed non-invasively. Several previous studies have reported significant associations between HbA1c levels and SAF [30, 31]. Fluorescence spectrometry is widely used in biochemical and molecular biology and involves electronic transitions between the lower and excited energy states, during which molecules absorb and re-emit energy at longer wavelengths. A broad band of the fluorescence spectrum is formed by the relaxation of excited molecules through the internal conversion of electronic energy in the molecule to rotational and vibrational modes [32]. The fluorescence phenomenon takes place at 10^{-9} s, at which the fluorophore emits the light it absorbed at longer wavelengths, thus forming specific excitation and emission peaks [33]. Therefore, fluorescence techniques can be used to investigate the structure and composition of biological tissues. The intrinsic fluorophore pentosidine and cross link emits light at specific wavelengths after being excited with ultraviolet (UV) rays [34]. This technique was developed to help in quantifying the accumulated levels of AGEs in the human body. Despite the presence of several confounding factors, skin autofluorescence (SAF) is a safe non-invasive technique for measuring the accumulated levels of AGEs. Although AGEs are non-fluorescent, a positive correlation between SAF and AGEs has been reported in a previous study [35]. In patients with chronic kidney disease, the SAF value has been found to be correlated with the severity of cardiovascular disease (CVDs) and an increased risk of death from CVD [36]. Thus, it can be inferred that SAF is a non-invasive and convenient tool for not only the evaluation of renal failure, but also for several other age- and diabetes-related disorders.

A complex cascade of events including overexpression of the glucose transporter 1 (GLUT1) in the vascular endothelial cells and renal mesangial cells induces cellular damage and malfunctioning. These events also include the formation of AGEs. The elevated glucose levels lead to glycation, which is a non-enzymatic method that results in the formation of covalent adducts of glucose with plasma proteins. With a constant high supply of glucose in diabetes, plasma proteins and collagen become glycated. These early glycated products (known as Schiff's bases and Amadori products) form irreversible cross-links with protein moieties in the connective tissue of skin with time. These AGEs are known to be involved in causing diabetic micro and macrovascular complications.

Advanced glycated products are well known for causing endothelial dysfunction in patients with type 2 diabetes. Therefore, it is being targeted as an independent factor for predicting the development and progression of CVDs through its ability to accelerate atherosclerosis and inflammation [37, 38].

However, research regarding AGEs as predictive markers for CVD risk in the Korean diabetic population is limited. Therefore, this study aimed to evaluate the levels of AGEs in Korean patients with T2DM, their use in the prevention and treatment of diabetic vascular complications, and to highlight the role of AGEs as therapeutic markers and cardiovascular risk factors.

2. Materials and methods

2.1. Research subject and period

A total of 310 subjects (177 men and 133 women) were invited to participate in this observational cross-sectional study. Patients with T2DM aged 20–80 years and receiving outpatient treatment at Jeong hospital in Gyeonggi-do were eligible for this study. An informed consent form was signed from the patients agreeing to the conditions of the study. Patients with psychiatric and malignant diseases, bedridden patients, Fitzpatrick skin types V and VI, and injuries in the forearm skin were excluded. SAF can only be measured in subjects with skin phototype I–IV, where the reflectance value is above 6%. When the absorbance was below 94%, compromised SAF values were obtained; this was seen in patients with a high melanin content or dark skin. The study lasted for 1 year and 6 months (January 1, 2017 to June 30, 2018), during which the patients; data were collected (from surveys and medical records) and analyzed. After the anamnesis, anthropometric measurements and SAP were conducted in a laboratory at room temperature (22–25 °C), humidity levels of 55–60%, and at the same time of the day. The study was subject to approval by the Korean ethical committe of Changwon National University.

Based on the SAF values, the patients were divided into Group I (SAF value $\leq$ 2.2), Group II (2.2 $\leq$ SAF value $\leq$ 2.7), and Group III (SAF values $>$ 2.7).

2.2. Anthropometric data and clinical assessment

The age, sex, duration of diabetes, and smoking status of each patient were analyzed. Through the evaluation of medical records, the diabetes complications of the patients were classified into microvascular and macrovascular complications. Microvascular complications included kidney diseases, diabetic neuropathy, diabetic nephropathy, and diabetic retinopathy. Meanwhile, macrovascular complications included diabetic

foot, coronary artery disease, cerebral infarction, and dementia. Height and weight were measured up to one decimal point using an electronic scale with the patients wearing light clothing. Anthropometric data were used to calculate the body mass index (BMI): the measured body weight (kg) divided by the height squared (m^2). Patients with a BMI >25 kg/m^2 was considered obese, according to the **World Health Organization criteria specifically for Asia**. The percentage of body fat and muscle mass composition were determined using In Body 720 (BiospaceCo, Ltd, Seoul Korea). The systolic and diastolic blood pressures were measured using a standard electronic manometer (FT 500, Korea) with the patient at rest.

2.3. Measurement of advanced glycation end products (AGEs)

SAF is a non-invasive method for measuring the level of AGEs using an AGE reader (Diagnostics Bv, Groningen, Netherlands). Due to the characteristic fluorescent properties of AGEs help in their detection, and their accumulated levels are quantified using a desktop device known as an autofluorescence reader (AFR). Guarded from surrounding light, a skin surface area of 1 cm^2 is illuminated by a light source. According to Mulder et al. [39], Meerwaldtet et al. [40], and Koetsier et al. [41], AGEs have an excitation wavelength between 300 and 400 nm, with a peak of 360 nm. The spectrometer uses a 200 mm glass fiber to measure the emitted and reflected wavelengths from the illuminated skin section. The value of SAF was calculated by taking the ratio between the emitted and reflected light multiplied by 100. The resulting value was expressed in arbitrary units. The lower arm area approximately 5–10 cm below the elbow was selected and cleaned with 70% alcohol, dried, and properly positioned on the spectrometer. The patients were seated, and the measurements were taken at room temperature. Portions of the skin with dermatitis, bruises, or pigmentation disorders; arterial venous fistulas; and malignancies were avoided. Only normal skin sites were selected for analysis. Autofluorescence was calculated automatically and was observer-independent. Three measurements were performed to obtain an average score.

2.4. Biochemical analysis

A biochemical analysis was conducted using the Green Cross Medical Foundation. Levels of biochemical indicators including fasting blood glucose (FBS), HbA1c, triglycerides, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol, and creatinine were measured. Urinalysis was performed to measure the levels of excreted proteins in urine.

2.5. Arterial pulse wave rate and blood vessel age

Arterial pulse wave velocity (PWV), which is the rate at which aortic wall expansion and relaxation is transmitted to the arteries in the form of waves, was measured using an automatic waveform analyzer (PV-1000, Colin Co., Komaki, Japan). Moreover, blood vessel age was also measured using an automatic waveform analyzer (PV-1000, Colin Co., Komaki, Japan). The measurement was taken after the patient had rested for 5 min or more in a lying position. The time interval for determining the pulse wave conduction rate and the distance between the measurement points was automatically calculated using an automatic waveform analyzer.

2.6. Statistical analysis

Statistical analyses were performed using SPSS version 24 (IBM Corporation NY, USA). The primary goal was to evaluate the relationship between SAF (AU) and clinical as well as biochemical risk factors of CVD. ANOVA was performed to analyze the general characteristics, anthropometric measurements, biochemical indicators, advanced glycated products, arterial pulse wave conduction rate, and blood vessel age. All results were expressed as means and standard deviation. The chi squared test was used to evaluate complications, and Pearson's correlation coefficient was used to evaluate the indicators associated with the advanced glycated product. General linear models (GLMs) were used to identify the independent effects of AGEs after adjusting the cardiovascular risk factors for some covariates (age, weight, and duration of diabetes). An unadjusted P-value $\leq$0.05 was considered significant.

Table 1. Descriptive and anthropometric data of patients with type 2 diabetes.

	Group I (n = 53)	Group II (n = 123)	Group III (n = 134)	*P* value
Male (n,%)	33 (62.3)	66 (53.7)	78 (58.2)	0.538
Age (years)	50.8 ± 9.6[a]	57.1 ± 10.1[b]	63.7 ± 10.1[c]	<0.001
Height (cm)	165.1 ± 9.7	162.5 ± 10.5	162.0 ± 8.3	0.129
Weight (kg)	72.7 ± 14.8[a]	69.3 ± 13.4[a,b]	65.8 ± 10.8[c]	0.002
BMI (kg/m^2)	26.6 ± 4.2[a]	26.3 ± 5.5[a,b]	25.0 ± 3.4[c]	0.033
Duration of diabetes (years)	3.9 ± 3.1	5.7 ± 5.0	9.3 ± 6.9	0.001
Body fat (kg)	21.8 ± 9.2	21.1 ± 7.7	20.0 ± 6.7	0.273
SMM (kg)	28.1 ± 6.0	26.6 ± 6.1	24.7 ± 4.9	<0.001
SBP (mmHg)	124.2 ± 13.4	126.7 ± 14.1	128.3 ± 15.9	0.224
DBP (mmHg)	75.2 ± 10.9[a]	75.7 ± 11.3[a,b]	71.0 ± 11.4[c]	<0.001
Smoking	36 (17)	78 (45)	82 (52)	0.690
Complications				
Macroangiopathy	47 (6)	107 (16)	90 (43)	<0.001
Microangiopathy	41 (77.4)	77 (62.6)	55 (41.4)	<0.001
Retinopathy	1 (1.9)	10 (8.1)	6 (4.5)	
Nephropathy	4 (7.5)	11 (8.9)	13 (9.8)	
Neuropathy	7 (13.2)	25 (20.3)	59 (44.4)	

Values are shown as means ± standard deviation or number (percentage).
BMI, body mass index; SMM, skeletal muscle mass; SBP, systolic blood pressure; DBP, diastolic blood pressure. Means in the same row not sharing a common letter are significantly different, with a *P* value < 0.05.

3. Results

3.1. General characteristics and body measurements of the subject

The general characteristics and anthropometric data of 310 patients with T2DM (177 men, 133 women) enrolled in this study are shown in Table 1. The mean ages were 50.8 ± 9.6 years for Group I, 57.1 ± 10.1 for Group II, and 63.7 ± 10.1 years for Group III; the differences between the three groups were significant. The mean BMI of each group was 26.6 ± 4.2 kg/m^2, 26.3 ± 5.5 kg/m^2, and 25.8 ± 4.5 kg/m^2 for Groups I, II, and III, respectively; the differences between the three groups were significant. The differences in diastolic blood pressure were also significant between the three groups: 75.2 ± 10.9, 75.7 ± 11.3, and 71.0 ± 11.4 for Groups I, II, and III, respectively. There were no significant differences in smoking between the groups. In this study, 147 patients had no diabetes complications (47.6%), and 163 (52.5%) diagnosed with complications. Major vascular complications such as coronary heart disease, cerebrovascular disease, and peripheral artery diseases were present in 43.9% of the patients. Meanwhile, microvascular complications including retinopathy, nephropathy, and neuropathy were present in 21.1% of the patients.

3.2. Biochemical indicators

The biochemical indicators of the patients are shown in Table 2. The mean FBS was 146.4 ± 62.3, 133.9 ± 54.8, and 156.3 ± 68.3 mg/dL for Groups I, II, and III, respectively, with significant differences between the three groups. FBS was higher in Group III than in the

Table 2. Biochemical parameters in patients with type 2 diabetes.

Variable	Total (n = 310)	Group I (n = 53)	Group II (n = 123)	Group III (n = 134)	*P* value
FBS (mg/dL)	145.7 ± 62.8	146.4 ± 62.3[a,b]	133.9 ± 54.8[b]	156.3 ± 68.3[c]	0.017
HbA1c (%)	7.5 ± 4.2	7.2 ± 1.8	6.9 ± 1.3	8.1 ± 6.1	0.089
TG (mg/dL)	148.5 ± 115.9	166.2 ± 182.6	144.1 ± 90.5	145.63 ± 102.8	0.524
Total Chol (mg/dL)	164.4 ± 47.3	171.0 ± 55.8	165.6 ± 38.8	160.6 ± 50.6	0.377
LDL Chol (mg/dL)	86.8 ± 35.2	171.0 ± 55.8	165.5 ± 38.8	83.55 ± 34.9	0.436
HDL Chol (mg/dL)	50.0 ± 13.4	46.63 ± 12.6	50.5 ± 12.5	50.9 ± 14.4	0.169
Creatinine (mg/dL)	1.5 ± 9.5	0.9 ± 0.2	2.2 ± 15.0	1.0 ± 0.4	0.510
Proteinuria (mg/dL)	44.0 ± 82.8	37.8 ± 98.8	37.1 ± 66.4	52.7 ± 89.1	0.317

All the values are means ± standard deviation analyzed using ANOVA.
FBS, fasting blood sugar; HbA1c, glycated hemoglobin; TG, triglyceride; Total Chol, total cholesterol; LDL Chol, low-density lipoprotein cholesterol; HDL Chol, high-density lipoprotein cholesterol. The means in the same row not sharing a common letter are significantly different, with a P value < 0.05.

other groups. The HbA1C level was 7.2 ± 1.8%, 6.9 ± 1.3% and 8.1 ± 6.1% for Groups I, II, and III, respectively; the differences were not significant. There was no significant difference in HDL cholesterol, LDL cholesterol, creatinine, triglycerides, and proteinuria between the three groups.

3.3. Skin Autofluorescence (SAF) as a marker of accumulation of AGEs, arterial pulse wave conduction rate, and blood vessel age

The AGEs, arterial pulse wave conduction rate, and blood vessel age measurements are shown in Table 3. The mean autofluorescence level was 2.7 ± 0.5 AU. AGE values were found to be significantly different between the groups. Moreover, the mean pulse wave conduction rates for both right and left arms were found to be significantly different between the groups. Additionally, the mean vein ages were significantly different and were highest in Group III. Figure 1(a,b,c,d) shows the associations between SAF the duration, pulse wave velocity and vein age.

3.4. Correlation between advanced glycated products by SAF and related indicators

The mean AGE value assessed using SAF was 2.7 ± 0.5 AU (both male and female patients). The estimated vascular age of the patients was 68.12 ± 13.565 years. The potentially correlated indicators related to AGEs are shown in Table 4. SAF levels were significantly higher in Group III than in Groups I and II. As expected, the levels of AGEs tended to increase significantly with age ($P < 0.001$). Moreover, the longer the duration of diabetes, the higher the SAF level of AGEs ($P < 0.001$). Covariates including height, weight, BMI, and skeletal muscle mass were positively correlated to AGEs. However, fat mass was not related to final AGE levels ($P = 0.507$).

3.5. Independent association of AGEs with cardiovascular risk factors

Table 5 shows the association between AGEs and cardiovascular risk factors in patients with diabetes; the significance of the covariates (age, weight, and duration of diabetes) was assessed. Independent associations for AGEs with FBS and proteinuria ($P < 0.001$) were observed. PWV and vein age were independently associated with AGEs (P 0.001).

4. Discussion

Arterial stiffness and atherosclerosis can be predicted by examining the relationship between early markers of vascular dysfunction, such as PWV and SAF [42]. This study aimed to investigate the level of AGEs to estimate the association between the advanced glycated product and PWV and SAF measurements in Korean patients with type 2 diabetes. The results of SAF and the arterial pulse wave conduction rate of Korean patients with T2DM were compared with those of other studies. Lutgers et al. [43] reported a final level of AGEs of 2.79 ± 0.8 AU in 973 patients with type 2 diabetes. Our results showed a similar value of 2.7 ± 0.5 AU for advanced glycated product measurements.

Many previous studies have reported the association between AGEs and an increase in PWV in healthy individuals as well as patients with hypertension, T2DM, and end-stage renal failure [44, 45]. AGEs cause arterial stiffness by two main mechanisms: interaction with arterial walls, which cause functional and structural changes that lead to an over production or cross-linking of collagen, a decreased level of elastin, and therefore increased arterial stiffness [46]; or by interacting with RAGEs, thereby activating different signaling pathways, which lead to the enhanced production of pro-inflammatory cytokines and vascular adhesion molecules responsible for atherosclerosis [47].

Previous studies have shown an arterial pulse wave rate of 1483.0 ± 320.1 cm/s on the right hand and 1483.1 ± 757.5 cm/s on the left hand in patients without hormonal therapy. In our study, the readings were 1597.3 ± 301.5 cm/s on the right hand and 1606.5 ± 316.0 cm/s on the left hand. Moreover, the pulse wave conduction rate was found to be lower in healthy subjects than patients with diabetes. Our study showed similar results to a study conducted by Saeko et al. [48] that involved 193 Japanese patients with diabetes and 24 control subjects, wherein the pulse wave conduction rate was 1275 ± 138.0 cm/s in the control group and 1719.0 ± 458 cm/s in the patients with diabetes. In addition, levels of advanced glycated products measured using SAF in Japanese patients with diabetes was found to be higher than those in our study. This was attributed to the fact that the average disease duration was 13.7 ± 10.3

Table 3. AGEs, PWV, and correlation with age in patients with type 2 diabetes.

Variable		Total (n = 310)	Group I (n = 53)	Group II (n = 123)	Group III (n = 134)	*P* value
AGEs (AU)		2.7 ± 0.5	2.1 ± 0.2	2.5 ± 0.1	3.2 ± 0.4	<0.001
PWV (cm/s)	Right	1597.3 ± 301.5	1439.3 ± 223.4	1532.7 ± 262.1	1721.5 ± 315.8	<0.001
	Left	1606.5 ± 316.0	1429.6 ± 229.6	1550.1 ± 297.7	1731.6 ± 315.0	<0.001
Vein age (years)		68.1 ± 13.6	58.5 ± 12.8	65.4 ± 12.4	74.9 ± 11.4	

All values are expressed as means ± standard deviation. Analyses were performed using ANOVA.
AGEs, advanced glycation end products; PWV, pulse wave velocity.

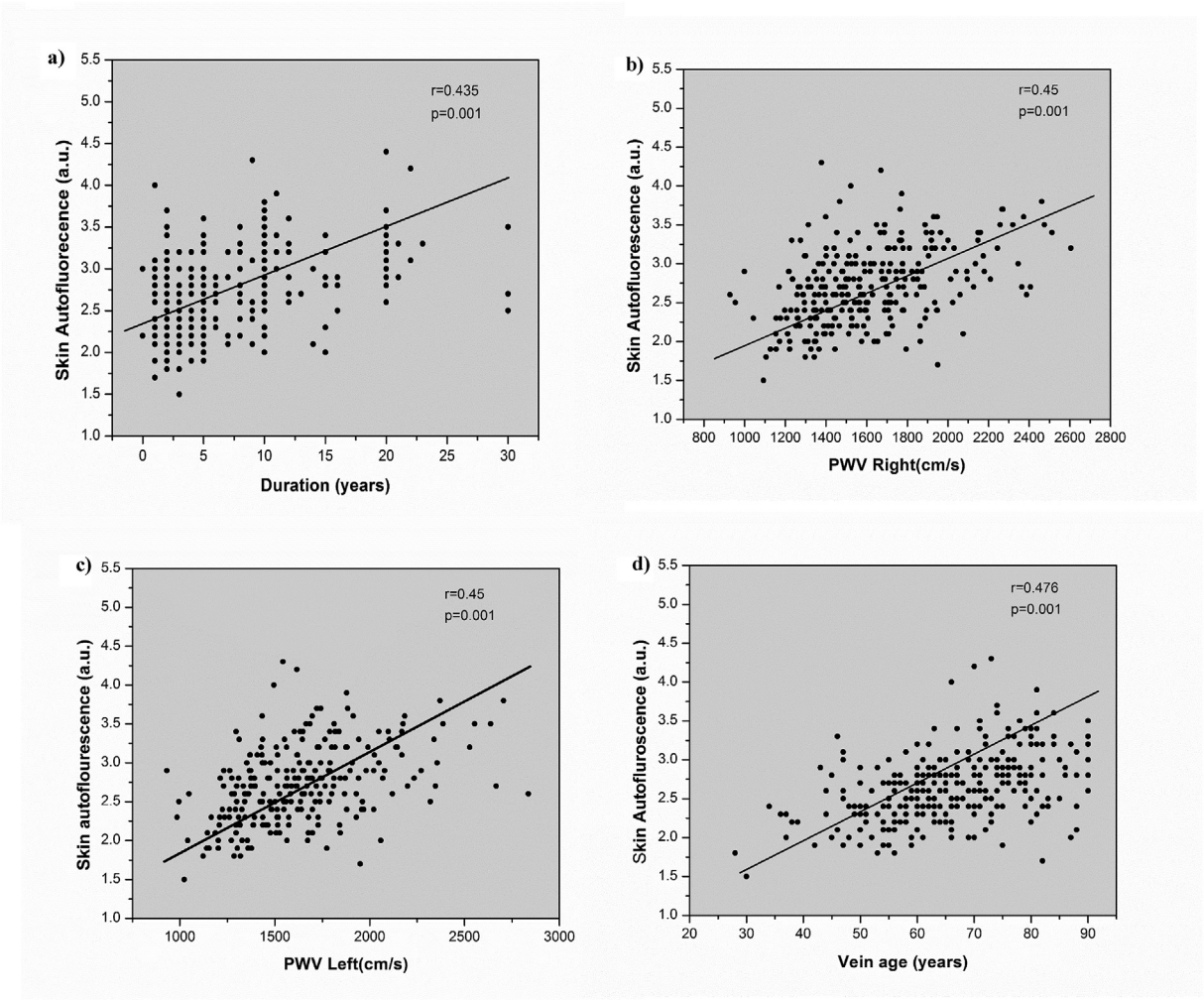

Figure 1. Skin autofluorescence as a function of duration (a) pulse wave velocity (PWV), right; (b) pulse wave velocity (PWV), left (c); and vein age (d). Moderate and positive correlations between variables. The regression lines represent both the average of the skin auto fluorescence level and the average of the disease duration, PWV, and age. The regression was moderate, positive, and statistically significant ($P < 0.05$).

Table 4. Correlations between AGEs and parameters in patients with type 2 diabetes.

Variable	AGEs (n = 310)	
	R	*P* value
Age	0.476	<0.001
Duration	0.435	<0.001
Height	-0.115	0.044
Weight	-0.191	0.001
BMI	-0.124	0.029
SMM	-0.238	<0.001
Fat mass	-0.038	0.507

r: Pearson's correlation coefficient.
AGEs measured using SAF (skin autofluorescence), advanced glycation end products; BMI, body mass index; SMM, skeletal muscle mass.

Table 5. Independent association of AGEs with cardiovascular risk factors.

Variables	FBS	Proteinuria	PWVR	PWVL	Vein age
AGEs	24.00 ± 7.98*	42.06 ± 10.33**	171.18 ± 37.10**	182.54 ± 38.81**	7.41 ± 1.33**
Age	-1.22 ± 0.36**	-	7.00 ± 1.59**	6.69 ± 1.77**	0.66 ± 0.06**
Weight	-	1.23 ± 0.38*	-	-1.91 ± 1.32	-
Duration			5.26 ± 2.85	5.55 ± 2.99	-
Intercept	152.23 ± 22.25**	154.12 ± 42.54**	688.20 ± 103.58**	814.46 ± 169.23**	9.18 ± 3.68*
R^2	0.04	0.08	0.26	0.27	0.48

The values are expressed regression coefficients ±standard error of estimate (SEE).
AGEs, advanced glycation end products; FBS, fasting blood sugar; PWVR, pulse wave velocity right; PWVL, pulse wave velocity left.
*$P < 0.01$; **$P < 0.001$.

years, whereas it was 6.9 ± 6.0 years in our study. These results confirm the strong correlation between the level of AGEs, measured using SAF and diabetes duration. Previous literature [49, 50, 51, 52, 53, 54, 55] also confirmed that the accumulation of advanced glycated products through skin collagen is related to the duration and severity of hyperglycemia and long-term angina. AGEs reportedly reflect metabolic changes, such as glucose levels that in turn contribute to arterial stiffness and atherosclerosis. As shown in our study, there is a positive association between PWV and SAF levels of AGEs; this is a predictive and a representative method for measuring atherosclerosis. In a representative large scale study by Lutgers et al. [43], the levels of advanced glycated products measured using SAF, reflected macro- and micovascular diseases, and thus, is related to the severity of diabetes complications. Similarly,

we found a direct association of AGEs with diabetes complications in Korean patients.

AGEs play an essential role in endothelial dysfunction and vascular inflammation via multiple mechanisms. AGEs may accumulate and cause a decrease in vasodilation by lowering nitric oxide levels and an increase in vasoconstriction by elevating endothelin-1 levels [56]. The binding of AGEs to RAGEs can lead to metabolic and phenotypic changes in different cells together with the production of pro inflammatory cytokines and activation of nuclear transcription factors that cause the development of atherosclerosis [57, 58]. In a study by Singh et al. [23] and Nakamura et al. [59], the advanced glycated product was found to be a risk factor for endothelial dysfunction in patients with T2DM. Studies have also shown that glycated skin collagen is a better target for assessing diabetic complications than HbA1c in patients with type 1 diabetes [52, 60]. Therefore, it can be assumed that the measurement of advanced glycated end products can be a meaningful risk predictor for patients with T2DM.

The limitation of this study is the lack of a comparison of the study patients with healthy individuals, especially since we included only Korean patients with type 2 diabetes. Data related to the intake of dietary advanced glycation products were insufficient. In the future, studies on the status of advanced glycated products in patients with and without diabetes and their relationship to diet and lifestyle are needed. Moreover, in-depth studies on dietary advanced glycated products are needed.

Through the results of this study, the level of advanced glycated products in Korean patients with diabetes was identified. We also observed the possibility of its use as an indicator in clinical diagnosis, prediction of complications, and diabetes management in these patients.

5. Conclusions

Our results show that increases in the level of AGEs in Korean patients with type 2 diabetes is associated with PWV and vein age, i.e., measures of arterial stiffness. Consequently, the levels of AGEs were correlated with cardiovascular risk factors in Korean patients with type 2 diabetes. AGE levels can thus be used as an indicator for CVDs in clinical diagnosis.

Declarations

Author contribution statement

Lee-Seoul Choi: Conceived and designed the experiments; Performed the experiments.

Jung-Eun Yim: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Kainat Ahmed: Performed the experiments; Wrote the paper.

Young-Seol Kim: Analyzed and interpreted the data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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