

# Assessment of skin autofluorescence as a marker of advanced glycation end product accumulation in type 1 diabetes

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## KEY WORDS

advanced glycation end products, autofluorescence, diabetes

## ABSTRACT

**INTRODUCTION** Advanced glycation end products (AGEs) are important in the pathogenesis of atherosclerosis and reflect the risk of cardiovascular mortality. AGE levels are significantly higher in patients with diabetes.

**OBJECTIVES** The aim of the study was to compare AGE accumulation in the skin of patients with type 1 diabetes and nondiabetic population as well as to assess its association with disease duration and metabolic control. We also aimed to assess the potential usefulness of this method in the monitoring of diabetes control, especially in a long-term follow-up.

**PATIENTS AND METHODS** The study included 140 type 1 diabetes patients (mean age  $30.4 \pm 9.7$  years; mean disease duration  $13.6 \pm 8.5$  years) and 57 nondiabetic subjects. AGE accumulation in the skin was assessed noninvasively with the AGE Reader device, which measures autofluorescence (AF) that occurs because some AGEs have fluorescent properties.

**RESULTS** Mean AF in the diabetes group was  $2.13 \pm 0.55$  and it was significantly higher than in controls ( $AF\ 1.70 \pm 0.27$ ,  $P < 0.05$ ). A significant positive correlation between AF and the age of patients was found for the whole study population ( $P < 0.05$ ). In diabetic subjects, we observed a significant positive correlation between AF and diabetes duration ( $P < 0.05$ ), and between AF and glycated hemoglobin ( $HbA_{1c}$ ) ( $P < 0.05$ ).

**CONCLUSIONS** AF measurement is a simple and noninvasive method of assessing AGE accumulation in the skin. It may be useful as a secondary method of assessing metabolic control, as it reflects glycemic control over a longer period of time than that reflected by  $HbA_{1c}$  levels.

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**INTRODUCTION** Advanced glycation end products (AGEs) are an important element linking accelerated atherosclerosis with diabetes. AGEs may be generated in human serum and tissues in several distinct pathways. The Maillard reaction, which occurs as a result of hyperglycemia, leads to the glycation of proteins.<sup>1</sup> Another mechanism of AGE formation is oxidative stress, which results in the autoxidation of glucose.<sup>2</sup> AGEs can also be derived from exogenous sources such as diet or smoking.<sup>3,4</sup> Moreover, their accumulation can be caused by decreased renal clearance of glycosylated proteins in renal insufficiency.<sup>5</sup>

It has been proved that AGEs play an important causative role in the pathogenesis of atherosclerosis.<sup>6,7</sup> Moreover, their levels predict the risk of cardiac death.<sup>8</sup> Those findings are particularly important in patients with type 1 and type 2 diabetes, who have significantly higher AGE levels compared with healthy population.<sup>9,10</sup> It has been observed that AGE levels in diabetic patients are associated not only with macrovascular but also with microvascular complications, such as retinopathy, nephropathy, or neuropathy.<sup>9-11</sup>

AGE levels can be measured using several methods. Plasma levels can be assessed in whole blood

samples. Tissue accumulation can be calculated with biochemical and immunochemical assays, but these methods are no longer in use.<sup>2</sup> Some AGEs have fluorescent properties, which makes it possible to measure their levels in tissue biopsies. Recently, a new, noninvasive method based on autofluorescence (AF), has been developed.<sup>12</sup>

AGE levels are also significantly higher in numerous conditions other than diabetes, for example in cardiovascular diseases. Elevated levels of skin AF have been observed in myocardial infarction and intensive care patients, which is probably related to acute inflammation and oxidative stress. In this setting, AF measurements have been shown to have prognostic value.<sup>13,14</sup> Increased skin AF has also been reported in patients with systemic lupus erythematosus; it correlated with disease duration.<sup>15</sup> As mentioned above, decreased renal clearance of AGEs results in their accumulation in the body, which is possibly the cause of elevated skin AF in patients with end-stage renal disease.<sup>16</sup> It has also been reported that AGE accumulation in tissue might correlate with vascular stiffness, which occurs in such pathological conditions as diabetes or isolated systolic hypertension.<sup>17</sup> AGEs are also related to metabolic memory and take part in the overproduction of reactive oxygen species.<sup>18</sup>

The aim of our study was to compare skin AF between patients with type 1 diabetes and non-diabetic subjects. It was measured noninvasively with the Autofluorescence Reader device. We also investigated the association between skin AF and diabetes duration and metabolic control. Moreover, we aimed to assess the usefulness of this method in monitoring diabetes control, especially in a long-term follow-up.

**PATIENTS AND METHODS** **Study population** Our study involved 140 type 1 diabetes patients (disease duration of at least 1.5 years), who were hospitalized in the Department of Internal Medicine and Diabetology in Poznań, Poland. A complete medical history with detailed clinical information about diabetes was taken. Patients underwent physical examination including the measurement of body weight, height, waist and hip circumference, and blood pressure. The control group included 57 nondiabetic subjects, matched for age, sex, and smoking history. All subjects were Caucasian.

Diabetic patients were classified into subgroups according to diabetes duration: less than 5 years ( $n = 21$ ), 5–10 years ( $n = 28$ ), more than 10 years ( $n = 91$ ), and according to glycosylated hemoglobin ( $HbA_{1c}$ ) levels: below 7% ( $n = 25$ ), 7%–8% ( $n = 26$ ), and above 8% ( $n = 89$ ).

All subjects were informed about the aim of the study and provided their written consent. The study was approved by the Ethics Committee of Poznań University of Medical Sciences.

**Skin autofluorescence** Skin AF was measured by Autofluorescence Reader (AGE Reader; Diagnostics,

Groningen, the Netherlands, Type 214D00102). It is a noninvasive device used to assess the accumulation of fluorescent AGEs in tissues. The tool has an excitation light source and optic spectrometer. First, it lights up the skin with ultraviolet light (300–420 nm). Subsequently, the light emitted by the skin is assessed with a spectrometer in the range of 300–600 nm. This measurement is divided by the range of light emitted by the light source, thus giving the AF score.<sup>12</sup>

In each patient, AF was measured 3 times in room temperature on the ventral site of lower arm, about 5 cm distally to the antecubital space. The AF score for each patient was calculated as the average of these 3 measurements. None of the subjects had tattoos or skin lesions.

**Laboratory analyses** Blood samples were assessed after 8-hour fasting.  $HbA_{1c}$  was measured with high-performance liquid chromatography with the reference value below 6%. All the other blood tests were performed according to the standard laboratory procedures.

**Statistical analysis** Statistical analysis was performed using STATISTICA StatSoft 7.0. The diabetic group and controls were compared using the Mann-Whitney test for continuous variables. Categorical variables were compared using the  $\chi^2$  test with the Yates' correction. The Spearman correlation coefficient was performed to evaluate associations between the factors. A one-way analysis of variance was used to compare mean skin AF between the subgroups of diabetic patients divided according to disease duration and  $HbA_{1c}$  levels.

The factors potentially associated with skin AF levels were studied using a multivariate linear-regression analysis including sex, body mass index (BMI), diabetes duration,  $HbA_{1c}$ , smoking, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol.  $P < 0.05$  was considered statistically significant. Data were expressed as mean  $\pm$  standard deviation or percentage.

## RESULTS Characteristics of the study population

Characteristics of the study population are shown in TABLE 1. Diabetic subjects and controls were similar in age, height, and hip circumference. Also, there were no statistically significant differences between both groups in terms of sex and smoking status. Diabetic patients had significantly higher weight, BMI, and waist circumference compared with controls. Also mean systolic blood pressure was significantly higher in diabetic patients. Diastolic pressure was similar in both groups. Diabetic subjects were more likely to have hypertension history (22.1% vs. 1.8%,  $P < 0.05$ ) and they reported treatment with angiotensin-converting enzyme inhibitors more often (30% vs. 1.8%,  $P < 0.05$ ).

In the diabetes group, disease duration was  $13.6 \pm 8.5$  years and  $HbA_{1c}$  was  $8.6 \pm 1.7\%$ . All diabetic

**TABLE 1** Characteristics of the study group

	Diabetes group	Control group	P
n	140	57	–
age, y	30.4 ± 9.7	27.1 ± 5.4	0.070
diabetes duration, y	13.6 ± 8.5	–	–
sex, % (women)	54.3	61.4	0.361
body mass, kg	71.5 ± 15.6	65.6 ± 12.4	0.017
height, cm	171.3 ± 9.2	172.6 ± 8.9	0.046
BMI, kg/m <sup>2</sup>	24.2 ± 4.2	21.9 ± 2.8	<0.001
waist circumference, cm	84.7 ± 12.5	76.9 ± 10.5	<0.001
hip circumference, cm	93.2 ± 9.0	93.2 ± 7.5	0.983
WHR	0.90 ± 0.12	0.82 ± 0.09	<0.001
smoking, %	22.9	14.0	0.163
SBP, mmHg	117.2 ± 14.6	111.9 ± 10.8	0.029
DBP, mmHg	72.8 ± 9.8	70.9 ± 9.2	0.196
history of hypertension, % (positive)	22.1	1.8	<0.001
ACEI, %	30.0	1.8	<0.001
FPG, mmol/l	9.91 ± 4.16	5.28 ± 0.57	<0.001
AF	2.1 ± 0.6	1.7 ± 0.3	<0.001
functional insulin therapy, %	91.4	–	–
insulin dose, units/kg body weight/day	0.66 ± 0.20	–	–
HbA <sub>1c</sub> , %	8.6 ± 1.7	–	–
CRP, mg/l	4.1 ± 11.8	1.6 ± 0.7	0.563
TG, mmol/l	1.20 ± 0.55	0.85 ± 0.46	0.051
LDL cholesterol, mmol/l	2.72 ± 0.76	2.69 ± 0.96	0.821
HDL cholesterol, mmol/l	1.64 ± 0.42	1.57 ± 0.53	0.671
creatinine, mg/dl	0.85 ± 0.20	0.66 ± 0.15	0.069
creatinine clearance, ml/min	101.1 ± 23.7	148.5 ± 68.9	0.231
macrovascular complications	cardiovascular disease, n (%)	5 (3.6)	–
	stroke incidence, n (%)	1 (0.7)	–
	peripheral arterial disease, n (%)	3 (2.1)	–
microvascular complications	retinopathy, n (%)	54 (38.6)	–
	neuropathy, n (%)	30 (21.4)	–
	nephropathy, n (%)	18 (12.9)	–

Data are presented as mean ± standard deviation or incidence (percentage)

Abbreviations: ACEI – angiotensin-converting enzyme inhibitors, AF – autofluorescence, BMI – body mass index, CRP – C-reactive protein, DBP – diastolic blood pressure, FPG – fasting plasma glucose, HbA<sub>1c</sub> – glycated hemoglobin A<sub>1c</sub>, HDL – high-density lipoprotein, LDL – low-density lipoprotein, SBP – systolic blood pressure, TG – triglycerides, WHR – waist-to-hip ratio

subjects were treated with insulin; the majority received functional insulin treatment (91.4%). Treatment and medication details are presented in **TABLE 1**.

#### Autofluorescence in diabetic subjects and controls

Mean AF was significantly higher in the diabetic group than in controls (2.13 ± 0.55 vs. 1.70 ± 0.27;  $P < 0.05$ ; **FIGURE 1**).

#### Associations between autofluorescence and other factors

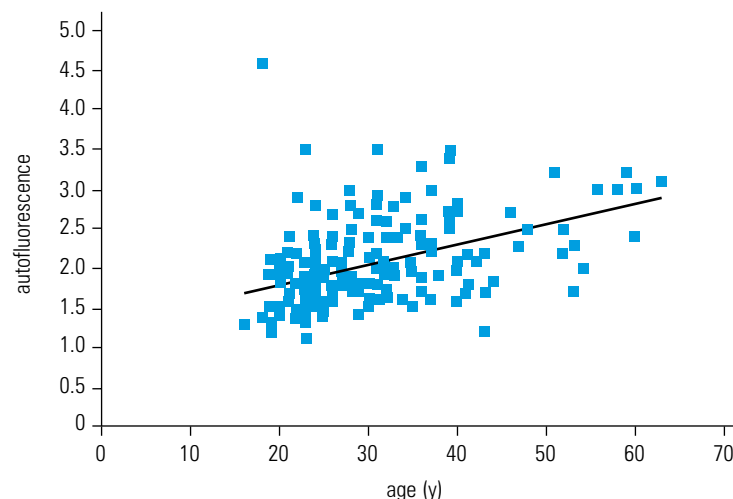
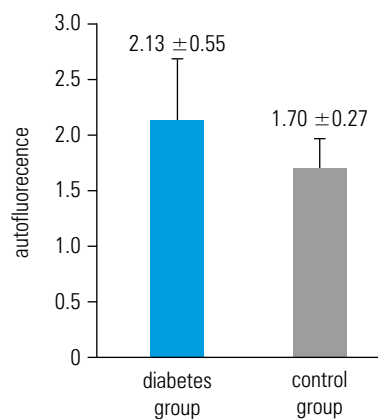
A significant positive correlation between AF and patients' age was observed for the whole study population ( $P < 0.05$ , **FIGURE 2**) and for each subgroup. A significant positive correlation between AF and diabetes duration was

observed in the diabetes group ( $P < 0.05$ , **FIGURE 3**). Mean AF levels differed significantly between the subgroups of patients divided according to diabetes duration (<5 years: 1.83 ± 0.38; 5–10 years: 2.01 ± 0.61; >10 years: 2.23 ± 0.53;  $P = 0.002$ ).

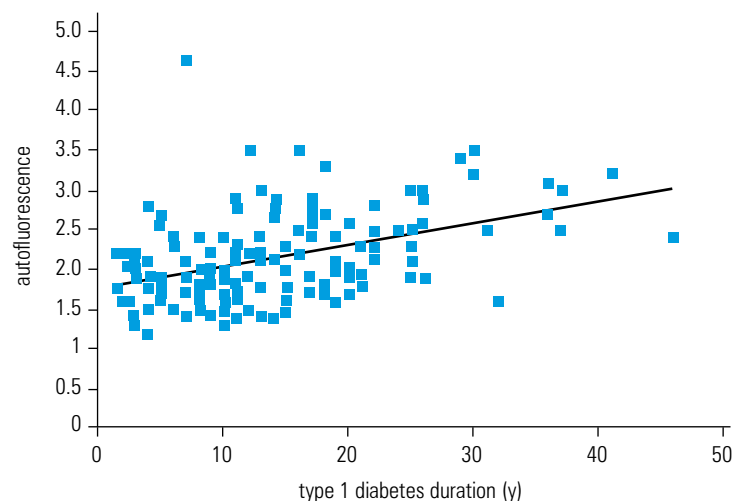
There was a statistically significant correlation between AF and HbA<sub>1c</sub> ( $P < 0.05$ , **FIGURE 4**). It did not differ significantly between the subgroup with HbA<sub>1c</sub> below 7% and the subgroup with HbA<sub>1c</sub> of 7% to 8% ( $P = 0.41$ ), but it was significantly higher in the subgroup with HbA<sub>1c</sub> above 8% compared with the 2 other subgroups ( $P = 0.01$ ).

No significant correlations between lipid parameters (LDL cholesterol, HDL cholesterol, triglycerides) and AF were observed.

**FIGURE 1** Comparison of skin autofluorescence in the diabetes group and the control group ( $P < 0.001$ )



**FIGURE 2** Correlation between autofluorescence and age in the whole study group ( $r = 0.45$ ,  $P < 0.05$ )



**FIGURE 3** Correlation between autofluorescence and diabetes duration in the diabetes group

A multivariate linear-regression model including sex, BMI, diabetes duration,  $HbA_{1c}$ , smoking, LDL cholesterol, and HDL cholesterol showed that diabetes duration and  $HbA_{1c}$  level were independently correlated with high AGE levels (TABLE 2).

**DISCUSSION** AGE Reader used in our study to assess AGE accumulation in the skin is a recently

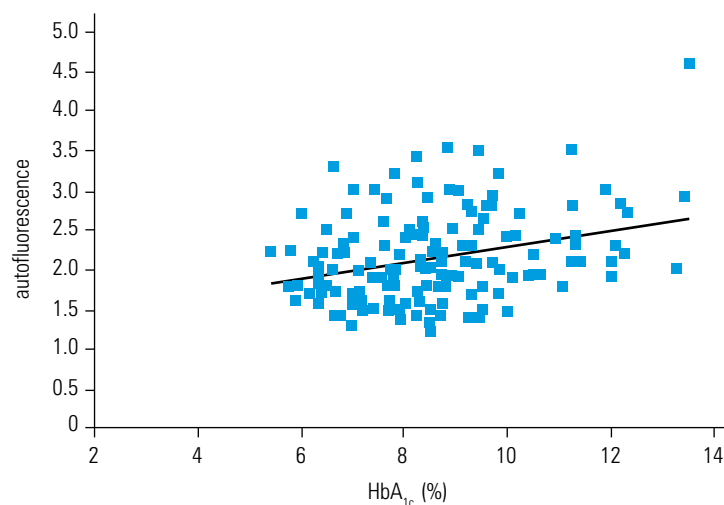
developed, simple, noninvasive device. As described by Meerwaldt et al.,<sup>12</sup> it is an alternative to invasive methods and can be useful in studying conditions in which accumulation of AGEs may be expected. Kasznicki et al.<sup>18</sup> also suggested use of this method, in line with the recent findings on the role of AGEs in metabolic memory.<sup>18</sup> All subjects in our study confirmed that the procedure was completely painless. They did not report any skin lesions or pain in the area exposed to the light emitted by the device. The examination took about 1 minute in each patient and the result was available directly after assessment. We did not assess skin covered with tattoos or lesions.

Our study confirmed a positive correlation between AF levels and age, which is in line with the results of Monami et al.<sup>19</sup> and Lutgers et al.<sup>10</sup>, who observed the correlation both in type 2 diabetes patients and in healthy subjects. In addition, Meerwaldt et al.<sup>12</sup> observed that an increase in AF associated with age was more than two-fold higher in diabetic patients (both type 1 and 2) than in nondiabetic subjects.<sup>12</sup> A study by Monnier et al.<sup>9</sup> also revealed a correlation between AGE levels and age, although it was assessed with a different method (by measuring collagen-linked fluorescence of skin biopsy specimens).<sup>9</sup> In our study, the correlation was significant in both diabetic patients and control subjects.

We observed significantly higher AF in patients with type 1 diabetes than in controls. Moreover, it correlated positively with disease duration. These results are in line with the study by Monnier et al.,<sup>9</sup> although these authors used a different method for AF assessment.<sup>9</sup> Lutgers et al.<sup>10</sup> and Meerwaldt et al.<sup>20</sup> also reported elevated AF levels in type 2 diabetes compared with healthy population, and, similarly, AF levels correlated with disease duration. Meerwaldt et al.<sup>20</sup> observed that AF was higher in type 1 and type 2 diabetes patients than in controls. Additionally, comparison between both groups of diabetic patients showed that AF was significantly higher in patients with type 2 diabetes. However, it was hypothesized that this finding might be associated with more advanced age and higher  $HbA_{1c}$  levels in type 2 diabetes patients.<sup>12</sup>

As discussed above, we also observed a significant positive correlation between diabetes duration and AF. Significant differences were found between the subgroups divided according to disease duration.

It has been proved that AGE levels are associated with metabolic control of diabetes, which could be partially due to the pathogenesis of glycated proteins. Hyperglycemia leads to nonenzymatic glycation of proteins as a result of the Maillard reaction.<sup>1</sup> Several studies have confirmed a positive correlation between AF and  $HbA_{1c}$  in patients with type 2 diabetes.<sup>10,19</sup> We also found a positive correlation between AF and  $HbA_{1c}$ , assessed during 1 week before AF measurement. However, when we divided our study population into 3 subgroups with  $HbA_{1c}$  of less than 7%, 7% to



**FIGURE 4** Correlation between autofluorescence and HbA<sub>1c</sub> in the diabetes group ( $r = 0.28$ ,  $P < 0.05$ )

Abbreviations: see [TABLE 1](#)

**TABLE 2** Variables related to autofluorescence levels (above and below 1.7) in a multivariate logistic regression

	AF	
	<i>P</i>	OR (95% CI)
sex	0.80	0.87 (0.31–2.47)
BMI	0.37	0.94 (0.83–1.07)
diabetes duration	0.004	1.11 (1.03–1.19)
smoking	0.12	3.11 (0.73–13.1)
HbA <sub>1c</sub>	0.04	1.37 (1.00–1.88)
LDL cholesterol	0.72	0.99 (0.97–1.01)
HDL cholesterol	0.76	1.00 (0.97–1.03)

Abbreviations: CI – confidence interval, OR – odds ratio, others – see [TABLE 1](#)

8%, and more than 8%, the difference between the first 2 subgroups was not statistically significant. It may reflect a feature of AF, which was suggested by Lutgers et al.,<sup>10</sup> namely, that AF might reflect metabolic control of diabetes over a longer period of time compared with HbA<sub>1c</sub>.<sup>10</sup> On the other hand, Meerwaldt et al.<sup>12</sup> reported a positive association between AF and mean HbA<sub>1c</sub> levels assessed repeatedly during the year preceding the study in type 1 and type 2 diabetes patients.<sup>12</sup> HbA<sub>1c</sub> is widely used to monitor glycemic control although its levels are not always clinically reliable.<sup>21</sup> Thus, it is possible that AF may become a useful secondary method of assessing metabolic control in diabetes.

**Conclusions** Noninvasive measurement of skin AF is a quick, straightforward, and comfortable method of assessing the accumulation of fluorescent AGEs in the skin. The result probably reflects long-standing glycemic control, possibly over a longer period of time compared with HbA<sub>1c</sub>. Thus, measurement of skin AF could become a useful secondary method of assessing metabolic control in diabetes.

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