Skin autofluorescence is associated with past glycaemic control and complications in type 1 diabetes mellitus

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Abstract

As skin autofluorescence (AF) can assess subcutaneous accumulation of fluorescent advanced glycation end-products (AGEs), this study aimed to investigate whether it was linked to glycaemic control and complications in patients with type 1 diabetes mellitus (T1DM). Using the AGE Reader™, AF was measured in T1DM patients referred to Haut-Levêque Hospital (Bordeaux, France); data on their HbA1c levels measured every 6 months as far back as the last 5 years were also collected. The association of AF with the patients' past glucose control, based on their latest HbA1c values, and the means of the last five and 10 HbA1c values, and with diabetic complications was also examined by linear regression analysis. The sample included 300 patients: 58% were male; the mean age was 49 (SD 17) years and the mean diabetes duration was 21 (SD 13) years. The median skin AF measurement was 2.0 [25th–75th percentiles: 1.7–2.4] arbitrary units (AU), and this was associated with age (β = 0.15 per 10 years, P < 0.001) and diabetes duration (β = 0.17 per 10 years, P < 0.001). After adjusting for age and estimated glomerular filtration rate (eGFR), the skin AF measurement was also related to the means of the last five and 10 HbA1c values (β = 0.10 per 1% of HbA1c, P = 0.005, and β = 0.13 per 1% of HbA1c, P = 0.001, respectively). In addition, the skin AF was associated with retinopathy (P < 0.001), albuminuria (P < 0.001) and decreased eGFR (P < 0.001). In conclusion, the skin AF is related to the long-term glucose control and diabetic complications.

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Keywords: Type 1 diabetes; Skin autofluorescence; Advanced glycation end-products; HbA1c; Microvascular complications

Résumé

L’autofluorescence cutanée est associée à l’acarien glycémique et aux complications diabétiques chez les patients ayant un diabète de type 1.

L’autofluorescence cutanée pouvait évaluer l’accumulation des produits fluorescents de glycation avancée, l’objectif de notre étude était d’analyser la relation avec le contrôle glycémique et les complications des patients présentant un diabète de type 1. En utilisant l’AGE Reader™, nous avons mesuré l’autofluorescence cutanée chez les patients ayant un diabète de type 1 suivi tous les six mois sur cinq ans à l’hôpital Haut-Levêque (Bordeaux, France). Nous avons étudié sa relation avec le contrôle glycémique : la dernière HbA1c, la moyenne des cinq et dix dernières HbA1c, et avec l’existence de complications, par régression linéaire. Les 300 patients (58% d’hommes) avaient une moyenne d’âge de 49 ± 17 ans et une durée de diabète moyenne de 21 ± 13 ans. L’autofluorescence cutanée médiane était de 2.0 [1.7–2.4] unités arbitraires (UA), associée à l’âge et à la durée de diabète (β = 0.15 pour 10 ans, P < 0.001 et β = 0.17 pour 10 ans, P < 0.001). Après ajustements pour l’âge et le débit de filtration glomérulaire estimé (DFGe), elle était liée à la moyenne des cinq dernières HbA1c (β = 0.10 pour 1% d’HbA1c, P = 0.005) et des dix dernières HbA1c (β = 0.13 pour 1% d’HbA1c, P = 0.001). L’autofluorescence cutanée était associée avec la présence d’une rétinopathie diabétique (P < 0.001), d’une micro- ou macroalbuminurie (P < 0.001) et d’une réduction du DFGe (P < 0.001). L’autofluorescence cutanée est liée au contrôle glycémique à long terme et aux complications (néphropathie et rétinopathie) chez les patients présentant un diabète de type 1.

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Mots clés : Diabète Type 1 ; Autofluorescence cutanée ; Produits de glycation avancée : HbA1c ; Complications microvasculaires

Abbreviations: AGEs, Advanced glycation end-products; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; HbA1c, Glycated haemoglobin; BMI, Body mass index; eGFR, Estimated glomerular filtration rate; AU, Arbitrary units.

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1. Introduction

Chronic hyperglycaemia is critical for the development of micro-angiopathic complications of type 1 diabetes mellitus (T1DM) via the increased production of advanced glycation end-products (AGEs) [1,2]. Their levels in skin biopsies were predictive of later retinopathy and nephropathy in the Diabetes Control and Complications Trial (DCCT) cohort [3]. In clinical practice, AGEs can only be indirectly assessed by the regular assay of HbA1c levels and systematic screening for diabetes complications.

However, the fluorescent properties of AGEs can help to improve their evaluation by measuring skin autofluorescence with an AGE Reader™ [4]. Studies of type 2 diabetes mellitus (T2DM) have indicated that skin autofluorescence is associated with diabetes complications [5] and predictive of cardiovascular events [6]. In T1DM, skin autofluorescence was higher in cases of retinopathy, although this relationship did not persist after adjusting for nephropathy [7]. Because nephropathy can be a consequence as well as a cause of AGE accumulation [8], the weak association with retinopathy questions the specificity of skin autofluorescence as a surrogate marker of the risk of diabetic complications. For this reason, the primary objective of the present study was to examine the association between skin autofluorescence on the forearm and glycaemic control over the past 5 years in adult patients with T1DM. A secondary objective was to investigate the association between skin autofluorescence and diabetic complications (retinopathy and nephropathy).

2. Patients and methods

2.1. Subjects

All consecutive patients with T1DM (n = 303) referred to the Nutrition-Diabetology unit of the University Hospital Haut-Levêque (Bordeaux, France) during 2009 were interviewed and examined. Of these patients, 300 gave their informed consent to participate in the study.

2.2. Skin autofluorescence measurement

The accumulation of AGEs was estimated from skin autofluorescence as measured by the AGE Reader (DiagnOptics Technologies B.V., Groningen, Netherlands) and expressed in arbitrary units (AU) [9]. Autofluorescence was calculated by automated observer-independent analysis by dividing the average light intensity of the emission spectrum (300–600 nm) by the average light intensity of the excitation spectrum (300–420 nm). Reproducibility was indicated by a mean coefficient of variation of around 5%. The measurements were performed at a skin site on the forearm that was free of scars, lichenification and other abnormalities. In some subjects, the measurements on the contralateral forearm (n = 22), calf (n = 51) and forehead (n = 26) were also taken for comparisons. Subjects with Fitzpatrick skin phototypes V and VI could not be evaluated due to their skin pigmentation with ultraviolet (UV) reflectance < 10%.

2.3. Clinical data

Age, gender, body mass index (BMI), smoking habits (smoker vs never-smoker), duration of diabetes (years) and complications were recorded at the time of skin autofluorescence measurement. Retrospectively, the latest HbA1c value (n = 300; blood sampled 10 days before skin autofluorescence measurement) as well as the last five (n = 243) and 10 (n = 200) HbA1c values were noted in the patients who had the corresponding follow-ups. As specialized visits are scheduled every 6 months at our centre, these measurements reflected the patients’ glucose control over the previous 3 months, 2.5 years and 5 years, respectively, prior to the skin autofluorescence measurement.

Using an independent ophthalmological examination, including retinal photography, fundoscopy and angiography when necessary, retinopathy was scored as either absent, background (micro-aneurysms, microhaemorrhages, hard exudates) or severe (pre-proliferative or proliferative). Micro-albuminuria was defined as an albumin excretion rate between 30 and 300 mg/24 h, and macro-albuminuria at a rate >300 mg/24 h. Reduced estimated glomerular filtration rate (eGFR) was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [10] and defined as a value < 60 mL/min/1.73 m².

2.4. Statistical analysis

The results were expressed as percentages, medians and interquartile ranges or as means and standard deviations (SD) where appropriate. The comparisons of skin autofluorescence between sites (forearm vs contralateral forearm, calf and forehead), between men and women, and between smokers and never-smokers were performed by non-parametric tests. Spearman’s correlation coefficients were calculated to analyze the relationship between skin autofluorescence values in forearm 1 and forearm 2, in forearm 1 and calf, and in forearm 1 and forehead. Separate univariable linear regression analyses were used to test the associations between skin autofluorescence (continuous variable) and complications: in the first model, retinopathy (three classes) was an independent variable, followed by reduced eGFR (three classes) and finally increased albuminuria (two classes). Multivariable logistic regression was performed to assess the association between retinopathy (dependent variable dichotomized as absent vs background or severe) and skin autofluorescence measurements (continuous) adjusted for age (continuous) and eGFR (three classes). Also, the comparisons of skin autofluorescence between classes of complications were performed by non-parametric tests. In addition, the associations between forearm skin autofluorescence (dependent variable) and independent variables (glucose control at various times) were tested by univariable models, while multivariable linear models were adjusted for age (continuous) and eGFR (three classes). Separates analyses were carried out respectively in patients with a single HbA1c measurement (cross-sectional analysis, n = 300), with five past HbA1c measurements (n = 243) and with 10 past HbA1c measurements.
### Table 1
Characteristics of type 1 diabetes patients recruited at the Haut-Levêque Hospital (Bordeaux, France) according to HbA1c values.

<table>
<thead>
<tr>
<th></th>
<th>Latest HbA1c values (n = 300)</th>
<th>Last five HbA1c values (n = 243)</th>
<th>Last 10 HbA1c values (n = 200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n, %)</td>
<td>174 [58%]</td>
<td>143 [58%]</td>
<td>120 [60%]</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.2 [17.4]</td>
<td>50.9 [16.5]</td>
<td>52.8 [15.3]</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.7 [1.5]</td>
<td>7.8 [0.9]</td>
<td>7.8 [0.9]</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.7 [4.2]</td>
<td>24.9 [4.2]</td>
<td>25.3 [4.3]</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>21.7 [13.7]</td>
<td>23.8 [12.5]</td>
<td>25.5 [12.1]</td>
</tr>
<tr>
<td>Albumin excretion rate (mg/24 h)</td>
<td>90 [287]</td>
<td>97 [305]</td>
<td>76 [239]</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>87 [57]</td>
<td>91 [61]</td>
<td>88 [51]</td>
</tr>
<tr>
<td>Retinopathy (n; when information available, % of n)</td>
<td>285</td>
<td>236</td>
<td>196</td>
</tr>
<tr>
<td>No</td>
<td>136 [48%]</td>
<td>98 [42%]</td>
<td>73 [37%]</td>
</tr>
<tr>
<td>Background</td>
<td>95 [33%]</td>
<td>87 [37%]</td>
<td>77 [39%]</td>
</tr>
<tr>
<td>Severe</td>
<td>54 [19%]</td>
<td>51 [21%]</td>
<td>46 [24%]</td>
</tr>
</tbody>
</table>

Categorical variables are expressed as n [percentage], and continuous variables are expressed as means [SD].

(n = 200). A value of P < 0.05 was considered significant. All the analyses were conducted using R statistics version 2.14.2 [11].

### 3. Results

#### 3.1. Study population characteristics

A total of 300 T1DM patients were included in the study. Table 1 presents the main clinical and biochemical characteristics of the patients.

#### 3.2. Skin autofluorescence in the forearm, calf and forehead

There were no differences in skin autofluorescence between forearms or between forearm and calf (Table 2). However, skin autofluorescence was lower on the forehead than on the forearm (P < 0.001). Also, forearm values correlated with those from the other sites (r = 0.93 with other forearm, P < 0.001; r = 0.75 with the calf, P < 0.001; and r = 0.59 with the forehead, P < 0.01).

In addition, skin autofluorescence was slightly higher in women than in men (P = 0.05), but did not differ between smokers and never-smokers (P = 0.7).

#### 3.3. Skin autofluorescence and diabetes complications

Skin autofluorescence on the forearm was higher in 50 patients who had micro-albuminuria or macro-albuminuria compared with those without albuminuria (Fig. 1a; Table 2). Furthermore, skin autofluorescence was associated with albumin excretion rate (β = 0.008 per 1 mg/day of albuminuria, P < 0.001), plasma creatinine level (β = 0.004 per 1 μmol/L of serum creatinine, P < 0.001) and with eGFR, as measured by CKD-EPI (β = −0.01 per 1 mL/min/1.73 m² of GFR, P < 0.001). Skin autofluorescence values increased with the decrease of eGFR (Fig. 1b). Retinopathy was present in 149 patients (52%), and skin autofluorescence was higher in the 95 patients who had background retinopathy vs no retinopathy and in the 54 with pre-proliferative or proliferative retinopathy (Fig. 1c). The presence of retinopathy was significantly associated with skin autofluorescence [odds ratio (OR): 2.45 for 1 AU, 95% confidence interval (CI): 1.38–4.32; P < 0.01] independent of age and eGFR. Interestingly, skin autofluorescence was also greater when retinopathy was present in

### Table 2
Skin autofluorescence (in arbitrary units, AU) in type 1 diabetes patients recruited at the Haut-Leveque Hospital (Bordeaux, France).

<table>
<thead>
<tr>
<th></th>
<th>Skin autofluorescence</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forearm, n = 300</td>
<td>2.0</td>
<td>[1.7–2.4]</td>
</tr>
<tr>
<td>Contralateral forearm, n = 22</td>
<td>2.0</td>
<td>[1.8–2.3]</td>
</tr>
<tr>
<td>Albumin excretion rate &lt; 30 mg/24 h, n = 231</td>
<td>2.0</td>
<td>[1.7–2.4]</td>
</tr>
<tr>
<td>≥ 30 mg/24 h, n = 50</td>
<td>2.4</td>
<td>[1.9–2.8]</td>
</tr>
<tr>
<td>Estimated GFR (CKD-EPI) ≥ 60 mL/min/1.73 m², n = 242</td>
<td>2.0</td>
<td>[1.7–2.4]</td>
</tr>
<tr>
<td>59–30 mL/min/1.73 m², n = 28</td>
<td>2.6</td>
<td>[2.3–2.9]</td>
</tr>
<tr>
<td>&lt; 30 mL/min/1.73 m², n = 8</td>
<td>2.9</td>
<td>[2.7–3.7]</td>
</tr>
<tr>
<td>Men, n = 174</td>
<td>2.0</td>
<td>[1.7–2.4]</td>
</tr>
<tr>
<td>Women, n = 126</td>
<td>2.1</td>
<td>[1.8–2.6]</td>
</tr>
<tr>
<td>Retinopathy:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No, n = 136</td>
<td>1.9</td>
<td>[1.7–2.2]</td>
</tr>
<tr>
<td>Background, n = 95</td>
<td>2.1</td>
<td>[1.8–2.6]</td>
</tr>
<tr>
<td>Severe, n = 54</td>
<td>2.4</td>
<td>[2.3–2.7]</td>
</tr>
</tbody>
</table>

Data are presented as medians [25th–75th percentiles]; GFR: glomerular filtration rate; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration.
patients with normal eGFR: no retinopathy (n = 119), autofluorescence = 1.9 AU, 95% CI: 1.7–2.2; background retinopathy (n = 78), autofluorescence = 2.1 AU, 95% CI: 1.8–2.5; and severe retinopathy (n = 34), autofluorescence = 2.3 AU, 95% CI: 1.9–2.5 (P < 0.001).

3.4. Skin autofluorescence and glucose control

Skin autofluorescence on the forearm was associated with age (Fig. 2a) and duration of diabetes (β = 0.15 per 10 years, P < 0.001, and β = 0.17 per 10 years, P < 0.001, respectively; Fig. 2b), and with the means of the last five HbA1c (β = 0.10 per 1% of HbA1c, P < 0.05) and last 10 HbA1c (β = 0.12 per 1% of HbA1c, P < 0.01) values, but not with the latest HbA1c value (P = 0.64).

After adjusting for age as a continuous variable and eGFR divided into three categories, skin autofluorescence was found to be related to glucose control over the past 2.5 and 5 years (mean of last five HbA1c values: β = 0.10 per 1% of HbA1c, P = 0.005; mean of last 10 HbA1c values: β = 0.13 per 1% of HbA1c, P = 0.001), whereas the relationship with the latest HbA1c level did not reach significance (P = 0.26; Table 3).

4. Discussion

Our main findings were the significant relationships between skin autofluorescence and: (1) long-term glucose control, as reflected by the means of the last five and 10 HbA1c values; and (2) diabetes complications, including retinopathy and nephropathy.

In the DCCT, skin biopsies taken 1 year before the end of the study showed increased skin glycated collagen variables in 215 patients with T1DM, while the levels were even higher in patients with poor glycaemic control [3]. In our present patients, the poor relationship between skin autofluorescence and the latest HbA1c value was consistent with the results of studies of T2DM showing non-significant (NS) [4] or weak [5,12] correlations. Statistical significance was also not reached in the two studies that included patients with T1DM [4,7], except when HbA1c was > 8%, as described in the recent report by Samborski et al. [13]. Turk et al. [14] reported a correlation (r = 0.77) between HbA1c and haemoglobin AGEs in 75 patients with diabetes of both types that was weaker (r = 0.37) in those with
HbA1c < 8%. However, the close relationship observed in our study between skin autofluorescence and the means of the last five and 10 HbA1c values suggests that skin autofluorescence can provide additional information on longer-term glucose memory. Closer correlation of skin autofluorescence with the means of previous HbA1c values at 1 year of follow-up was also noted by Meerwaldt et al. [4] in 25 patients with T1DM. This was probably due to the long half-life of subcutaneous collagen to which AGEs link.

Accumulation of AGEs is a time-dependent process that increases with chronic hyperglycaemia [15], but also with other mechanisms, involving oxidative stress, reduced eGFR [16], hepatic function [17] and blood lipid levels [18]. Accordingly, skin autofluorescence increases with age [9] and with reduced GFR [8]. The high levels of skin autofluorescence noted in our patients with diabetic complications could therefore have been due to their older age and more severe nephropathy; renal insufficiency is, by itself, associated to high skin autofluorescence even in non-diabetic subjects [16]. To discriminate among these influences, our patients were classified according to their severity of kidney injury, with results adjusted for age and eGFR.

Skin autofluorescence was higher in patients who had diabetic complications, with marked differences in the small group with advanced complications: +25% with pre-proliferative or proliferative retinopathy; and +30% with decreased eGFR. Such relationships have already been confirmed in patients with T2DM [19,20], and shown to be predictive of vascular events and mortality [6]. Three studies, involving 48 [21], 69 [22] and 133 [7] patients, respectively, found a relationship between skin autofluorescence and complications of T1DM. In the pioneering study by Meerwaldt et al. [21], skin autofluorescence was associated with coronary heart disease (OR: 7.8, n = 13), micro-albuminuria and hypertension. More recently, Chabroux et al. [7] noted an association between skin autofluorescence and nephropathy, and our present study has confirmed its association with macro-albuminuria and a reduced eGFR. Renal insufficiency is itself associated with high skin autofluorescence even in non-diabetic subjects [16], and mortality and skin autofluorescence are associated in dialysis patients [23]. In diabetic nephropathy, AGEs accumulate and lead to the release of proinflammatory cytokines and activate nuclear factor (NF)-κB through binding to its receptor [16,24]. The absence of a link between retinopathy and skin autofluorescence, as mentioned by Chabroux et al. [7], was probably due to their smaller sample size; interestingly, they found elevated skin autofluorescence in 24 patients with severe retinopathy, although the contribution of retinopathy was no longer significant after adjusting for nephropathy. In our present study, skin autofluorescence increased with the severity of diabetic retinopathy even in patients with normal renal function. Nephropathy was not, therefore, the only explanation for the high skin autofluorescence in our patients with retinopathy.

The main limitation of our study was its retrospective aspect, as skin autofluorescence would be of greater value if it could predict the complications of T1DM, as has already been seen in T2DM [5]. However, further follow-up of our patients should provide more information in that respect. Also, the fact that diabetic retinopathy was assessed by an ophthalmological examination that did not systematically include retinal photography is another limitation that may have led to an underestimation of retinopathy. In our present patients, 52% had retinopathy after 22 years of diabetes, which is close to the 40% after 17 years reported by Chabroux et al. [7], who similarly evaluated retinopathy, and comparable to the 46% retinopathy rate after 15 years of diabetes in the EURODIAB study, which used retinal photography [25]. More work including more sensitive ophthalmological evaluations, especially for maculopathy, and also systematic evaluations of diabetic neuropathy are now required to further explore the links between skin autofluorescence and diabetic complications.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Authors contributions: M.G. collected and analyzed data, and wrote and revised the manuscript. A.V. analyzed data and revised the manuscript. C.G. revised the manuscript. C.R. revised the manuscript. P.B.G. helped interpret the data, and wrote and revised the manuscript. H.G. collected and analyzed data, and reviewed/edited the manuscript. V.R. collected and analyzed data, and wrote and revised the manuscript.

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