



Original article

Advanced glycation end products assessed by skin autofluorescence in type 1 diabetics are associated with nephropathy, but not retinopathy

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Abstract

Aims. – Advanced glycation end products (AGEs) are thought to play a central role in the pathogenesis of diabetes complications. For this reason, a non-invasive tool using skin autofluorescence (AF) quantification that correlates with levels of tissue AGEs has been developed. The present study aimed to assess whether or not skin AF is associated with microvascular complications in patients with type 1 diabetes (T1D).

Methods. – All consecutive patients with T1D ($n = 133$) had three AF measures taken on the forearm, using illumination with a fluorescent tube, all on the same day after breakfast or lunch. Potential associations between skin AF levels and microvascular complications, age, diabetes duration and health status were then assessed using a multivariate linear-regression model.

Results. – On age-adjusted analyses, diabetes duration, retinopathy, nephropathy and neuropathy were significantly associated with skin AF levels (all $P < 0.001$). AF levels increased significantly with severity in both retinopathy and nephropathy ($P < 0.001$). After adjusting for age, diabetes duration, HbA_{1c}, smoking, retinopathy, nephropathy and neuropathy, the association of AF levels remained significant with nephropathy and neuropathy, but not with retinopathy and diabetes duration.

Conclusion. – This study suggests an independent association between skin AF levels and diabetic nephropathy and neuropathy, but not retinopathy, in T1D patients. Prospective studies are needed to confirm the ability of skin AF levels to predict microangiopathy.

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Keywords: Type 1 diabetes; Complications; Retinopathy; Nephropathy; Neuropathy; Advanced glycation end products; AGE; Skin autofluorescence

Résumé

Produits de la glycation avancée cutanés par autofluorescence et microangiopathie au cours du diabète 1.

Objectifs. – Les produits de la glycation avancée (AGE) jouent un rôle important dans la survenue des complications diabétiques. Une méthode non invasive est disponible pour une quantification des AGE cutanés par la mesure de l'autofluorescence (AF). L'objectif était d'étudier les associations potentielles entre AF et microangiopathie chez des patients diabétiques de type 1.

Méthodes. – Un groupe de 133 diabétiques de type 1 vus de façon consécutive ont eu trois mesures successives de l'AF cutanée sur l'avant-bras. Pour chaque patient, l'analyse a été réalisée après le petit déjeuner ou le déjeuner et a été corrélée avec l'âge, la durée du diabète et l'état clinique.

Résultats. – Après ajustement sur l'âge, la durée du diabète, l'existence d'une rétinopathie, d'une néphropathie ou d'une neuropathie étaient associés au niveau d'AF cutané ($P < 0,001$). Après ajustement sur l'âge, la durée du diabète, l'HbA_{1c}, le tabac, la rétinopathie, la néphropathie et la neuropathie, les associations entre AF et néphropathie et neuropathie persistaient mais non celles avec la rétinopathie et la durée du diabète.

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Conclusion. – Notre étude suggère une association indépendante entre AF cutanée, néphropathie diabétique et neuropathie mais non avec la rétinopathie chez les diabétiques de type 1. Des études prospectives semblent nécessaires pour confirmer la valeur prédictive de la mesure de l'AF cutanée dans la survenue d'une complication microangiopathique chez les diabétiques de type 1.

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Mots clés : Diabète de type 1 ; Complications ; Rétinopathie ; Néphropathie ; Neuropathie ; Produits de la glycation avancée ; AGE ; Autofluorescence cutanée

1. Introduction

Diabetes causes a variety of end-stage organ complications. Hyperglycaemia appears to be the major contributing factor in proliferative retinopathy, and is a necessary – but not the only – factor in the development of diabetic nephropathy [1,2]. Advanced protein glycation in diabetes as a result of chronic hyperglycaemia as well as oxidative stress have been postulated to play central roles in diabetic complications involving the accumulation of advanced glycation end products (AGEs) in tissues and arterial walls [3,4]. The individual capacity to modulate AGE tissue content may explain the possible discrepancies between patients, despite comparable disease duration and glycaemic control. The Diabetes Control and Complications Trial (DCCT) clearly demonstrated the importance of blood glucose control in reducing the risk of microangiopathy in type 1 diabetes (T1D) patients [1], along with carotid intima-media thickness [5]. Moreover, its long-term observational follow-up study – the Epidemiology of Diabetes Interventions and Complications (EDIC) study [6] – also indicated that skin AGE levels are associated with long-term complications, and that this association persists after adjustment for HbA_{1c} [7,8]. This observation supports the 'glycaemic memory' hypothesis for diabetic complications. Until recently, measuring AGE accumulation required invasive sampling with tissue biopsy. However, a non-invasive method was recently described that can assess tissue AGEs by measuring skin autofluorescence (AF). The method uses the fluorescent properties of AGEs and collagen-linked fluorescence (CLF), which are then correlated with tissue AGEs [9,10]. The aim of the present study was to assess whether or not skin AF is associated with microvascular complications in patients with T1D.

2. Patients and methods

2.1. Patients

Consecutive patients ($n=133$) with T1D who had been referred to the Edouard Herriot Hospital in Lyon, France, from June to October 2008 were interviewed and examined by the same investigator (C.T.). All patients with clinical or biological signs of infection were excluded.

2.2. Data collection

Incipient nephropathy was defined as repeated early-morning urinary samples with albumin concentrations of 30–299 mg/L, and overt nephropathy as levels greater than 300 mg/L. Patients with creatinine clearance greater than 30 mL/min and/or requiring dialysis were considered to have severe renal failure. Retinal

lesions were screened using standardized photographs (Topcon TRC-NW6S, Tokyo, Japan), including three fields for each eye and dilatation when appropriate, which were scored by an independent ophthalmologist as absent, background or moderate (grades 1/2), or severe (grades 3/4). The presence of peripheral neuropathy was assessed by stringent clinical criteria using a monofilament and a graduated tuning fork. Gender, age, body mass index (BMI), diabetes duration, smoking habits and HbA_{1c} at the time of skin AF measurement were systematically recorded. All patients gave their informed consent to participate in the study.

2.3. AGE measurement

Skin AF in our T1D patients was assessed using the AGE ReaderTM (DiagnOptics BV, Groningen, the Netherlands) by the same investigator (C.T.). Three measurements per patient were taken at room temperature on the forearm, at around 10 cm below the elbow fold, with patients in a sitting position. All measurements were done on the same day after either breakfast or lunch, and were then correlated with age, diabetes duration and health status. Skin AF was expressed in arbitrary units (AU) and multiplied by 100 to obtain the mean of the three separate measures. Normal AF levels for each given patient was defined according to the distribution of AF levels in a non-diabetic control group of the same age, as established by the manufacturer of the AGE ReaderTM and automatically made available.

2.4. Statistical analysis

Factors potentially associated with skin AF levels were studied using a bivariate linear-regression model, with skin AF level as the dependent variable. The model was systematically adjusted for age. The multivariate analyses included adjustments for age, diabetes duration, HbA_{1c}, smoking, retinopathy, nephropathy and neuropathy. Continuous data are presented as means (SD) or medians (interquartile range [IQR] = 75th percentile minus 25th percentile) as appropriate. All comparisons were two-sided and a P value < 0.05 indicated a statistically significant difference. Data were analyzed using STATATM Version 9.1 statistical software (StataCorp 2003, Release 9.1, College Station, TX, USA).

3. Results

3.1. Patients' characteristics

A total of 133 T1D patients – 63 men (47.4%) and 70 women (52.6%) – were included in the present study. Median age was

Table 1

Factors associated with levels of advanced glycation end products (AGEs), as assessed by skin autofluorescence, in age-adjusted analyses.

			AGE (median, IQR)	Coefficient (95% CI) ^a	P
Gender	Men	63 (47.4)	1.95 (0.67)	Reference	–
	Women	70 (52.6)	2.17 (0.87)	0.09 (–0.10 to 0.28)	0.33
Non-smoker or ex-smoker		98 (75.4)	1.96 (0.78)	Reference	–
Current smoker		32 (24.6)	2.13 (0.5)	0.18 (–0.04 to 0.39)	0.10
BMI (kg/m ² , mean [SD])		24.2 (4.2)		–0.02 (–0.05 to –0.01)	0.14
Diabetes duration (years, median [IQR])		17 (15)	–	0.02 (0.01 to 0.03)	<0.001
HbA _{1c} (%; mean [SD])		8.3 (1.84)		0.03 (–0.02 to 0.09)	0.26
Retinopathy	None	80 (60.2)	1.87 (0.54)	Reference	–
	Moderate	29 (21.8)	2.30 (0.54)	0.40 (0.18 to 0.62)	<0.001
	Severe	24 (18.0)	2.91 (1.15)	0.66 (0.40 to 0.91)	<0.001
Nephropathy:	None	108 (81.8)	1.95 (0.61)	Reference	–
	Incipient	9 (6.8)	2.6 (0.62)	0.53 (0.23 to 0.83)	0.001
	Overt or renal failure	15 (11.4)	3.1 (1.25)	1.05 (0.81 to 1.29)	<0.001
Neuropathy:	No	116 (90.6)	1.99 (0.67)	Reference	–
	Yes	12 (9.6)	3.33 (0.66)	1.09 (0.81 to 1.37)	<0.001

Continuous variables are expressed as means (SD) or medians (IQR) as appropriate; categorical variables are expressed as *n* (%); IQR: interquartile range; CI: confidence interval; BMI: body mass index.

^a Estimated by age-adjusted linear regression.

30 years (IQR = 23), diabetes duration was 17 years (IQR = 15) and HbA_{1c} was 8.0% (IQR = 1.5). Microvascular complications were present in 41.3% of the patients, including retinopathy in 39.9% (*n* = 53), nephropathy in 18.2% (*n* = 24) and neuropathy in 9.4% (*n* = 12). In patients without complications (*n* = 80), the median age was 24 years (IQR = 15), diabetes duration was 12 years (IQR = 9; minimum = 2, maximum = 46) and HbA_{1c} was 7.8% (IQR = 1.7). In patients with microangiopathy (*n* = 53), the median age was 38 (IQR = 22), diabetes duration was 23 years (IQR = 15; minimum = 8, maximum = 52) and HbA_{1c} was 8% (IQR = 1.4). The median skin AF level was 2.07 AU (IQR = 0.79).

3.2. Factors associated with skin autofluorescence

As expected, age was positively associated with skin AF ($P < 0.001$). On age-adjusted analyses, diabetes duration, retinopathy, and incipient and overt nephropathy as well as neuropathy were each separately associated with increased AF levels (all $P < 0.001$; Table 1). Moreover, skin AF increased with the severity of nephropathy (without, incipient, overt and severe nephropathy) and retinopathy (without, moderate, severe) (P value for trend < 0.001). In contrast, gender, smoking status, BMI and HbA_{1c} were not significantly associated with increased skin AF levels. Also, AF levels increased systematically between patients without renal or retinal complications (1.86, IQR = 0.52), patients with retinopathy only (2.30, IQR = 0.73), and patients with both retinal and renal complications (2.94, IQR = 1.25) (P value for trend < 0.001 ; Fig. 1). Within the subgroup of T1D patients free of microvascular complications, only 53% (8/15) had normal skin AF levels after 20 years of diabetes, but 100% (6/6) did after 40 years of diabetes. On multivariate analyses, age, nephropathy and neuropathy – but neither retinopathy nor diabetes duration – remained significantly and independently associated with increased skin AF (Table 2). After adjustments for confounders and compared with patients without nephropathy, mean AF

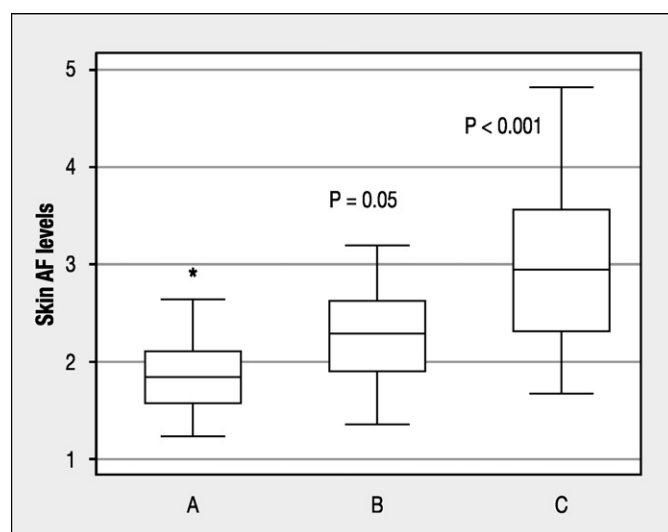


Fig. 1. Median and interquartile range (25th to 75th percentile) of advanced glycation end product (AGE) levels, as assessed by skin autofluorescence (AF), according to retinal and renal complications in patients with no complications (A, *n* = 80), with retinopathy only (B, *n* = 29), and with both retinopathy and nephropathy (C, *n* = 24). *Age-adjusted linear regression.

levels were 0.37 AU greater (95% CI: 0.03–0.70; $P = 0.03$) in patients with incipient nephropathy, and 0.58 AU greater (95% CI: 0.18–0.99; $P = 0.005$) in patients with overt or severe nephropathy. The association between retinopathy and skin AF disappeared after adjusting for nephropathy, as did the association with diabetes duration after adjusting for retinopathy. There was no significant interaction between retinopathy and nephropathy or between diabetes duration and retinopathy.

4. Discussion

Our results indicate that age, nephropathy and neuropathy – but not retinopathy – were independently associated with skin AF levels in patients with T1D.

Table 2

Factors associated with advanced glycation end products (AGEs), as assessed by skin autofluorescence, on multivariate analyses.

		Coefficient (95% CI) ^a	P
Age (years) ^b		0.013 (0.006 to 0.02)	0.001
Diabetes duration (years) ^b		0.009 (−0.002 to 0.02)	0.09
HbA _{1c} (%)		0.016 (−0.03 to 0.06)	0.47
Non-smoker or ex-smoker		Reference	–
Current smoker		0.06 (−0.13 to 0.25)	0.54
Retinopathy	Without	Reference	–
	Moderate	0.08 (−0.14 to 0.30)	0.46
	Severe	0.12 (−0.17 to 0.42)	0.40
Nephropathy	Without	Reference	–
	Incipient	0.37 (0.03 to 0.70)	0.03
	Overt and/or severe	0.58 (0.18 to 0.99)	0.005
Neuropathy	No	Reference	–
	Yes	0.46 (0.02 to 0.91)	0.04

CI: confidence interval.

^a Estimated by multivariate linear regression adjusted for age, diabetes duration, HbA_{1c}, smoking, retinopathy, nephropathy and neuropathy.^b For an increase of 1 year.

Long-term glycaemic control and diabetes duration are well-known risk factors for the development of diabetic microangiopathy [1]. Given a sufficient diabetes duration, retinopathy is nearly universal, whereas not all patients develop nephropathy. The relatively short turnover time of haemoglobin remains the limiting factor in the use of HbA_{1c} as a long-term glycaemic index; it is also an Amadori product, not an AGE [11]. Therefore, additional markers that reflect long-term exposure to glucose are needed. In the present study, there was no association between skin AF levels and HbA_{1c} at the time of analysis. Our data are consistent with two previous studies in type 2 diabetes (T2D) patients that monitored skin AF [9] and serum low-molecular-weight AGE [12], while another study found a modest correlation between the two markers using skin AF [10]. The DCCT/EDIC study of T1D [9] and, more recently, the United Kingdom Prospective Diabetes Study (UKPDS), a post-monitoring study of T2D [13], have underscored the importance of early glycaemic control. The so-called glycaemic memory effect – also called the “legacy effect of hyperglycaemia” – may correspond to the accumulation of AGEs [14].

Glycation end products are a complex group of compounds formed by the non-enzymatic reaction of reducing sugars and amine residues on proteins, lipids and nucleic acids. Some of the best chemically characterized AGEs in humans include pentosidine and *N*-(carboxymethyl)lysine (CML). AGEs accumulate at most sites of diabetic complications, including the kidney, retina and atherosclerotic plaques [15,16]. Despite the heterogeneity of AGE structures, a common consequence of their formation is a covalent cross-linking formation and increased stiffness of the protein matrix [17,18]. Clinical studies have demonstrated that increased serum levels of AGEs are seen within 2–5 years of diabetes onset in relation to glycaemic levels, and that circulating AGEs may be linked to various diabetes complications [19]. AGE assays of blood are more accessible, whereas plasma assays have yet to be shown to be directly related to tissue AGE content, and also require sophisticated and expensive laboratory techniques that have many sources of interference [20]. These technical aspects as well as the absence of standards have limited

the use of such measurements in the clinic. Nevertheless, early studies have shown that skin collagen, CML, pentosidine and fluorescence were increased by up to twofold in T1D patients compared with non-diabetic controls [21]. Skin AGE levels may also provide a more long-term ‘memory’ of glycaemic stress and, therefore, may be more appropriate for predicting complications. The DCCT substudy has already demonstrated in T1D that skin collagen glycation and glycoxidation products are better predictors for diabetes complications than are mean HbA_{1c} levels [7,8], which has been confirmed by 30 years of monitoring [22]. However, the need for skin biopsy is a serious limitation in clinical monitoring, making non-invasive techniques such as skin AF measurement of greater interest.

The present study results indicate that nephropathy is associated with increased skin AF. It is important to bear in mind that a small, but consistent, proportion of T1D patients do not develop severe complications, suggesting that hyperglycaemia is necessary, but not the only factor, especially in the case of nephropathy [2,8]. The kidney is a target for AGE-mediated damage and, as a major site of clearance, it is also an important contributor to circulating AGE concentrations in diabetes [23]. The positive and independent association found between AF levels and nephropathy is concordant with previous studies of T2D [10,24,25]. As for T1D, our present results are also consistent with the associations observed between serum AGEs, microalbuminuria and overt nephropathy [26]. The prospective DCCT/EDIC study found that AGE levels, as assessed by skin biopsy, were independently predictive of the progression of nephropathy in T1D after 10 years of follow-up [8]. On the other hand, the significant link between skin AF and retinopathy in our present study disappeared after adjusting for nephropathy. Similarly, no independent association between AF level and retinopathy was found in a study of T2D patients using the same protocol [19]. This may be a reflection of the lack of sufficient statistical power to detect a true, but small, association. Another point of consideration is that retinopathy results from a specific accumulation of AGEs [24]. CML and other AGEs have been localized to retinal blood vessels in patients with T2D,

and found to correlate with the degree of retinopathy [15,27]. In addition, as additional pathogenic pathways are involved, the expression of vascular growth factors could be interrelated [28]. Furthermore, the possibility that fluorescent skin proteins may underestimate levels of retinal AGEs cannot be excluded. Follow-up studies have already demonstrated the usefulness of skin AF as a new marker for macrovascular morbidity and mortality [25,29]. A small pilot study has shown that transplanted T1D patients failed to normalize AF levels despite optimal blood glucose control (data not shown). This may have been due to the high cardiovascular risk usually observed in such a population. In contrast to previous skin AF studies in T2D, we could find no association with smoking habits, female gender or BMI, all of which have been previously attributed to the increased formation of advanced lipoxidation end products in T2D patients [9]. Although AGEs can originate from exogenous sources such as tobacco smoke [30] and diet [31], we could find no positive correlation between skin AF and smoking.

The present study has several strengths. First, although several studies have studied the link between AGE levels and diabetes-related complications in T2D patients, this was the first study designed to evaluate the association between AGE levels, as assessed by skin AF, and microangiopathy in T1D patients. In contrast to T2D, involving complex interactions between AGEs, microangiopathy, macroangiopathy and cardiovascular risk, the results in T1D strengthen the connection between AGEs and microangiopathy. Second, our present study used a non-invasive skin AF tool that has been shown to correlate with several skin AGEs measured by skin biopsy (CLF, *N*-CML, pentosidine and *N*-[carboxyethyl]lysine) [9], and also allows routine and repeated measurements. The question of factors associated with resistance to microvascular complications in patients with extreme disease duration is also important. It has been reported that some diabetic patients never develop severe complications, suggesting that they may possess factors that neutralize the adverse effects of hyperglycaemia [32]. Interestingly, none of our non-complicated patients had increased skin AF levels, despite long-term disease duration. Although a larger population is needed before definite conclusions can be drawn, this observation has important clinical consequences.

Nevertheless, several limitations of the present study should also be mentioned. First, the design of the study was cross-sectional, with clinical characteristics and AF levels recorded at the same time. This suggests that the causality link between clinical characteristics and skin AF need to be interpreted with caution. Second, patients were not examined while in a fasting state. A recent study suggests that skin AF measurement increased after meals by 11% in a small group of T2D patients 2–4 h; the 594 Kcal meal was 59% fat, and had a calculated AGE content of 8.518 kU [33]. However, the chances of our having overestimated AF levels in the present study are minimal, as the composition of meals is different in real life. Also, it is unlikely that this could have introduced a bias, as there were no cut-off values for AF levels, and none of our patients were tested in the fasting state.

In conclusion, our present findings suggest that AGE accumulation, as assessed by a non-invasive tool for measuring skin

AF, is independently associated with nephropathy and neuropathy – but not retinopathy – in patients with T1D. Prospective data are now needed to assess the role of high levels of skin AF in such patients as a predictor of the subsequent development of microangiopathy. In any event, skin AF is a novel and promising way for clinicians to approach diabetic complications.

Conflicts of interest

The authors declare no potential conflicts of interest relevant to this article.

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