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Elevated skin autofluorescence is strongly associated with foot ulcers in patients with diabetes: a cross-sectional, observational study of Chinese subjects

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Abstract: Objective: This study was designed to evaluate the association between skin autofluorescence (AF), an indicator of advanced glycation end-products (AGEs), and foot ulcers in subjects with diabetes. Methods: In this study, 195 Chinese diabetic subjects were examined. Their feet were examined regardless of whether an ulcer was present or not. Skin AF was measured with an AGE reader. Demographic characteristics and blood data were recorded. Results: The mean values of skin AF were 2.29±0.47 for subjects without foot ulcers, and 2.80±0.69 for those with foot ulcers, a significant difference (P<0.05). Skin AF was strongly correlated with age and duration of diabetes. After adjusting for these factors, multivariate logistic regression showed that skin AF was independently associated with foot ulcerations. Conclusions: Skin AF is independently associated with diabetic foot ulcerations. It might be a useful screening method for foot ulceration risk of diabetic patients.

Key words: Advanced glycation end-products (AGEs), Diabetic foot ulcerations, Screening
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1 Introduction

Meerwaldt *et al*. (2004) proposed the assessment of skin autofluorescence (AF) as a new method for measuring of advanced glycation end-products (AGEs) in humans. This simple, non-invasive method was designed based on the fluorescent properties of AGEs. Their first clinical trial showed a positive correlation between collagen-linked fluorescence and AGEs content in skin biopsies (Meerwaldt *et al*., 2004; 2005b). The assessment of skin AF has some advantages over traditional measurements of plasma AGEs (Dorrian *et al*., 1998), including being inexpensive, easy to conduct, non-invasive, with a more reproducibility and a higher correlation to tissue contents of AGEs (Monami *et al*., 2008).

Diabetic foot ulcerations are associated with increased morbidity and mortality in diabetes mellitus. The majority of non-healing wounds of the foot lead to amputation, increasing the direct costs of diabetic care and rehabilitation, and losing productivity (Jeffcoate and Harding, 2003). Major mechanisms involved in the development of diabetic foot ulcerations are diabetic peripheral arterial disease (PAD) and diabetic sensory neuropathy (Boulton *et al*., 2004; Hoyt, 2004; Prompers *et al*., 2008).

Nonenzymatic glycation leading to AGEs formation is one of the important biochemical pathways involved in the development of long-term complications of diabetes (Brownlee, 2001). Basic researches have reported that the binding of AGEs to the receptor

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of AGEs (RAGE) on cells induces a signaling cascade with nuclear factor kappa beta (NF-κB) as a key signaling factor. Extracellular AGEs may bind several proteins, including lipoproteins and collagen (Meerwaldt *et al*., 2008). Through these pathways, AGEs induce both cell dysfunction and extracellular matrix degeneration (Mišur *et al*., 2004; Meerwaldt *et al*., 2008; Peppa *et al*., 2009). Higher skin AF is also associated with diabetic nephropathy, neuropathy, and cardiovascular disease in cross-sectional studies (Meerwaldt *et al*., 2005a; 2007; Chabroux *et al*., 2010). However, little clinical evidence is available focusing on the evaluation of the role of skin AF in determining foot ulceration risk in diabetes.

This study was designed to evaluate the association between skin AF and foot ulcers in subjects with diabetes, and identify other factors affecting its role in determining foot ulcer risk.

2 Subjects and methods

2.1 Study populations

From February 2009 to October 2009, a consecutive series of 195 Chinese patients with diabetes mellitus were enrolled in our study. These patients were previously diagnosed and treated for diabetes at the outpatient diabetic center of our hospital. The exclusion criteria were those who have impaired fasting glucose and impaired glucose tolerance. After informed consent, subjects received a thorough inspection and examination for foot ulcerations.

2.2 Medical history information

Medical history was collected in accordance with the practical guideline of the International Working Group on the Diabetic Foot (IWGDF) (the latest version 2007) (Apelqvist *et al*., 2008). This guideline encompasses a complete medical history with a list of detailed information on the diabetic foot, including ischemic symptoms (claudication and rest pain), loss of sensation, foot deformities, limited joint mobility, and previous ulcerations and amputations. Demographic characteristics including age, sex, duration of diabetes, current pharmacological treatment, and alcohol intake were also recorded. Self-reported smoking habits were calculated and recorded as a smoke index (cigarettes per day×smoke years). All patients underwent a physical examination, during which their weight, height, and waist circumference (WC) were recorded. Their body mass index (BMI) was also calculated and recorded.

2.3 Foot inspection and examination

Complete foot inspections and examinations were conducted according to the practical guideline of IWGDF (Apelqvist *et al*., 2008). Inspections include foot wear, skin intact/ulcer, abnormal pressure, deformity, callus and bony prominences, vascular status, and pedal pulsation. When an ulcer was found, its conditions, including depth, likely cause, and infection were recorded. Examinations included both neural and vascular examinations: standard 10 g Semmes-Weinstein monofilament test, vibration perception threshold (VPT) assessment with a biothesiometer (Biomedical Instrument Co., Newbury, Ohio, USA) (Garrow and Boulton, 2006), and standard ankle-brachial index [ABI, including right ABI (RABI) and left ABI (LABI)] measurement (using the Dopplex Assist Range, HUNTLEIGH Co., Ltd., UK) (Vierron *et al*., 2010).

2.4 Skin autofluorescence measurement

Skin AF was measured with an AGE reader (DiagnOptics BV, Groningen, the Netherlands), as previously described (Meerwaldt *et al*., 2004). The measurement steps were carried out in strict accordance with the product instructions. Briefly, the instrument illuminates a 1 cm^2-skin surface of the forearm, guarded by a rubber pad against surrounding light, with a wavelength of 300–420 nm. Light both passively reflected and automatically emitted by the measured skin was collected with a spectrometer in the 300–600 nm range. AF is automatically calculated as the average light intensity per nanometer in the 420–600 nm range divided by the average light intensity per nanometer in the 300–420 nm range. The mean of three measurements taken from three different sites and 5 min apart was used for calculations (Monami *et al*., 2008).

2.5 Other laboratory procedures

Blood samples were drawn in the morning after overnight fasting for the determination of blood urea nitrogen (BUN), creatinine (Cr), C-reactive protein (CRP), and lipid profile including triglyceride (TG),

high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Serum glycosylated hemoglobin (HbA1c), fasting blood glucose (FBG), and postprandial blood glucose (PBG) levels were also recorded for reflecting the patient's glucose control.

2.6 Subject grouping

After complete inspections and examinations, subjects were classified into two groups: those without foot ulcers and those with foot ulcers.

2.7 Statistical analysis

Continuous variables are expressed as mean± standard deviation (SD). Enumeration data are expressed as percentages. P value <0.05 was chosen to indicate statistical significance. We first checked the normality and homogeneity of the continuous variables, and then adopted an independent sample t-test or nonparametric independent sample test to analyze the differences between variables of both groups, depending on the distribution of the variables. Bivariate correlation was conducted to analyze correlation between skin AF and other continuous variables. Finally, the accumulative logistic regression was performed to summarize the factors that had a significant, independent association with diabetic foot ulcers. Statistical analysis was conducted using SPSS 11.5 for WINDOWS.

3 Results

3.1 Basic characteristics

A total of 195 consecutive patients with diabetes, including 111 men and 84 women, were enrolled in our study. Among them, 25 subjects were found to have foot ulcers, ranging from superficial epidermal blisters to half-foot gangrene. Their basic characteristics are shown in Table 1.

The value of skin AF of each group was: 2.29±0.47 for subjects without foot ulcers; 2.80±0.69 for subjects with foot ulcers. The skin AF values were significantly higher in those with foot ulcers than in those without foot ulcers (P<0.01). In addition to skin AF, other variables including age, duration of diabetes, FBG, Cr, TG, HDL, LDL, LABI, CRP, RABI, and VPT of subjects with foot ulcers were also found to be significantly different from those of subjects without foot ulcers.

Table 1 Subject characteristics

Variable	Value	
	Subjects without foot ulcers	Subjects with foot ulcers
Case number	170	25
Male number	96 (56.50%)	15 (60.00%)
Age (year)	57.01±14.50	68.20±12.26**
Skin AF (AU)	2.29±0.47	2.80±0.69**
BMI (kg/m^2)	23.57±3.65	22.92±3.46
WC (cm)	86.12±10.68	87.44±10.03
Smoke index	274.58±437.22	200.00±365.15
Duration of diabetes (year)	6.71±5.77	11.07±6.04**
FBG (mmol/L)	10.43±4.62	9.65±6.51*
PBG (mmol/L)	15.60±6.21	13.69±3.46
HbA1c (%)	9.47±2.54	9.25±2.14
BUN (mmol/L)	5.59±2.35	8.16±5.80
Cr (μmol/L)	67.65±41.15	123.47±181.71*
TG (mmol/L)	2.16±2.10	1.45±0.74*
HDL (mmol/L)	1.27±0.30	1.15±0.33*
LDL (mmol/L)	3.01±0.99	2.47±0.74**
LABI	1.08±0.14	0.90±0.31**
CRP (mg/L)	9.42±22.39	35.57±49.69**
RABI	1.08±0.13	0.92±0.24**
VPT	17.29±9.67	25.69±12.63**
Previous ulcer or amputation number	0 (0%)	2 (8%)

Values are expressed as mean±SD or n (%). * P<0.05, ** P<0.01, with foot ulcers vs. without foot ulcers. AF: autofluorescence; AU: arbitrary unit; BMI: body mass index; WC: waist circumference; FBG: fasting blood glucose; PBG: postprandial blood glucose; HbA1c: glycosylated hemoglobin; BUN: blood urea nitrogen; Cr: creatinine; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reactive protein; ABI: ankle-brachial index; RABI: right ABI; LABI: left ABI; VPT: vibration perception threshold

3.2 Correlation between skin autofluorescence and other variables

The correlation between skin AF and the other variables was further analyzed by the bivariate correlation method. As shown in Table 2, skin AF was significantly correlated with age, WC, duration of diabetes, BUN, CRP, and VPT (Table 2). After adjusting for these factors, the skin AF of subjects with foot ulcers remained significantly higher than that in those without foot ulcers (P<0.05).

Table 2 Correlations between skin AF and other variables

Variable	Pearson correlation coefficient	*P* value
Age	0.27	<0.01**
BMI	0.02	0.75
WC	0.19	0.02*
Smoke index	0.07	0.36
Duration of diabetes	0.35	<0.01**
FBG	−0.06	0.39
PBG	−0.12	0.97
HbA1c	−0.06	0.41
BUN	0.20	<0.01**
Cr	0.10	0.19
TG	0.01	0.87
HDL	−0.15	0.045*
LDL	−0.06	0.41
CRP	0.25	<0.01**
LABI	−0.02	0.82
RABI	−0.03	0.64
VPT	0.27	<0.01**

* $P<0.05$; ** $P<0.01$. All the abbreviations are the same as those shown in Table 1

3.3 Single variate binary logistic regression

We adopted single variate binary logistic regression to assess the contribution of each variable to diabetic foot ulcerations separately. As listed in Table 3, skin AF, age, duration of diabetes, BUN, Cr, CRP, TG, HDL, LDL, RABI, LABI, and VPT each had a significant association with diabetic foot ulcers.

Table 3 Single variate binary logistic regression

Variable	Wald value	OR	95% CI of OR	*P* value
Skin AF	11.72	3.08	[1.62, 5.80]	0.01*
Age	11.88	1.06	[1.03, 1.10]	0.01*
BMI	0.17	0.98	[0.87, 1.10]	0.68
WC	0.59	1.02	[0.97, 1.07]	0.44
Smoke index	0.09	1.00	[0.99, 1.01]	0.76
Duration of diabetes	11.15	1.13	[1.05, 1.21]	0.01*
FBG	0.90	0.95	[0.87, 1.05]	0.34
PBG	1.60	0.95	[0.88, 1.03]	0.21
HbA1c	0.16	0.97	[0.81, 1.15]	0.69
BUN	9.61	1.20	[1.07, 1.34]	0.02*
Cr	4.24	1.01	[1.00, 1.02]	0.04*
TG	4.00	1.00	[0.33, 0.99]	0.045*
HDL	4.12	0.22	[0.05, 0.95]	0.04*
LDL	6.94	0.45	[0.25, 0.82]	0.01*
CRP	9.45	1.02	[1.01, 1.03]	<0.01**
LABI	15.01	0.01	[0.00, 0.11]	<0.01**
RABI	11.45	0.02	[0.00, 0.17]	<0.01**
VPT	15.04	1.07	[1.04, 1.11]	<0.01**

* $P<0.05$; ** $P<0.01$. All the abbreviations are the same as those shown in Table 1

For the variables found to be significantly associated with foot ulcers in single variable regression, multivariate analysis was then conducted by backward step-wise accumulative binary logistic regression method. One variable was removed out of the equation according to the maximum *P* value (the largest P & $P>0.05$) at each step, until all the variables were significant in the final equation ($P<0.05$). The removed variable of each step is listed in Table 4. Skin AF, TG, BUN, RABI, and CRP are variables retained in the final equation of accumulative regression, indicating their independent associations with foot ulcers (Table 5).

Table 4 Variable removed in each step

Step	Variable removed	Wald value	*P* value
1	Duration of diabetes	0.22	0.64
2	Age	0.21	0.65
3	LABI	0.29	0.59
4	HDL	0.64	0.43
5	Cr	0.40	0.53
6	LDL	1.71	0.19
7	VPT	1.68	0.20

All the abbreviations are the same as those shown in Table 1

Table 5 Variables left in the final equation

Variable left	Wald value	OR	95% CI of OR	*P* value
AF	4.77	2.55	[1.10, 5.91]	0.03*
TG	6.94	0.31	[0.13, 0.74]	<0.01**
BUN	4.61	1.22	[1.02, 1.46]	0.03*
RABI	14.56	0.001	[0.000, 0.04]	<0.01**
CRP	4.66	1.02	[1.001, 1.03]	0.03*

* $P<0.05$; ** $P<0.01$. All the abbreviations are the same as those shown in Table 1

We further analyzed the correlations between variables left in the final equation and the variables removed from the equation that had significant association with diabetic foot ulcerations in univariate logistic regression. The results showed that each variable removed from the equation had a significant correlation with at least one variable retained in the final equation (Table 6).

4 Discussion

This single center, cross-sectional study demonstrated that skin AF, which reflects the AGEs' accumulation, was significantly elevated in diabetic

Table 6 Correlations between variables removed and retained in the equation

Variable	AF		RABI		CRP		BUN		TG	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Age	0.27	<0.01**	−0.11	0.12	0.34	<0.01**	0.16	0.03*	−0.06	0.41
Duration of diabetes	0.35	<0.01**	−0.04	0.55	0.17	0.03*	0.21	<0.01**	−0.02	0.78
LABI	−0.02	0.82	0.75	<0.01**	0.09	0.28	−0.20	<0.01**	0.00	0.99
HDL	−0.15	0.045*	−0.03	0.64	−0.06	0.45	−0.10	0.20	−0.20	<0.01**
Cr	0.10	0.19	0.03	0.66	0.14	0.07	0.40	<0.01**	0.13	0.07
LDL	−0.06	0.41	−1.22	0.91	0.07	0.38	0.18	0.02*	0.55	<0.01**
VPT	0.27	<0.01**	−0.06	0.41	0.29	<0.01**	0.18	0.01*	−0.08	0.29

* $P<0.05$; ** $P<0.01$. *r*: correlation coefficient. All the abbreviations are the same as those shown in Table 1

subjects with foot ulcers. Although some of the other variables, including age and duration of diabetes, had a significant correlation with skin AF, after adjusting for these, skin AF of those with foot ulcer was still significantly higher than that of those without foot ulcer. Path-analysis using multivariate logistic regression had unveiled that skin AF was one of the variables that had a significant, independent association with diabetic foot ulcerations. Although other variables such as age, duration of diabetes, and lipid profiles were not directly associated with the diabetic foot ulcers, they had a strong correlation with skin AF.

Some previous studies had suggested that skin AF is an independent predictor of diabetic microvascular complications, cardiovascular risk, and diabetic renal failure (Meerwaldt *et al*., 2005b; 2007; Lapolla *et al*., 2007). Meerwaldt *et al*. (2005a) showed that skin AF was increased and correlated with the Wagner score in diabetics with neuropathy. Skin AF correlated inversely with nerve conduction velocity and amplitude. Lapolla *et al.* (2007) found that AGE levels were higher in type 2 diabetics with PAD compared to those without PAD; AGE contents were correlated inversely to ABI, even after correction for other cardiovascular risk factors. Our study, to our knowledge, is the first to focus on the role of skin AF in determining foot ulceration risk in diabetics. According to the results of our study, beside ulcer, other factors, including nutrition status (e.g., WC), renal function (e.g., BUN), infection or inflammation (e.g., CRP), and lipid profile (e.g., HDL), also had significant correlations with skin AF. It was also worthwhile to notice that AF was significantly correlated with age of patients and duration of diabetes in our study. This strongly supports the theory of Dyer *et al.* (1993) that time plays a vital role in the deposition and accumulation of AGEs in tissue. Recently, Holman *et al.* (2008) on behalf of the United Kingdom Prospective Diabetes Study (UKPDS) group, proposed that AGE probably is a carrier of the so-called metabolic memory or legacy. Our finding agreed with the result of Meerwaldt *et al*. (2005a) that tissue AGE accumulation may represent the long-term effects of a final common pathway for various risk factors. Thus, based on the present work, skin AF might integrate these factors and is more informative than the actual levels of these risk factors themselves.

According to Liu *et al.* (2010), the prevalence of vascular complication, neuropathy, and diabetic foot ulcers in Chinese urban diabetic outpatients was 30.1%, 17.8%, and 0.8%, respectively. Although this prevalence might not be as high as in Western countries, the absolute number of patients is very large. Our findings suggest that skin AF could provide useful information in screening for diabetic foot ulceration risk. Advantages of skin AF measurement are its simplicity, wide availability, non-invasiveness, and cost effectiveness.

However, our study had some limitations. First, only 25 subjects were found to have foot ulcers, and their ulcer severity ranged from superficial epidermal blister to half-foot gangrene, which made it impossible to analyze them within subgroups. On the other hand, we should be cautious about the positive results provided by this study, since their statistic power might be limited by sample size. Second, our study was conducted in a central hospital in East China, with a referral function for local hospitals. Hence, patients were usually referred with an established diagnosis and medical or insulin intervention before they came to our center. The glucose and HbA1c levels, presented in this study, might poorly reflect the real status of blood glucose control over longer periods. Large multi-center prospective studies are needed to further confirm these findings.

5 Conclusions

Skin AF has a significant, independent association with diabetic foot ulcerations. Although other factors like age and duration of diabetes strongly correlated with skin AF, after adjusting for these factors, the association between skin AF and foot ulceration remained significant. Our findings suggest that skin AF might provide useful information in screening for diabetic foot ulceration risk. Large multi-center prospective studies are needed to further confirm these findings.

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