DIABETICMedicine

DOI: 10.1111/j.1464-5491.2011.03448.x

Article: Complications

Skin autofluorescence is associated with severity of vascular complications in Japanese patients with Type 2 diabetes

K. Tanaka, Y. Tani, J. Asai, F. Nemoto, Y. Kusano, H. Suzuki, Y. Hayashi, K. Asahi, M. Nakayama, T. Miyata[1] and T. Watanabe

Department of Nephrology, Hypertension, Diabetology, Endocrinology and Metabolism, Fukushima Medical University, Fukushima and [1]United Centers for Advanced Research and Translational Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

Accepted 6 September 2011

Abstract

Aims Skin autofluorescence, a non-invasive measure of the accumulation for advanced glycation end products, has been reported to be a useful marker for diabetic vascular risks in the Caucasian population. The aim of this study was to evaluate associations between skin autofluorescence and vascular complications in non-Caucasian patients with Type 2 diabetes.

Methods Subjects in this cross-sectional study comprised 130 Japanese patients with Type 2 diabetes. Skin advanced glycation end products were assessed by skin autofluorescence using an autofluorescence reader. Association between skin autofluorescence and severity of vascular complications was evaluated.

Results Of the 130 patients, 60 (46.2%) had microvascular complications such as diabetic retinopathy, neuropathy and nephropathy, 10 (7.7%) had macrovascular complications and 63 (48.5%) had micro- and/or macrovascular complications. Skin autofluorescence increased with severity of vascular complications. Independent determinants of skin autofluorescence were age ($\beta = 0.24$, $P < 0.01$), mean HbA_{1c} in previous year ($\beta = 0.17$, $P = 0.03$), microvascular complications ($\beta = 0.44$, $P < 0.01$) and macrovascular complications ($\beta = 0.27$, $P < 0.01$). Multiple logistic regression analysis revealed that diabetes duration (odds ratio 1.15, $P < 0.01$), systolic blood pressure (odds ratio 1.04, $P = 0.01$), skin autofluorescence (odds ratio 3.62, $P = 0.01$) and serum albumin (odds ratio 0.84, $P < 0.01$) were independent factors for the presence of vascular complications in these patients.

Conclusions Skin autofluorescence had independent effects on vascular complications in Japanese patients with Type 2 diabetes. This indicates that skin advanced glycation end products are a surrogate marker for vascular risk and a non-invasive autofluorescence reader may be a useful tool to detect high-risk cases in non-Caucasian patients with diabetes.

Diabet. Med. 29, 492–500 (2012)

Keywords advanced glycation end products, diabetes, vascular

Introduction

Diabetes and its associated vascular complications have become a public health problem of considerable magnitude. The Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study have indicated that hyperglycaemia is the initiating cause of diabetic vascular complications and is an independent risk factor for these complications in patients with Type 1 and Type 2 diabetes [1–3]. The precise mechanism by which hyperglycaemia causes these complications remains unclear, although the accumulation of advanced glycation end products (AGEs), caused by hyperglycaemia and oxidative stress, is thought to play a role in the pathogenesis of diabetic vascular complications [4,5].

In a Diabetes Control and Complications Trial substudy, Monnier *et al.* reported that tissue autofluorescence is related to advanced glycation end product accumulation and progression of diabetic vascular complications, after evaluating tissue autofluorescence using skin biopsy specimens [6]. As tissue accumulation of advanced glycation end products is a long-term process with low turnover, and thereby may reflect 'metabolic

Correspondence to: Kenichi Tanaka, Department of Nephrology, Hypertension, Diabetology, Endocrinology and Metabolism, Fukushima Medical University, Hikarigaoka 1, Fukushima City 960-1295, Japan.
E-mail: kennichi@fmu.ac.jp

memory', advanced glycation end product accumulation could be a useful marker for chronic diabetic vascular complications. However, skin biopsy is an invasive and time-intensive method and is not feasible in daily practice for outpatients.

An autofluorescence reader (AGE reader; Diagnoptics, Groningen, the Netherlands) is able to non-invasively assess advanced glycation end product accumulation using skin autofluorescence under ultraviolet light. Skin autofluorescence is reportedly associated with progression of vascular complications and is an independent predictor of microvascular complications and cardiac mortality in Caucasian patients with diabetes [7,8]. However, the relationship between skin autofluorescence and the severity of diabetic microvascular complications, such as grade of diabetic retinopathy and nephropathy (amount of proteinuria), has not been closely examined. Moreover, skin autofluorescence has not been sufficiently evaluated in non-Caucasian patients with diabetes, despite the fact that skin autofluorescence was shown to be related to cardiovascular disease in Japanese patients with chronic kidney disease (CKD) and end-stage renal disease [9,10]. The present cross-sectional study therefore aimed to clarify the contributing factors to skin autofluorescence and their relationships with vascular complications in Japanese (non-Caucasian) patients with Type 2 diabetes.

Patients and methods

Study population

This cross-sectional study included 130 patients with Type 2 diabetes and 70 control subjects who visited Fukushima Medical University Hospital or Tani Hospital between December 2008 and August 2009. Patients with Type 1 diabetes and patients receiving dialysis therapy were excluded from this study. Twelve patients with skin reflectance (R%) below 10% were excluded because of the autofluorescence reader's limitation to measure accurately in non-Caucasians with dark skin type [11]. The control subjects were outpatients who visited Fukushima University Hospital or Tani Hospital because of various diseases that did not affect accumulation of advanced glycation end products, such as gastrointestinal diseases, respiratory diseases and neurological diseases, and were age and sex matched to patients with Type 2 diabetes. In the control subjects, patients with diabetes mellitus, renal disease and macrovascular complications were excluded by the criteria described below, and by measurement of serum creatinine levels (< 88.4 μmol/l), respectively. The study protocol complied with the Declaration of Helsinki and was approved by the ethics committees at Fukushima Medical University. All patients received an explanation of the procedures and possible risks of this study and provided written informed consent to participate. All patients were of Japanese ethnicity (non-Caucasian). Patients with acute/chronic inflammatory disease and active malignancy were excluded. The characteristics of patients with Type 2 diabetes are summarized in Table 1.

Table 1 Clinical characteristics of patients with Type 2 diabetes

Variable	
n	130
Age (years)	68.5 (58.8–76.0)
Gender (male)	51 (39.2%)
History of smoking	56 (43.1%)
Body mass index (kg/m^2)	24.3 (22.1–27.4)
Duration of diabetes (years)	9.0 (4.0–14.3)
Systolic blood pressure (mmHg)	136 (123–145)
Diastolic blood pressure (mmHg)	73 (66–80)
Skin autofluorescence (AU)	2.22 (1.82–2.49)
eGFR (ml min^{-1} 1.73 m^{-2})	63.4 (48.1–80.2)
HbA_{1c} (mmol/mol; IFCC)	50 (45–56)
HbA_{1c} (%; NGSP)	6.8 (6.3–7.2)
Albumin (g/l)	41 (38–44)
Haemoglobin (g/l)	131 (116–142)
LDL cholesterol (mmol/l)	2.8 (2.3–3.3)
HDL cholesterol (mmol/l)	1.4 (1.2–1.7)
Microvascular complications	60 (46.2%)
Retinopathy	45 (34.6%)
Neuropathy	19 (14.6%)
Nephropathy	40 (30.8%)
Macrovascular complications	10 (7.7%)
Micro- and/or macrovascular complications	63 (48.5%)

Values are expressed as medians (interquartile range).
AU, arbitrary units; eGFR, estimated glomerular filtration rate; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; NGSP, National Glycohemoglobin Standardization Program.

Data collection

Blood pressure was taken as a single seated measurement using an aneroid device, obtained after 5 min of rest. Blood samples were collected at the clinic by venipuncture from every patient in a non-fasting state. Serum creatinine was measured using an enzyme-based method and LDL cholesterol was measured using a direct method. Serum albumin, haemoglobin, HDL cholesterol and HbA_{1c} were measured according to the automated standardized laboratory techniques in the clinical laboratories of each participating institution. Mean HbA_{1c} levels were calculated from monthly HbA_{1c} values in the previous 1 year from the skin autofluorescence measurement in 130 patients with diabetes. The value of HbA_{1c}, which was equivalent to the internationally used HbA_{1c} defined by the National Glycohemoglobin Standardization Program (NGSP), was calculated by adding 0.4% to the HbA_{1c} (%) defined by the Japan Diabetes Society, in accordance with the current revision of notation for the international standardization of HbA_{1c} [12]. The corresponding International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) standardized values were calculated using the relationship: IFCC value (in mmol/mol) = (NGSP value − 2.152)/0.09148 [13,14]. All HbA_{1c} data are given as NGSP standardized values and IFCC values. Estimated glomerular filtration rate (eGFR) was calculated using the estimation equation for Japanese patients

with chronic kidney disease. This equation calculates GFR from serum creatinine, age and gender using the following formula [eGFR (ml/min/1.73 m^2) = 194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$ (× 0.739 for women)] [15].

Definition of micro- and macrovascular complications

Diabetic retinopathy was determined by independent ophthalmologists based on retinal photography, which was scored as non-diabetic retinopathy, simple diabetic retinopathy or proliferative diabetic retinopathy. Neuropathy was diagnosed based on the presence of one or more neuropathic symptoms, such as numbness or reduced ability to feel pain, and abnormal vibration perception threshold, measured by biothesiometers on the big toe and on the medial malleolus, and the absence of two or more ankle or knee reflexes. Nephropathy was defined as positive dipstick results for proteinuria (≥ 1 +). We defined 'microvascular complication score' in this study as follows: patients without any microvascular complications such as diabetic retinopathy, neuropathy and nephropathy were scored as '0', those with one of the three complications were scored as '1', those with two of the three complications were scored as '2' and those with all three complications (triopathy) were scored as '3'.

A history of macrovascular complications was classified as at least one of the following events occurring before the time of skin autofluorescence measurement: acute myocardial infarction as a result of clinical and electrocardiographic or laboratory changes; angina pectoris based on clinical characteristics; coronary artery disease documented by coronary angiography; cerebral infarction verified by computed tomography (CT), magnetic resonance imaging (MRI) and/or the course of neurological disorders; and aortic disease including dissection and aneurysm verified by computed tomography and/or magnetic resonance imaging and peripheral artery disease. The definition of peripheral artery disease included patients with intermittent claudication (Fontaine's stage II), ischaemic rest pain (stage III) or ulcer, necrosis or a history of amputation (stage IV).

Diabetes was defined as glucose values ≥ 11.1 mmol/l at any time, fasting glucose values ≥ 7.0 mmol/l or the use of insulin or oral hypoglycaemic drugs.

Skin autofluorescence

Skin advanced glycation end product levels were assessed based on skin autofluorescence using the AGE Reader, as described previously [16–18]. Measurement of autofluorescence was defined as the average light intensity per nanometer in the range between 420 and 600 nm, divided by the average light intensity per nanometer in the range between 300 and 420 nm. Autofluorescence was expressed in arbitrary units (AU). The amount of ultraviolet light exposure is small and the autofluorescence reader has already been tested in several studies without any adverse effects [7–10,16,17,19–23]. All measurements were performed at room temperature (25 °C) with the patient in a seated position, at the volar side of the lower arm, approximately 10–15 cm below the elbow fold. Care was taken to perform the measurement at a normal skin site without visible vessels, scars, lichenification or other skin abnormalities. The intra- and inter-day assay precision expressed as coefficients of variation for autofluorescence reader measurements were 2.5% (n = 10) and 4.6% (n = 12), respectively. Autofluorescence was calculated offline by automated analysis and was observer-independent.

Statistical analysis

Statistical analysis was performed using PASW Statistics version 18.0 software (SPSS Japan, Tokyo, Japan). All variables are expressed as median [interquartile range (IQR)]. Spearman's rank correlation test was used to estimate relationships between variables. The Kruskal–Wallis test was applied to compare differences between groups. Multiple linear regression analysis was performed to determine the independent relationship between variables with skin autofluorescence. Independent effects of variables on the presence of vascular complication were assessed by forward stepwise logistic regression analysis ($P < 0.05$ for entry and $P \geq 0.10$ for removal). Differences were considered significant at the $P < 0.05$ level.

Results

Clinical and biochemical characteristics

The clinical characteristics of the 130 patients with diabetes are shown in Table 1. Median age was 68.5 years, median duration of diabetes was 9.0 years and 39.2% of the subjects were men. Skin autofluorescence was significantly higher in patients with Type 2 diabetes when compared with control subjects [median 2.22 arbitrary units (interquartile range 1.82–2.49; range 1.20–3.86) vs. 1.93 arbitrary units (interquartile range 1.74–2.26; range 1.02–3.42), respectively, $P < 0.01$]. Microvascular complications were present in 60 patients (46.2%) (retinopathy 34.6%, neuropathy 14.6% and nephropathy 30.8%). Ten patients (7.7%) had histories of macrovascular complications (ischaemic heart disease 0.8%, cerebral infarction 3.1%, peripheral artery disease 3.1% and aortic disease 0.8%) and 63 patients (48.5%) had micro- and/or macrovascular complications. Eleven per cent of patients were treated with insulin and 89% were treated with non-insulin therapies (diet and/or oral hypoglycaemic drugs).

Correlations between skin autofluorescence and other variables in patients with Type 2 diabetes

Skin autofluorescence was significantly correlated with age (r = 0.24, $P < 0.01$), BMI (r = −0.22, P = 0.01), eGFR (r = −0.36, $P < 0.01$), serum albumin (r = −0.40, $P < 0.01$), haemoglobin (r = −0.30, $P < 0.01$), microvascular complication score (r = 0.40, $P < 0.01$) and macrovascular complications

(r = 0.30, P < 0.01). Gender distribution, history of smoking, duration of diabetes, systolic blood pressure, diastolic blood pressure, mean HbA_{1c} in the previous year, LDL cholesterol and HDL cholesterol did not have any significant effect on skin autofluorescence. Multiple linear regression analysis showed that 35% (R^2) of the variance in skin autofluorescence could be predicted by age, mean HbA_{1c} in the previous year, microvascular complication score and macrovascular complications (Table 2). BMI, eGFR, serum albumin and haemoglobin were not significant contributors in this model. Mean HbA_{1c} in the previous year did not have a significant effect on skin autofluorescence on univariate analysis, but showed a significant and positive correlation with skin autofluorescence after multivariate adjustment.

Table 2 Determinants of skin autofluorescence in multiple regression analysis

Variable			
Dependent	Independent	β	P
Skin autofluorescence	Age	0.24	< 0.01
	HbA_{1c}	0.17	0.03
	Microvascular complication	0.44	< 0.01
	Macrovascular complication	0.27	< 0.01

Final results are given in the table. β is the standard coefficient; the multiple coefficient of determination (R^2) = 0.35.

Vascular complications and skin autofluorescence in patients with Type 2 diabetes

Diabetic retinopathy was not observed in 85 patients (65.4%), while 29 patients (22.3%) had simple retinopathy and 16 patients (12.3%) had proliferative retinopathy. Skin autofluorescence increased as the diabetic retinopathy stage advanced and was 16.1 and 31.7% higher in patients with simple and proliferative retinopathy, respectively, than in those without (Fig. 1a). Diabetic neuropathy was observed in 19 patients (14.6%) and skin autofluorescence was significantly higher in patients with diabetic neuropathy when compared with those without (Fig. 1b). Forty patients (30.8%) had diabetic nephropathy and

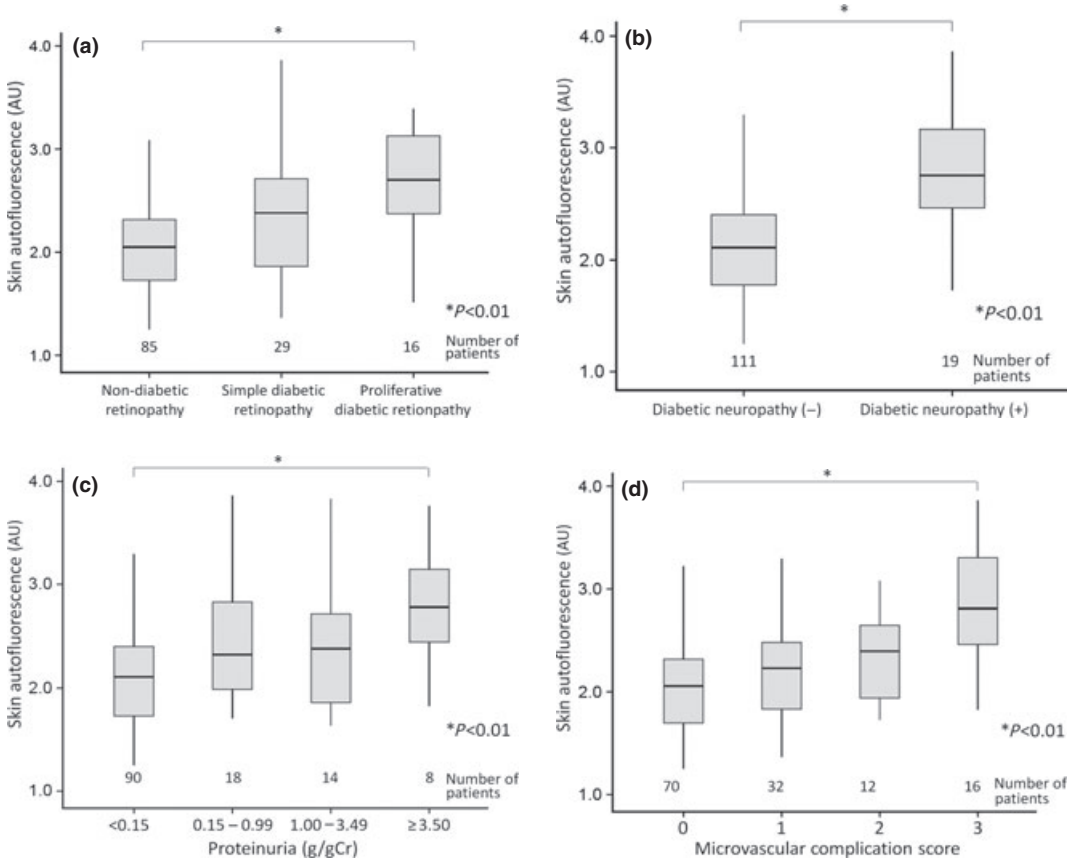

FIGURE 1 Skin autofluorescence and grade of diabetic retinopathy (a), neuropathy (b), nephropathy (c) and microvascular complication score (d). Microvascular complication score indicates the severity of microvascular complications, as described in 'Patients and methods'. Skin autofluorescence was significantly higher in patients with diabetic neuropathy than those without and significantly increased with grade of retinopathy, nephropathy and microvascular complication score (P < 0.01, respectively).

skin autofluorescence was significantly elevated as the amount of proteinuria increased. Skin autofluorescence was 31.8% higher in patients with proteinuria of nephrotic range than in those without overt proteinuria (Fig. 1c). Figure 1d shows the relationship between microvascular complication score and skin autofluorescence and reveals that skin autofluorescence increased with microvascular score and was markedly higher in patients with score 3 (triopathy). Skin autofluorescence was significantly higher in patients with macrovascular complications when compared with those without [median 2.16 (interquartile range 1.78–2.45) vs. 2.74 (2.41–3.33), respectively, $P < 0.01$].

Table 3 shows unadjusted odds ratios (ORs) for diabetic retinopathy, neuropathy, nephropathy and macrovascular complications in patients with Type 2 diabetes. Skin autofluorescence and serum albumin had significant correlations with all four complications. Duration of diabetes was significantly related to retinopathy and neuropathy and tended to be associated with macrovascular complications ($P = 0.08$). Because of the limited number of patients, multivariate adjustment was not performed in this situation; however, except for nephropathy, relationships between skin autofluorescence and each microvascular or macrovascular complication remained significant after adjustment for age and eGFR. Skin autofluorescence showed a correlation with nephropathy after adjustment for age and eGFR, but this was not significant ($P = 0.10$).

Comparison of data between patients with and without vascular complications

Skin autofluorescence was 18% higher in patients with micro- and/or macrovascular complications [median 2.39 arbitrary units (interquartile range 1.89–2.81)] than in those without [median 2.02 arbitrary units (interquartile range 1.67–2.31); $P < 0.01$].

Table 4 shows unadjusted and adjusted odds ratios for the presence of micro- and/or macrovascular complications in patients with Type 2 diabetes. Skin autofluorescence, history of smoking, duration of diabetes, systolic blood pressure, eGFR, serum albumin and haemoglobin were significantly related to vascular complications. Because of the limited sample size, we performed forward stepwise logistic regression analysis using vascular complications as the dependent variable and identified skin autofluorescence, duration of diabetes, systolic blood pressure and serum albumin as independently related to vascular complications.

Discussion

The present cross-sectional study found that skin autofluorescence was correlated with severity of diabetic retinopathy and amount of proteinuria and increased as microvascular complications advanced. Skin autofluorescence was higher in patients with diabetic vascular complications than in those without and showed independent effects on diabetic vascular complications after multivariate adjustment by logistic regression analysis. Skin autofluorescence has been evaluated in several studies performed with a Caucasian population; however, skin autofluorescence has not been studied in non-Caucasian patients with Type 2 diabetes. The present study is thus the first to show the relationships between skin autofluorescence and diabetic vascular complications in Japanese (non-Caucasian) patients with Type 2 diabetes.

Glycaemic control in our patients was fairly good; indeed, the medians of mean HbA_{1c} levels in the previous year were 51 mmol/mol (6.8%) and 76.2% of patients achieved the Japan Diabetes Society's recommended glycaemic control levels [HbA_{1c} < 52 mmol/mol (< 6.9%)]. However, 48.5% of the patients already had vascular complications. Moreover, skin autofluorescence had an independent relationship with diabetic vascular complications after adjustment in a multivariate model including HbA_{1c}. Skin advanced glycation end product accumulation was thought to reflect a more 'long-term memory' of glycaemic control because of its low turnover. Indeed, the Diabetes Control and Complications Trial substudy demonstrated that advanced glycation end product accumulation in skin collagen had a better relationship and predicting value for diabetic vascular complications compared with HbA_{1c} in Type 1 diabetes [6], and skin autofluorescence was reportedly an independent and strong predictor for micro- and macrovascular complications in Caucasians with Type 2 diabetes [7,8]. The present study suggests that skin autofluorescence, reflecting 'long-term metabolic memory', is a possible marker for chronic vascular complications in non-Caucasian, well-controlled patients with Type 2 diabetes.

Reduced GFR is a significant contributor to accumulation of advanced glycation end products [24,25] and our recent study showed an independent relationship between skin autofluorescence and eGFR in patients with chronic kidney disease [9]. Independent determinants of skin autofluorescence were age, current glycaemic control and vascular complications; however, eGFR was not selected as an independent factor for skin autofluorescence in the present study. This indicates that the contribution of decreased GFR to advanced glycation end product accumulation was not greater than other contributors, such as glycaemic control and oxidative stress, in patients with diabetes. Indeed, our recent study demonstrated that skin autofluorescence had an independent relationship to renal function in pre-dialysis patients with chronic kidney disease and this relationship was significant in patients with chronic kidney disease without diabetes, but not significant in patients with chronic kidney disease with diabetes [9]. In addition, overestimation of eGFR, which occurs as a result of hyperfiltration in patients with early stages of diabetic nephropathy, may also be taken into consideration. Therefore, further investigation is necessary in order to clarify the relationships between advanced glycation end product accumulation, GFR and the amount of microalbuminuria in patients with early stages of diabetic nephropathy.

Table 3 Unadjusted odds ratios for diabetic retinopathy, neuropathy, nephropathy and macrovascular complications in patients with Type 2 diabetes

	Retinopathy			Neuropathy			Nephropathy			Macrovascular complication		
Variable	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Skin autofluorescence (AU)	5.03	2.27–11.2	< 0.01	11.7	3.85–35.9	< 0.01	4.01	1.87–8.63	< 0.01	7.25	2.22–23.7	< 0.01
Age (years)	1.01	0.98–1.04	0.67	1.00	0.96–1.04	0.99	1.02	0.99–1.06	0.27	1.05	0.98–1.13	0.14
Gender (male)	1.60	0.77–3.35	0.21	1.88	0.71–5.05	0.20	1.41	0.66–3.01	0.37	4.03	0.99–16.4	0.05
History of smoking	2.51	1.20–5.26	0.02	2.61	0.95–7.14	0.06	2.01	0.95–4.28	0.07	6.00	1.22–29.5	0.03
Body mass index (kg/m^2)	0.94	0.86–1.03	0.19	0.84	0.73–0.98	0.02	0.90	0.81–1.00	0.04	1.00	0.86–1.17	0.98
Duration of diabetes (years)	1.13	1.06–1.20	< 0.01	1.11	1.03–1.19	< 0.01	1.03	0.98–1.09	0.27	1.08	0.99–1.18	0.08
Systolic blood pressure (mmHg)	1.03	1.01–1.05	< 0.01	1.03	1.01–1.06	0.02	1.03	1.01–1.06	< 0.01	1.00	0.96–1.03	0.93
Diastolic blood pressure (mmHg)	1.01	0.98–1.05	0.44	1.01	0.97–1.06	0.60	1.03	0.99–1.06	0.15	1.00	0.94–1.06	0.95
eGFR (ml min^{-1} 1.73 m^{-2})	0.97	0.96–0.99	< 0.01	0.95	0.93–0.97	< 0.01	0.93	0.91–0.96	< 0.01	0.98	0.96–1.01	0.12
HbA_{1c} (mmol/mol; IFCC)	1.00	0.96–1.04	0.89	1.04	0.99–1.09	0.13	0.98	0.94–1.03	0.40	0.97	0.90–1.05	0.49
HbA_{1c} (%; NGSP)	1.03	0.67–1.59	0.89	1.53	0.88–2.65	0.13	0.82	0.51–1.31	0.40	0.74	0.31–1.75	0.49
Albumin (gl)	0.88	0.82–0.95	< 0.01	0.89	0.82–0.96	< 0.01	0.75	0.67–0.85	< 0.01	0.89	0.81–0.97	< 0.01
Haemoglobin (g/l)	0.95	0.92–0.97	< 0.01	0.94	0.91–0.97	< 0.01	0.94	0.92–0.97	< 0.01	0.98	0.95–1.02	0.35
LDL cholesterol (mmol/l)	0.83	0.52–1.32	0.43	0.32	0.14–0.72	< 0.01	0.89	0.55–1.42	0.62	1.09	0.50–2.35	0.83
HDL cholesterol (mmol/l)	0.98	0.45–2.14	0.97	0.77	0.25–2.38	0.66	0.39	0.15–1.05	0.06	0.21	0.03–1.59	0.13

AU, arbitrary units; eGFR, estimated glomerular filtration rate; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; NGSP, National Glycohemoglobin Standardization Program.

Table 4 Unadjusted and adjusted odds ratios for micro– and/or macrovascular complications in patients with Type 2 diabetes by logistic regression analysis

	Univariate			Multivariate		
Variable	OR	95% CI	*P*	OR	95% CI	*P*
Skin autofluorescence (AU)	5.09	2.28–11.4	< 0.01	3.62	1.28–10.2	0.01
Age (years)	1.02	0.99–1.05	0.25			NS
Gender (male)	1.53	0.75–3.11	0.24			NS
History of smoking	2.40	1.18–4.88	0.02			NS
Body mass index (kg/m^2)	0.94	0.86–1.02	0.15			NS
Duration of diabetes (years)	1.12	1.06–1.19	< 0.01	1.15	1.07–1.24	< 0.01
Systolic blood pressure (mmHg)	1.03	1.01–1.05	< 0.01	1.04	1.01–1.07	0.01
Diastolic blood pressure (mmHg)	1.03	0.99–1.06	0.12			NS
eGFR (ml min^{-1} 1.73 m^{-2})	0.96	0.94–0.98	< 0.01			NS
HbA_{1c} (mmol/mol; IFCC)	1.00	0.96–1.04	0.99			NS
HbA_{1c} (%; NGSP)	1.00	0.66–1.50	0.99			
Albumin (g/l)	0.83	0.75–0.91	< 0.01	0.84	0.74–0.94	< 0.01
Haemoglobin (g/l)	0.95	0.92–0.97	< 0.01			NS
LDL cholesterol (mmol/l)	0.81	0.53–1.25	0.34			NS
HDL cholesterol (mmol/l)	0.61	0.28–1.33	0.21			NS

AU, arbitrary units; eGFR, estimated glomerular filtration rate; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; NGSP, National Glycohemoglobin Standardization Program; NS, not significant.

Serum albumin correlated with each micro- or macrovascular complication and had an independent relationship to vascular complication in the present study. Malnutrition is one of the major contributors for the progression of vascular complication in patients with chronic kidney disease or end-stage renal disease. However, prospective evaluations are needed to clarify whether serum albumin has an independent effect on progression of vascular complications and mortality in our patients with diabetes.

As progression of advanced glycation end product accumulation and vascular complications are time-dependent processes, our results could be biased by age. We always included age as a dependent variable in multivariate analysis to reduce the potential effects of such biases, and the present data nonetheless showed a significant correlation between skin autofluorescence and micro- and macrovascular complications.

There remain several limitations to the present study. First, patients in the present study were of Japanese ethnicity (non-Caucasian) and the autofluorescence reader is not reliable for individuals with very dark skin, as measurement of autofluorescence may be affected by skin colour and pigmentation because of the high absorption grade of excited light [17,26,27]. Patients with skin reflectance below 10% were excluded in the present study, in accordance with the recent report that the threshold value of skin reflectance (R%) below which skin autofluorescence becomes unreliable is R% = 10% in a non-Caucasian (Chinese) population [11]. However, the autofluorescence reader has not yet been sufficiently validated for non-Caucasian (Japanese) patients. Therefore, investigation of whether skin autofluorescence actually reflects accumulation of well-defined advanced glycation end products, such as pentosidine and *N*-carboxymethyllysine, in skin collagen and other organs in non-Caucasian populations remains necessary, although skin autofluorescence has been validated against evaluation of skin biopsy specimens among Caucasian patients [16,17,19]. Several recent studies have reported skin autofluorescence results in Japanese patients with end-stage renal disease [10,20,21], chronic kidney disease [9], rheumatoid arthritis, osteoarthritis and dialysis-related spondyloarthropathy [22] and cerebral infarction [23], and demonstrated its potential to be a useful marker in non-Caucasian subjects.

As blood samples were collected from every patient in a non-fasting state in the present study, influences of meals on cholesterol data should be considered. To minimize such influences, LDL cholesterol was measured using a direct method in the present study, although LDL cholesterol should be ideally calculated by the Friedewald formula using total cholesterol, HDL cholesterol and triglycerides in a fasting state. Peripheral artery disease was defined by Fontaine classification in the present study. However, ankle brachial index should be measured to make the definition of peripheral artery disease more precise. These points are limitations to the present study that should also be considered.

The other limitation is study design and sample size. As the present study included only a cross-sectional analysis of insufficient size, it remains to be confirmed whether autofluorescence is a relevant predictor of progression of diabetic vascular complications or mortality in a prospective study with sufficient size and better statistical methods.

Current glycaemic control may be necessary but not sufficient to prevent vascular complications and improve the quality of life (QOL) and mortality in patients with diabetes; therefore, accumulation of advanced glycation end products may have the potential to be an important target for therapeutic modalities to reduce 'metabolic memory' and improve outcome. Indeed, several studies have reported that some advanced glycation end

product breakers reduce accumulation of advanced glycation end products by some mechanisms, which include cleaving preformed advanced glycation end product cross-links, reducing accumulation of carbohydrate as well as lipid-derived advanced glycation end products and a potent free radical scavenging activity, and inhibit the development of vascular disease in experimental animals [28–30]. The autofluorescence reader may play a role as an important surrogate marker reflecting long-term glycaemic stress to monitor the effects of these treatments when these drugs are applied in clinical practice.

Skin accumulation of advanced glycation end products measured as skin autofluorescence is independently related to the severity of diabetic vascular complications in Japanese patients with Type 2 diabetes. Thus, the non-invasive and convenient autofluorescence readers may provide useful markers for risk assessment of diabetic vascular complications. Further investigation is needed into whether skin autofluorescence is a relevant marker for predicting progression of vascular complications and mortality.

Competing interests

Nothing to declare.

Acknowledgments

The authors wish to thank the staff of Tani Hospital for their efforts in collecting and analysing serum samples and measuring skin autofluorescence.

References

1 The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; **329**: 977–986.
2 UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with Type 2 diabetes (UKPDS 33). *Lancet* 1998; **352**: 837–853.
3 UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with Type 2 diabetes (UKPDS 34). *Lancet* 1998; **352**: 854–865.
4 Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005; **54**: 1615–1625.
5 Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF, Schmidt AM. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. *Glycobiology* 2005; **15**: 16R–28R.
6 Monnier VM, Bautista O, Kenny D, Sell DR, Fogarty J, Dahms W *et al.* Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of Type 1 diabetes: relevance of glycated collagen products versus HbA_{1c} as markers of diabetic complications. DCCT Skin Collagen Ancillary Study Group. Diabetes Control and Complications Trial. *Diabetes* 1999; **48**: 870–880.
7 Gerrits EG, Lutgers HL, Kleefstra N, Graaff R, Groenier KH, Smit AJ *et al.* Skin autofluorescence: a tool to identify Type 2 diabetic patients at risk for developing microvascular complications. *Diabetes Care* 2008; **31**: 517–521.
8 Meerwaldt R, Lutgers HL, Links TP, Graaff R, Baynes JW, Gans RO *et al.* Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. *Diabetes Care* 2007; **30**: 107–112.
9 Tanaka K, Tani Y, Asai J, Nemoto F, Kusano Y, Suzuki H *et al.* Skin autofluorescence is associated with renal function and cardiovascular diseases in pre-dialysis chronic kidney disease patients. *Nephrol Dial Transplant* 2011; **26**: 214–220.
10 Tanaka K, Katoh T, Asai J, Nemoto F, Suzuki H, Asahi K *et al.* Relationship of skin-autofluorescence to cardiovascular disease in Japanese hemodialysis patients. *Ther Apher Dial* 2010; **14**: 334–340.
11 Yue X, Hu H, Koetsier M, Graaff R, Han C. Reference values for the Chinese population of skin autofluorescence as a marker of advanced glycation end products accumulated in tissue. *Diabet Med* 2010; **28**: 818–823.
12 Seino Y, Nanjo K, Tajima N, Kadowaki T, Kashiwagi A, Araki E *et al.* The Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus; Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Invest* 2010; **1**: 212–228.
13 Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I *et al.* IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. *Clin Chem* 2004; **50**: 166–174.
14 Sacks DB; ADA/EASD/IDF Working Group of the HbA1c Assay. Global harmonization of hemoglobin A1c. *Clin Chem* 2005; **51**: 681–683.
15 Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K *et al.* Revised equations for eGFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; **53**: 982–992.
16 Meerwaldt R, Graaff R, Oomen PH, Links TP, Jager JJ, Alderson NL *et al.* Simple non-invasive assessment of advanced glycation end product accumulation. *Diabetologia* 2004; **47**: 1324–1330.
17 Meerwaldt R, Links T, Graaff R, Thorpe SR, Baynes JW, Hartog J *et al.* Simple noninvasive measurement of skin autofluorescence. *Ann N Y Acad Sci* 2005; **1043**: 290–298.
18 Mulder DJ, Water TV, Lutgers HL, Graaff R, Gans RO, Zijlstra F *et al.* Skin autofluorescence, a novel marker for glycemic and oxidative stress-derived advanced glycation endproducts: an overview of current clinical studies, evidence, and limitations. *Diabetes Technol Ther* 2006; **8**: 523–535.
19 Meerwaldt R, Hartog J, Graaff R, Huisman RJ, Links TP, den Hollander NC *et al.* Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J Am Soc Nephrol* 2005; **16**: 3687–3693.
20 Ueno H, Koyama H, Tanaka S, Fukumoto S, Shinohara K, Shoji T *et al.* Skin autofluorescence, a marker for advanced glycation end product accumulation, is associated with arterial stiffness in patients with end-stage renal disease. *Metabolism* 2008; **57**: 1452–1457.
21 Ueno H, Koyama H, Fukumoto S, Tanaka S, Shoji T, Emoto M *et al.* Advanced glycation end products, carotid atherosclerosis, and circulating endothelial progenitor cells in patients with end-stage renal disease. *Metabolism* 2010; **60**: 453–459.
22 Matsumoto T, Tsurumoto T, Baba H, Osaki M, Enomoto H, Yonekura A *et al.* Measurement of advanced glycation endproducts in skin of patients with rheumatoid arthritis, osteoarthritis, and dialysis-related spondyloarthropathy using non-invasive methods. *Rheumatol Int* 2007; **28**: 157–160.
23 Ohnuki Y, Nagano R, Takizawa S, Takagi S, Miyata T. Advanced glycation end products in patients with cerebral infarction. *Inter Med* 2009; **48**: 587–591.

24 Miyata T, Fu MX, Kurokawa K, van Ypersele de Strihou C, Thorpe SR, Baynes JW. Autoxidation products of both carbohydrates and lipids are increased in uremic plasma: is there oxidative stress in uremia? *Kidney Int* 1998; **54**: 1290–1295.
25 Sugiyama S, Miyata T, Ueda Y, Tanaka H, Maeda K, Kawashima S *et al*. Plasma levels of pentosidine in diabetic patients: an advanced glycation end product. *J Am Soc Nephrol* 1998; **9**: 1681–1688.
26 Gerrits EG, Smit AJ, Bilo HJ. AGEs, autofluorescence and renal function. *Nephrol Dial Transplant* 2009; **24**: 710–713.
27 Na R, Stender IM, Henriksen M, Wulf HC. Autofluorescence of human skin is age-related after correction for skin pigmentation and redness. *J Invert Dermatol* 2001; **116**: 536–540.
28 Joshi D, Gupta R, Dubey A, Shiwalkar A, Pathak P, Gupta RC *et al*. TRC4186, a novel AGE-breaker, improves diabetic cardiomyopathy and nephropathy in Ob-ZSF1 model of type 2 diabetes. *J Cardiovasc Pharmacol* 2009; **54**: 72–81.
29 Coughlan MT, Forbes JM, Cooper ME. Role of the AGE crosslink breaker, alagebrium, as a renoprotective agent in diabetes. *Kidney Int Suppl* 2007; **72**: S54–S60.
30 Forbes JM, Thallas V, Thomas MC, Founds HW, Burns WC, Jerums G *et al*. The breakdown of pre-existing advanced glycation end products is associated with reduced renal fibrosis in experimental diabetes. *FASEB J* 2003; **17**: 1762–1764.