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Correlation between diabetic retinopathy severity and elevated skin autofluorescence as a marker of advanced glycation end-product accumulation in type 2 diabetic patients

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ABSTRACT

Aims: We evaluated skin autofluorescence (AF) as a marker of tissue advanced glycation end-product (AGE) accumulation and examined whether it was related to the prevalence and severity of diabetic retinopathy (DR) and of diabetic macular edema (DME) in patients with type 2 diabetes mellitus (DM).
Methods: This study included 138 type 2 DM patients consisting of 31 patients with proliferative DR, 71 patients with non-proliferative DR, and 36 patients without retinopathy, in addition to 111 non-DM control subjects. At the time of skin AF and HbA1c measurement, self-assessed duration of DM was also determined. DR and DME stages were classified according to international guidelines.
Results: Skin AF was significantly increased in patients with DM as compared with non-DM controls. Furthermore, skin AF was correlated with the severity of DR, whereas single measurement of HbA1c and self-assessed DM duration were not. None of these 3 factors showed a correlation with DME prevalence or severity.
Conclusions: Skin AF levels, which can be measured non-invasively on a screening basis without skin biopsy or blood sampling, have a greater predictive ability for the presence and severity of DR than single measurement of HbA1c or self-assessed DM duration in patients with type 2 DM.

1. Introduction

Diabetic retinopathy (DR) is divided clinically into two main stages: non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). Vision deterioration in NPDR is caused primarily by diabetic macular edema (DME), which is the leading cause of legal blindness in developed countries (Saaddine et al., 2008). Vascular endothelial growth factor (VEGF) induces both vascular leakage and angiogenesis and is known to play a causative role in the development and progression of DR (Aiello et al., 1994; Murata et al., 1995, 1996). Retinal vascular leakage results in DME, while retinal neovascularization leads to the advancement of NPDR to PDR. We previously reported that advanced glycation end-product (AGE) accumulation in diabetic retinal vessels co-localized with VEGF overexpression at both NPDR and PDR stages (Murata et al., 1997). Since intracellular endothelial cell proteins, which include transcription factors and mitochondrial proteins, are also targets of non-enzymatic glycation, abnormal AGE accumulation can damage their functional properties and lead to vascular occlusions (Milne & Brownstein, 2013). Subsequent ischemia-induced VEGF overexpression in non-perfusion areas of the retina may then cause retinal neovascularization, which is the hallmark of PDR (Aiello et al., 1994; Murata et al., 1995, 1996; Vinores et al., 1997). Thus, AGE accumulation may play a causative role in vision deterioration either by DME in NPDR or by traction retinal detachment in PDR via VEGF overexpression. PDR is a leading cause of total blindness in the working age population due to traction retinal detachment or neovascular glaucoma (Grauslund, 2010).

The Diabetes Control and Complications Trial (DCCT) sub-study showed that skin AGEs are better predictors of retinopathy than HbA1c since they reflect hyperglycemia over a longer course (Monnier et al., 1999). However, the need for a skin biopsy has hampered routine clinical monitoring of skin AGEs. Although an association between serum AGEs and DR has also been reported (Milne & Brownstein, 2013), AGE assessment is rarely used in clinical practice because of technical impracticalities (Meerwaldt et al., 2007).

Skin autofluorescence (AF) is a recently developed non-invasive biomarker for AGE accumulation in skin tissues that makes use of the characteristic fluorescence pattern of AGEs (Mulder, Bieze, Graaff,

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Smit, & Hooymans, 2010). Skin AF correlates strongly with tissue levels of AGE, which include pentosidine, crosslines, and pyrropyridine, as well as with measures of long-term glycemic control (Meerwaldt et al., 2004, 2007; Mulder et al., 2010).

Although we earlier immunohistochemically identified AGE accumulation in the retinal vascular wall (Murata et al., 1997), there has been no method to quantitatively measure AGE accumulation in the retina to date. Recently, Januszewski et al. (2012) reported that ocular AF measured in the cornea correlated closely with skin AF. Both corneal and skin AF has also been shown to correlate with vascular dysfunction caused by AGE accumulation. These data suggested that skin AF may reflect AGE accumulation in diabetic retinal vessels as well and might serve as a predictor of DR severity in initial screenings.

Accumulation of AGEs in tissues is accelerated by the presence and duration of hyperglycemia (Milne & Brownstein, 2013; Murata et al., 1997), both of which are known major risk factors for DR (Grauslund, 2010; Klein, Klein, Moss, Davis, & DeMets, 1984). Most investigations on the association between DR prevalence and skin AF or AGE accumulation have been performed with type 1 diabetic patients; studies involving type 2 diabetes are rare. In type 1 diabetic patients, it has been established that skin AGE accumulation (Cleary et al., 2013; Fitzpatrick, 1988) and skin AF (Cleary et al., 2013; Monnier et al., 1986; Orchard et al., 2013) correlates with the prevalence of DR. Skin AGE accumulation is also predictive of future DR progression (Genuth et al., 2005). On the other hand, Chabroux et al. (2010) reported a lack of association between skin AF and DR prevalence. Meanwhile, in type 2 diabetic patients, Gerrits et al. (2008) found that skin AF did not have any predictive ability for the development of DR.

The aim of this study is to measure skin AF levels in type 2 diabetic patients with DR and examine whether they correlate with the prevalence and severity of DR or DME graded according to international clinical severity scales (Wilkinson et al., 2003).

2. Subjects and methods

This study was approved by the ethics committee of Shinshu University (approval number 2208) and was registered in the UMIN Clinical Trials Registry (registration number 000010741). The study was conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all subjects.

2.1. Subjects

This cross-sectional study was performed between March and July 2013 and included 138 consecutive new patients with type 2 DM who visited the outpatient clinic of the ophthalmology department of Shinshu University, Matsumoto, Japan. The inclusion and exclusion criteria were as follows:

Inclusion criteria

1) Patients who were diagnosed by a physician as having type 2 DM according to the guidelines of the Japanese Diabetes Association (JDA).

DR was diagnosed by ophthalmoscopy and contact lens slit lamp biomicroscopy following mydriasis by two independent ophthalmologists (YI and TM) who were blinded to HbA1c and skin AF findings. DR and DME severity were determined according to respective international clinical DR and DME severity scales (Wilkinson et al., 2003). Briefly, the severity of DR was categorized as no DR (NDR), mild, moderate, or severe NPDR, or PDR. DME severity was classified as mild, moderate, or severe DME. Macular thickening due to DME was assessed by spectral domain optical coherence tomography (SD-OCT) using a Cirrus® device (Carl Zeiss Meditec, Inc., Dublin, CA, USA) by measuring retinal thickness in the 9 standard early treatment diabetic retinopathy study (ETDRS) subfields. One hundred and eleven healthy age-matched controls without DM or apparent ocular diseases were asked to undergo the same examinations.

Data on age, gender, earlier HbA1C (NGPS; National Glycohemoglobin Standardization Program) levels, duration of DM, blood pressure, history of smoking, nephropathy, neuropathy, and diabetes management were collected from medical records. Additional careful interviewing was performed to more precisely identify DM duration. Skin AF was assessed on the ventral side of the lower arm with an AGE-Reader™ (DiagnOptics Technologies BV, Griningen, The Netherlands). The AGE-Reader™ is a desktop device that uses the characteristic fluorescence properties of certain AGEs to estimate overall AGE accumulation in the skin. (de Vos et al., 2013; Meerwaldt et al., 2007; Mulder et al., 2010). The most recent HbA1c value was obtained on the same day as skin AF measurement.

2.2. Cutoff point of skin AF values for prediction of DR prevalence and severity

The ability of skin AF to predict DR prevalence and severity in initial screenings was analyzed by receiver operating characteristic (ROC) curves. The areas under the ROC curves (AUC) were calculated and compared to determine the diagnostic accuracy of skin AF. We also selected the optimal cutoff AF value for each DR severity level that maximized sensitivity and specificity.

2.3. Statistics

Spearman's correlation coefficient was used to identify associations between DR severity and skin AF, HbA1c level, and DM duration measurements, as well as between severity of DME and skin AF. The Mann–Whitney U-test was employed to compare differences between two groups, and one-way ANOVA with Tukey's multiple comparison test was employed to compare differences among three groups or more. Categorical variables were analyzed using Fisher's exact test or the chi-square test.

Cleary et al. (2013) have reported that skin AF reflects not only long term hyperglycemia, but also age, smoking, and renal damage. To confirm the correlation between skin AF and DR after adjustment for these covariates, analysis of covariance (ANCOVA) was performed using the Statistical Package for Social Sciences (SPSS) version 18.0 (SPSS Inc., Chicago, IL, USA). Linear regression analysis was used to evaluate the partial correlation coefficient between skin AF and DR severity after adjusting for age, smoking, and renal damage (model 1) and after adjustment for all covariates listed in Table 1, i.e., sex, age, HbA1c in initial screenings, self-stated duration of diabetes, systolic and diastolic blood pressure, history of smoking, diabetic nephropathy, and diabetic neuropathy (model 2). A P-value of <0.05 was considered to be statistically significant.

3. Results

3.1. Subject characteristics

We enrolled a total of 138 (90 male, 48 female) diabetic patients of a (mean ± SD) age of 63.7 ± 12.2 years, HbA1c level of 7.5 ± 1.7% (58.4 ± 13.2 mmol/mol), and diabetes duration of 13.2 ± 9.9 years. We also examined 111 (33 male, 78 female) age-matched control subjects without DM who were 62.2 ± 15.4 years of age. Among the diabetic patients, 31 (22.4%) had PDR, 71 (51.4%) had NPDR, and 36 (26.1%) had no apparent DR. All patients had been diagnosed by a physician as having type 2 DM according to the guidelines of the JDA. All patients were of Japanese ethnicity and had a suitable skin pigmentation level for accurate AGE measurement (Meerwaldt et al., 2005). The characteristics of the type 2 diabetic patients and controls are listed in Table 1.

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