

Corneal Advanced Glycation End Products Increase in Patients With Proliferative Diabetic Retinopathy

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OBJECTIVE — To evaluate corneal advanced glycation end product (AGE) fluorescence in patients with diabetes and in healthy control subjects.

RESEARCH DESIGN AND METHODS — Corneal autofluorescence was measured in 26 eyes of 26 patients with type 2 diabetes (mean age 57.0 years; mean disease duration 12.2 years; mean HbA_{1c} 7.1%) and 13 eyes of 13 healthy age-matched control subjects (mean age 57.9 years). The patients with type 2 diabetes were divided into the following groups: patients without diabetic retinopathy (DR), patients without proliferative diabetic retinopathy (PDR), and patients with PDR. Corneal autofluorescence was measured by fluorophotometry with the wavelength that is characteristic of AGE fluorescence (excitation and emission 360–370 nm and 430–450 nm, respectively). We defined peak corneal autofluorescence levels as corneal AGE fluorescence values. We compared the corneal AGE fluorescence values in the four groups.

RESULTS — In the PDR group (11.9 ± 3.9 arbitrary units [mean \pm SD]), the corneal AGE fluorescence values were significantly higher compared with the control subjects (6.9 ± 1.3 arbitrary units), the patients without DR (7.4 ± 2.1 arbitrary units), and the patients without PDR (6.9 ± 2.2 arbitrary units) ($P < 0.05$).

CONCLUSIONS — We found that corneal AGEs may increase in patients with diabetes and PDR compared with control subjects, patients without DR, and patients without PDR. In the patients with PDR, increased corneal AGEs may play a role in diabetic keratopathy.

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The nonenzymatic glycation of proteins in a process known as the Maillard reaction eventually causes the formation of advanced glycation end products (AGEs) (1). AGEs have been implicated as causal factors in the complications of diabetes (2), including diabetic retinopathy (DR) (3–6), and have specific fluorescence characteristics (1,7,8).

We previously showed that corneal autofluorescence values measured using a

commercial fluorophotometer significantly increased in patients with proliferative diabetic retinopathy (PDR) (9). Moreover, we reported that lens autofluorescence values using our fluorophotometer were significantly correlated with AGE levels in extracted lenses in diabetic animal models (10).

In the present study, we measured corneal autofluorescence using our fluorophotometer, the wavelength of which

was characteristic of AGE fluorescence, and evaluated corneal AGEs in patients with type 2 diabetes and in healthy control subjects.

RESEARCH DESIGN AND METHODS

Corneal autofluorescence was measured in 26 eyes of 26 patients with type 2 diabetes and 13 eyes of 13 healthy age-matched control subjects. The patients with type 2 diabetes were divided into three groups: patients without DR, patients without PDR, and patients with PDR (Table 1). Eyes that had undergone surgery were excluded from this study. The study protocol was reviewed by the ethics committee of our institution. All procedures adhered to the tenets of the Declaration of Helsinki.

Corneal autofluorescence was measured by scanning with a lens measurement system (SpectRx) (11) that had been adjusted for corneal measurement. The system has a xenon lamp as an excitation source and a fluorescence detector. The noninvasive corneal autofluorescence measurements were performed at various distances along the optical axis of the cornea with the assistance of a pupil-tracking system. The pupil-tracking system, an automatic alignment system, was incorporated into the lens measurement system and works as follows. An infrared charged-coupled-device camera images the iris and the pupil, and the image is digitized. The position data derived from the digitized charged-coupled-device image are fed into a two-axis servomotor positioning system to maintain the x and y positions of the sample volume to within $\pm 100 \mu\text{m}$ of the programmed “lock” position (11). The sample volume in the optics of the lens measurement system was $\sim 300 \mu\text{m}$ long, $600 \mu\text{m}$ high, and $80 \mu\text{m}$ wide. We used color filters at wavelengths of 360–370 nm for excitation and 430–450 nm for emission, because 360–370 and 430–450 nm (excitation and emission, respectively) were reported to be characteristic of AGE fluorescence (i.e., crossline, etc.) in the tissue (1,7,8). We reported that there was a significant pos-

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Abbreviations: AGE, advanced glycation end product; BAB, blood-aqueous barrier; BRB, block-retinal barrier; CML, *N*-(carboxymethyl) lysine; DR, diabetic retinopathy; oxLDL, oxidized LDL; PDR, proliferative diabetic retinopathy; VEGF, vascular endothelial growth factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—Demographic data from patients with type 2 diabetes and healthy control subjects

	Number of individuals	Age (years)	Duration of diabetes (years)	HbA _{1c} (%)
Patients with diabetes	26	57.0 ± 9.2	12.2 ± 6.6	7.1 ± 1.2
NO DR group	8	59.9 ± 5.6	11.2 ± 7.6	6.9 ± 0.8
NO PDR group	6	59.5 ± 13.1	12.8 ± 7.6	8.1 ± 1.3
PDR group	12	53.9 ± 8.6	12.8 ± 5.7	6.9 ± 1.2
Healthy control subjects	13	57.9 ± 16.8	—	—

Data are means ± SD.

itive correlation between lens autofluorescence values and the AGE levels in the same extracted lenses in diabetic hamsters (10). We used the peak corneal autofluorescence level as the corneal autofluorescence value. The corneal autofluorescence values measured by our fluorophotometry are defined as corneal AGE fluorescence. The corneal AGE fluorescence values are expressed as arbitrary units.

We compared the corneal AGE fluorescence values in the four groups of patients and examined the relationship between these values and age, duration of disease, and HbA_{1c} levels in the patients with type 2 diabetes.

Data analysis

The results were expressed as means ± SD. The distribution of corneal AGE fluorescence values in these four groups was compared using a one-way of analysis variance and Scheffe's test. A *P* value of 0.05 or lower was considered significant. The Pearson correlation coefficient (*r*) was calculated to determine whether there were significant relationships between variables.

RESULTS

The results of the corneal AGE fluorescence values in the four groups of patients are shown in Fig. 1. The corneal AGE fluorescence values in the control subjects, the patients without DR, the patients without PDR, and the patients with PDR were 6.9 ± 1.3, 7.4 ± 2.1, 6.9 ± 2.2, and 11.9 ± 3.9 arbitrary units (mean ± SD), respectively. A significant difference was noted in the corneal AGE fluorescence values between the four groups (analysis of variance, *P* = 0.0001); corneal AGE fluorescence values were markedly higher in the patients with PDR than in the control subjects, the patients without DR, or the patients without PDR (Scheffe's test; *P* = 0.0006, *P* = 0.0053,

and *P* = 0.0078, respectively). In the patients with type 2 diabetes, no significant correlation was found between corneal AGE fluorescence values and age (*r* = 0.063), duration of disease (*r* = 0.21) (Fig. 2), or HbA_{1c} level (*r* = 0.38) (Fig. 3).

CONCLUSIONS

— In the present study, we measured corneal AGE fluorescence values using our fluorophotometry system, the wavelength of which was characteristic of AGE fluorescence (1,7,8). We previously reported that there was a correlation between lens autofluorescence values detected by our fluorophotometry system and AGE levels in the same lenses measured by an enzyme-linked immunosorbent assay in diabetic animals (10). The previous reported results showed a strong correlation between the value of lens autofluorescence measured with the 360- to 370-nm and 430- to 450-nm wavelengths (excitation and emission, respectively) and the AGE lev-

els measured by enzyme-linked immunosorbent assay in the same extracted lenses (10). It also has been reported that oxidized LDLs (oxLDLs), which are believed to increase in patients with diabetes, generate fluorescent products with emission at 430 nm when excitation is performed at 360 nm (12–14). That wavelength is characteristic of oxLDL fluorescence and similar to that of AGE fluorescence. However, there is much less autofluorescence of oxLDL than of AGEs (14). Thus, it is possible that corneal autofluorescence induced by these wavelengths as AGE fluorescence measured by our fluorophotometry might mainly reflect corneal AGE fluorescence.

Sady et al. (15), using a two-step high-performance liquid chromatography assay, reported the presence of pentosidine in the cornea. They also reported that those pentosidine levels correlated with the collagen-bound fluorescence and pentosidine levels and that collagen-bound fluorescence was higher in human diabetic corneas than in control corneas. Those authors suggested that AGEs in the cornea increase in patients with diabetes. Kaji et al. (16) reported that *N*-(carboxymethyl) lysine (CML) immunoreactivity was observed at the site of the epithelial basement membrane in the corneas of patients with diabetes. AGEs, which are produced by the nonenzymatic reaction of glucose and other aldoses with protein via the Maillard reaction and have specific fluorescence characteristics, seem to ac-

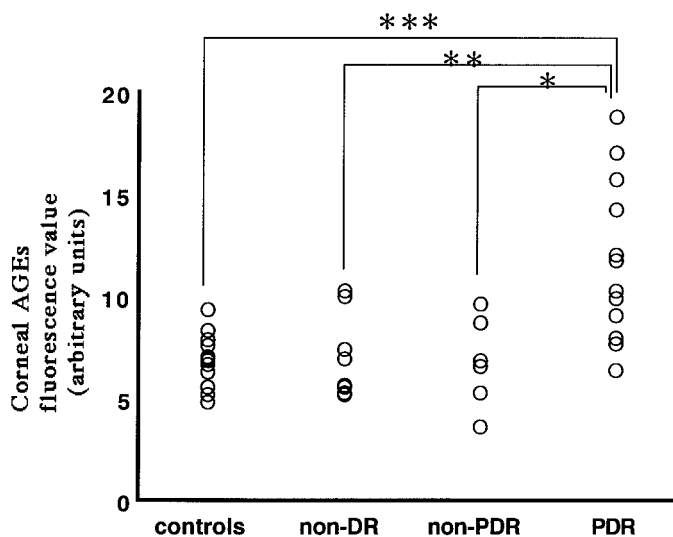


Figure 1—Corneal AGE fluorescence values in healthy control subjects, patients without DR (non-DR), patients without PDR (non-PDR), and patients with PDR (PDR) (Scheffe's test; * *P* = 0.0006, ***P* = 0.0053, **P* = 0.0078).**

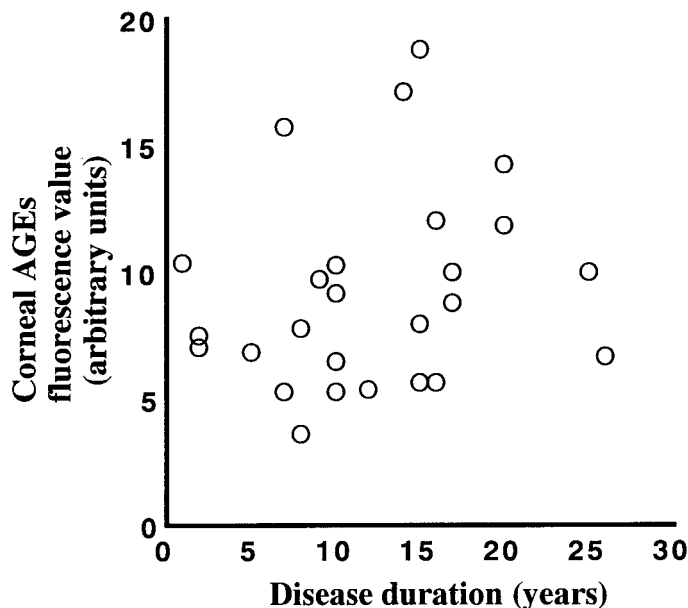


Figure 2—Relationship between duration of disease and corneal AGE fluorescence values in patients with type 2 diabetes. A statistically significant correlation was not found ($r = 0.21$, $P = 0.3105$).

accumulate in the corneas of patients with diabetes. In the present study, the corneal AGE fluorescence value in the group of patients with PDR was significantly higher than that in the control subjects, the patients without DR, and the patients without PDR. These results indicate that corneal AGEs increase in patients with PDR.

Using our fluorophotometry, corneal AGE fluorescence could not be broken into separate signals from the corneal epithelium, stroma, and endothelium. The pentosidine concentration in human corneal collagen is increased in patients with diabetes (15). These results suggest that pentosidine, which is an AGE, can cause increased fluorescence in the corneal

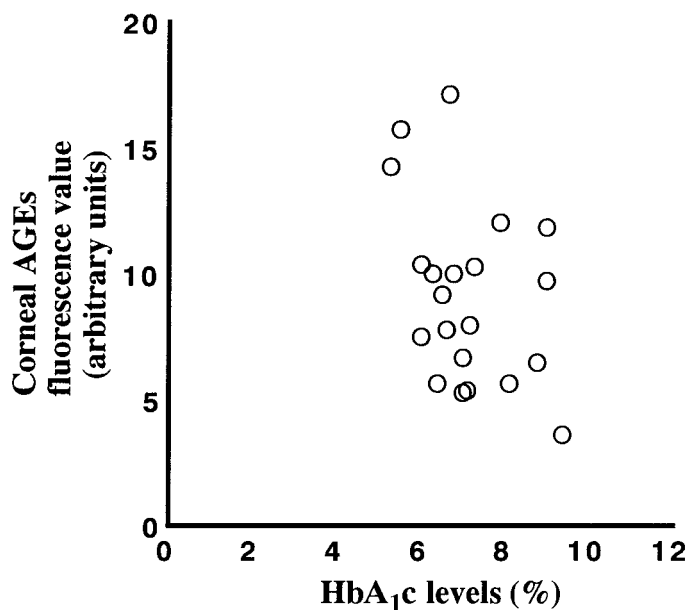


Figure 3—Relationship between HbA_{1c} levels (%) and corneal AGE fluorescence values in patients with type 2 diabetes. A statistically significant correlation was not found ($r = 0.38$, $P = 0.0893$).

stroma of patients with diabetes. However, CML, which is another AGE, was observed at the site of the epithelial basement membrane in corneas of patients with diabetes (16). The distribution of AGEs in the corneas of patients with diabetes is still unclear.

In the present study, there was no correlation between HbA_{1c} levels or duration of disease and corneal AGE fluorescence values in the patients with type 2 diabetes. However, corneal AGE fluorescence values increased only in the patients with PDR. The vitreous concentrations of AGEs and vascular endothelial growth factor (VEGF) were both elevated in the patients with PDR (5,17,18). VEGF plays a role in breakdown of the blood-retinal barrier (BRB) (19–21). Increased values of AGEs in the vitreous in patients with PDR seem to be related to breakdown of the BRB. Furthermore, some authors reported that permeability of the blood-aqueous barrier (BAB) increased in patients with PDR (22,23). We speculated that increased corneal AGEs in patients with PDR may be caused by an increase of AGEs in the aqueous humor and vitreous resulting from breakdown of the BRB and/or BAB.

Diabetic corneal epitheliopathy has been recognized clinically, especially after vitrectomy (24). Structural changes in the endothelium have been reported in patients with diabetes (25,26). In corneas of patients with diabetes, decreased sensation, neurotrophic ulceration, delayed wound healing, recurrent erosions, and epithelial edema have been found clinically. Histologically, thickening and multilayering of the epithelial basement membrane and morphologic changes of the corneal epithelial cells have been reported (27). These changes are assumed to be due to intracellular edema, which may result from activation of the sorbitol pathway. The aldose reductase inhibitor has been reported to be effective in treating corneal epithelial defects (28). However, the pathogenic mechanisms underlying those abnormalities are still unclear. In vitro, nonenzymatic glycation of laminin-attenuated adhesion and spreading of corneal epithelial cells and the presence of aminoguanidine, an inhibitor of AGE formation in the incubation mixture during glycation, inhibited CML formation and promoted adhesion and spreading of corneal epithelial cells (16). We hypothesized that one of the corneal complica-

tions of diabetes, especially in patients with PDR, could be related to the accumulation of AGEs in the cornea. We believe that this should be considered in the future.

In conclusion, these results demonstrate that corneal AGEs may increase in patients with PDR compared with control subjects, patients without DR, and patients without PDR. In patients with PDR, increased corneal AGEs may play a role in diabetic keratopathy.

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