CLINICAL STUDY

Diurnal variation of corneal autofluorescence in normal and diabetic eyes

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Abstract

Purpose **To examine the diurnal variations in corneal autofluorescence in normal and diabetic patients.**

Methods **We measured corneal autofluorescence using a fluorophotometer fitted with an anterior segment adapter. Corneal autofluorescence was measured 10 times at 3 min intervals to evaluate the reproducibility of this instrument in 4 eyes of 4 normal subjects. The diurnal variation in corneal autofluorescence was determined by measuring the fluctuations in 10 eyes in 10 normal subjects and one unoperated eye each of 10 patients with proliferative diabetic retinopathy (PDR). We performed five consecutive measurements at 1000, 1130, 1400, 1630 and 1900 hours. The mean value of five measurements, the variation range and the coefficient of variation were analysed.**

Results **The mean coefficient of variation in the measurement using this instrument was 8.6 ± 1.0%. In the patients with PDR, the mean corneal autofluorescence value was significantly higher ($p < 0.001$), the variation range was significantly wider ($p < 0.001$) and the coefficient of variation was significantly greater ($p < 0.01$) than in the normal subjects.**

Conclusions **The results of this study suggest that corneal autofluorescence changes over the course of a day in patients with diabetes. This may be caused by the breakdown of the blood–aqueous barrier that we reported previously.**

Key words Corneal autofluorescence, Diurnal variation, Diabetic patients

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The correlation between corneal autofluorescence and diabetic retinopathy was recently reported.[1–6] These reports showed increased corneal autofluorescence in diabetics compared with normal subjects. The source of the increased fluorescence is not known precisely, but previous studies[7,8] suggested that corneal autofluorescence is correlated with corneal metabolic changes, which can be indicators of corneal metabolism.

In general, corneal metabolism is mostly dependent on the aqueous humour. It is known that aqueous flare, an index of the blood–aqueous barrier, varies diurnally.[9] In addition to this, we previously reported the breakdown of the blood–aqueous barrier before the onset of diabetic retinopathy.[10] It was also reported that patients with severe proliferative retinopathy have greater blood–aqueous barrier dysfunction.[11] From these reports the corneal environment can be seen to fluctuate during the day, and there may be differences between normal subjects and patients with diabetes.

In the present study we investigated whether diurnal variations in corneal autofluorescence are present in normal subjects and patients with proliferative diabetic retinopathy (PDR).

Methods

Fluorophotometric scan

We measured corneal autofluorescence using a fluorophotometer (Fluorotron Master, OcuMetrics, USA) fitted with an anterior segment adapter for detailed scanning of the anterior ocular segment. The peak transmission of the excitation filters was 85% and that of the fluorescence filters 90% (bandpass width at half height: 430–490 nm and 530-630 nm, respectively).[12]

The autofluorescence value obtained, which was expressed in nanogram equivalents of fluorescein per millilitre, was the peak of the corneal measurements (Fig. 1).

Instrument reproducibility

To investigate the reproducibility of this instrument, corneal autofluorescence was measured 10 times at 3 min intervals in 4 eyes of 4 normal subjects 28–31 years of age (mean ± SD, 29.3 ± 1.3 years). The coefficient of variation (CV) (standard deviation/mean value × 100) was then calculated in each subject. The stability

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of the instrument was checked with a fluorescein disodium solution of known concentration before the measurements were performed.

Diurnal variation

The diurnal variation in corneal autofluorescence was determined in patients with PDR and age-matched normal controls. The PDR group consisted of 10 eyes of 10 non-insulin-dependent Japanese patients with diabetes mellitus aged 34–71 years (mean ± SD, 49.9 ± 11.6 years). The HbA1c was 6.5–10.2 (mean ± SD, 8.5 ± 1.4%) in the PDR group. Patients who had undergone procedures such as cataract or vitreoretinal surgery were excluded from the study. Patients who had undergone fluorescein angiography, fluorescein staining and fundus photocoagulation during the month preceding the start of the study were also excluded.

Ten eyes of 10 age-matched Japanese subjects aged 30–83 years (mean ± SD, 48.7 ± 20.9 years) served as controls. Each study eye was selected randomly. In the control group, no subject had ocular disease except for mild cataract and refractive errors. Individuals who wore contact lenses were also excluded.

After obtaining written informed consent from the subjects five consecutive measurements of corneal autofluorescence were performed at 1000, 1130, 1400, 1630 and 1900 hours. Three parameters – the mean value of five diurnal measurements, the variation range (the differences between the highest and the lowest levels of five diurnal measurements) and the coefficient of variation – were then calculated.

Statistical analysis

In the diurnal study, the parameters were analysed using the Mann–Whitney *U*-test. A p value of less than 0.05 was considered to be statistically significant.

Results

Instrument reproducibility

The coefficients of variation of 10 consecutive measurements in four normal subjects were 8.9%, 9.8%, 8.4% and 7.3%, respectively (mean ± SD, 8.6 ± 1%).

Diurnal variation

The patterns of the diurnal variations of corneal autofluorescence in the patients with PDR and the normal controls are shown in Fig. 2. There was no uniform pattern in either group.

In the patients with PDR, the mean corneal autofluorescence value ($\times 10^{-9}$ (g/ml); mean ± SD, 20.2 ± 7.7) was significantly higher than that of the controls (mean ± SD, 9.6 ± 2.0) ($p < 0.001$) (Fig. 3a).

The diurnal variation range ($\times 10^{-9}$ (g/ml); mean ± SD, 6.6 ± 2.7) was significantly wider than that of the controls (mean ± SD, 1.7 ± 0.7%) ($p < 0.001$) (Fig. 3b).

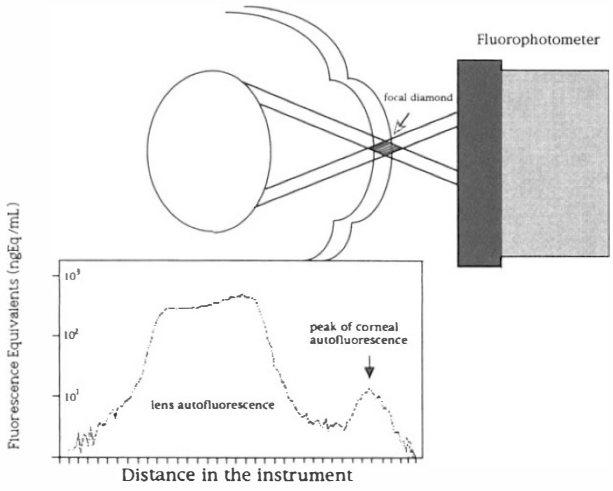

Fig. 1. *Upper part: Schematic representation of the scanning of corneal autofluorescence. The arrow indicates the measurement area (focal diamond). Lower part: Typical fluorophotometric scan in a human eye using the anterior segment adapter. The peak autofluorescence values corresponding to scans of the cornea (arrow) are the corneal autofluorescence values.*

The coefficient of variation (%; mean ± SD, 12.8 ± 3.3) was also significantly greater than that of the controls (mean ± SD, 7.2 ± 3.1) ($p < 0.01$) (Fig. 3c).

Discussion

In the study of instrument reproducibility, the coefficient of variation of corneal autofluorescence ranged between 7.3% and 9.8%. Therefore, instrument reproducibility should be considered when the diurnal changes in corneal autofluorescence are investigated.

The presence of higher corneal autofluorescence values in patients with PDR supports the results of previous studies.[1–6] Furthermore, the diurnal variation range in these patients was also significantly greater than in the controls. However, considering instrument error, higher values might have a wider variation range. Thus, we also compared the coefficient of variation in the two

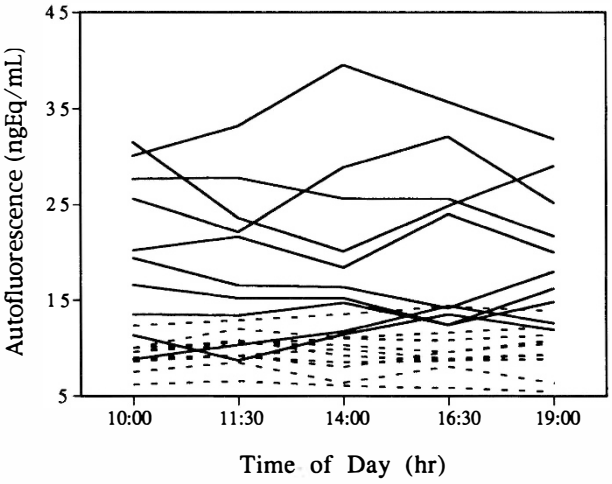

Fig. 2. *The patterns of diurnal corneal autofluorescence in normal subjects (broken lines) and patients with proliferative diabetic retinopathy (continuous lines).*

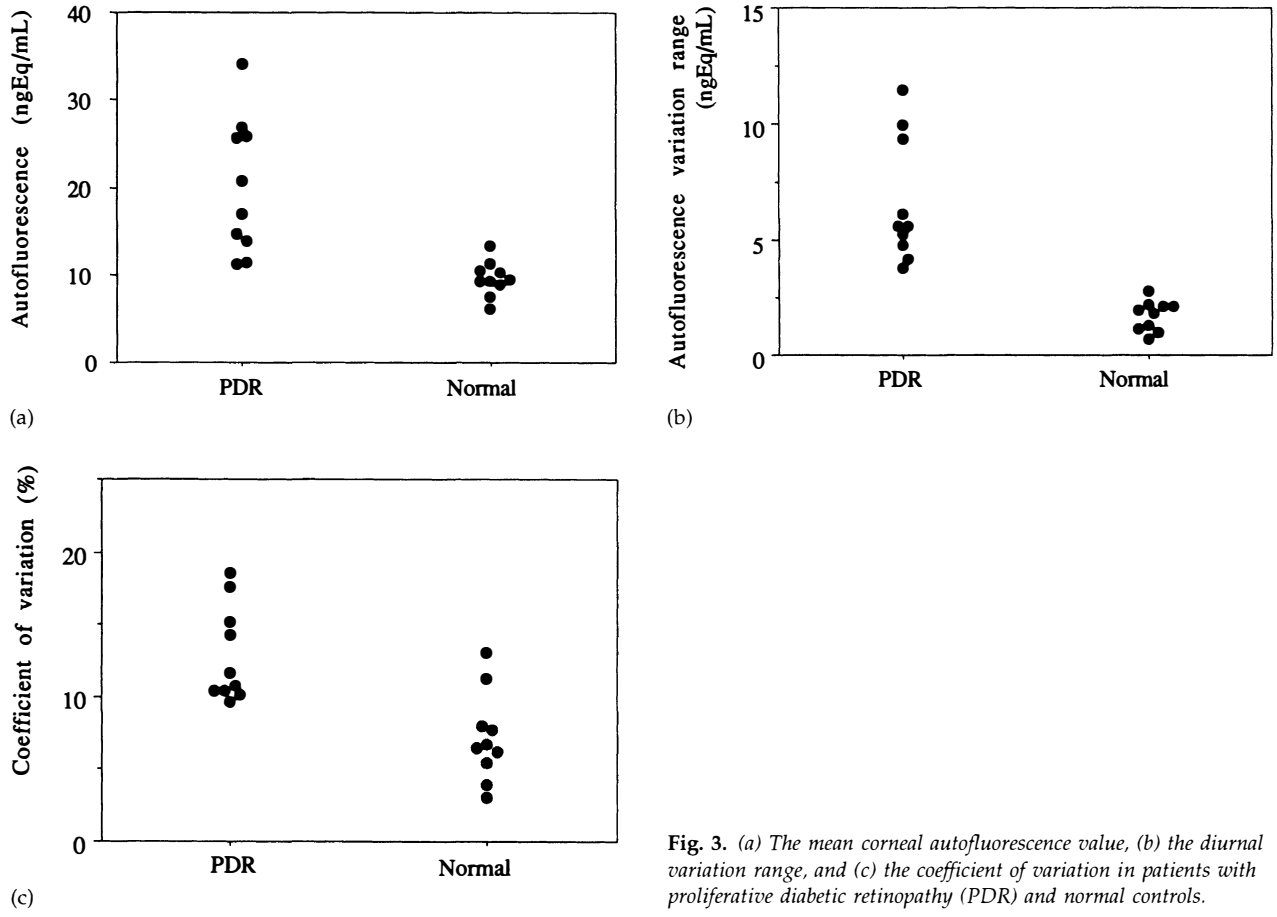

Fig. 3. *(a) The mean corneal autofluorescence value, (b) the diurnal variation range, and (c) the coefficient of variation in patients with proliferative diabetic retinopathy (PDR) and normal controls.*

groups. In the present study, the coefficient of variation in patients with PDR was also greater than in the controls. Our results suggested that corneal autofluorescence levels in patients with diabetes may fluctuate over the course of a day. There is one further possibility that we must not ignore. We did not correct corneal autofluorescence using lenticular fluorescence tailing because this contributed to corneal fluorescence tailing in this study. Thus, further consideration of the effect of lenticular fluorescence tailing on the coefficient of variation may be required.[13]

Corneal thickness varies over the course of a day,[14] and the greatest increases occur during sleep,[14] so the effect of this diurnal variation on corneal autofluorescence in the present study may be minimal because the measurements were performed during the day. Furthermore, we adopted the peak corneal value from the fluorophotometric scan as the corneal autofluorescence value. The fluorescence was scanned along the optical axis, and the measurement area of this instrument (the focal diamond) was approximately 700 μm long × 50 μm wide × 950 μm high (OcuMetrics, personal communication 1997), indicating that the focal diamond is not wholly in the cornea. If the fluorescence intensity were uniform in the cornea, the peak values would indicate the values when the focal diamond occupies the greatest volume in the cornea. Therefore, thick corneas might have more autofluorescence than thin corneas. However, most fluorescence originates in the epithelium,[2,5–7] so the effect of corneal thickness may be minimal. Corneas of patients with diabetes are thicker than those of normal subjects;[15] however, the mean corneal autofluorescence value in patients with PDR was twice that of the controls, so corneal swelling cannot explain our results.

Using fluorophotometry and laser photometry, the blood–aqueous barrier is more dysfunctional in patients with severe PDR,[11] which was the degree of PDR in our patients. Thus, the blood–aqueous barrier may be highly damaged in PDR patients. In an experimental study using an alloxan-induced diabetes model in rabbits, it was reported that the levels of glucose, myo-inositol and sorbitol in the aqueous humour from diabetic rabbits were significantly higher than those of controls. The glucose level in the aqueous humour also correlated linearly with the serum glucose level. Furthermore, glucose, sorbitol and myo-inositol increased in all corneal layers in diabetic rabbits.[16] Because corneal metabolism is supplied by the aqueous humour, the disrupted homeostasis around the cornea may produce these diurnal changes. Further investigation of a correlation between the degree of blood–aqueous barrier dysfunction and corneal autofluorescence is warranted. We recently reported that changes in corneal autofluorescence correlated significantly with changes in blood glucose in PDR patients.[17] In the present study, although the sample number was small, no pattern of diurnal variation was found in normal controls, and

corneal autofluorescence levels in patients with PDR varied greatly over a day. The results of the present study support those of our previous reports.[17]

Corneal autofluorescence also increases in other pathologies, for example open-angle glaucoma or ocular hypertension.[18] Eyes with branch retinal vein occlusion have significantly higher levels of corneal autofluorescence than fellow eyes and corneal autofluorescence may be correlated with retinal ischaemia.[19] We recently reported that corneal autofluorescence increases as the axial length elongates in myopia.[20]

The origin of corneal autofluorescence is uncertain, but in animal studies changes in corneal autofluorescence originated from flavoproteins and pyridine nucleotides as a result of metabolically impaired corneal mitochondrial respiration.[7,21,22] From the results of the present study, one factor causing corneal autofluorescence may fluctuate greatly in a short time. We speculate that corneal autofluorescence might be a good index in several diseases. Further biochemical studies are needed to elucidate its origins.

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