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Corneal Autofluorescence: An Indicator of Diabetic Retinopathy

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The metabolic disorder in diabetics often results in progressive retinopathy with severe visual impairment. Changes in metabolism can influence corneal autofluorescence. This has led to speculation that diabetic retinopathy might be associated with changes in corneal autofluorescence. Corneal autofluorescence of both eyes was determined by fluorophotometry in 94 insulin-dependent diabetes mellitus patients and in 46 healthy controls to evaluate its correlation with diabetic retinopathy. The modified Airlie House classification was used for grading diabetic retinopathy: (1) no or negligible retinopathy; (2) minimal background retinopathy; (3) background retinopathy; and (4) (pre-) proliferative retinopathy.

The corneal autofluorescence values of grade 1 retinopathy patients did not differ significantly from those of the healthy controls (mean ± standard deviation in ng equivalent fluorescein/ml: 11.6 ± 3.0 and 11.4 ± 2.8, respectively; $P = 0.8$). The means of grade 2, 3, and 4 retinopathy patients (mean ± standard deviation in ngEq fluorescein/ml: 16.2 ± 4.4, 16.7 ± 4.3, 20.9 ± 5.4, respectively) were significantly higher than the means of grade 1 patients and healthy controls ($P < 0.004$). The mean values of patients with grade 4 were significantly higher than those of patients with grades 2 and 3 ($P < 0.01$). The sensitivity and specificity of corneal autofluorescence as a screening test for diabetic retinopathy were 80% and 76%, respectively; the positive predictive value for the presence of retinopathy was 90%. The values for screening on (pre-) proliferative diabetic retinopathy were 68%, 72%, and 58%, respectively.

These data show corneal autofluorescence to be an adequate indicator of diabetic retinopathy. This noninvasive technique, requiring less than one minute, can be applied efficiently as a clinical diagnostic tool for retinopathy screening in diabetic patients. Invest Ophthalmol Vis Sci 32:92–97, 1992.

The incidence of diabetes mellitus varies from 1.5% to 5.0% per year, with a variation in prevalence of 7.8% to 45% in different racial and ethnic groups.[1] In diabetics, an average prevalence of 52% for diabetic retinopathy has been reported.[1] Diabetic retinopathy has been the leading cause of blindness in Great Britain, North America, Europe, and Scandinavia since 1974.[2] In the United States, current estimates of the annual number of new cases of blindness due to diabetic retinopathy range from 12,000 to 24,000.[3] Several risk factors for development of retinopathy in diabetic patients have been established.[4] So far, the duration of diabetes has been shown to be the most powerful predictor of diabetic retinopathy.[5]

The measurement of corneal autofluorescence is a noninvasive technique that can be performed in less than one minute. It recently has been shown to be a useful parameter in ophthalmology because an increased corneal autofluorescence was found in diabetic patients compared with healthy controls.[6] The presence and severity of diabetic retinopathy was not considered in that study.

In the present study, the corneal autofluorescence value was determined in insulin-dependent diabetes mellitus (IDDM) patients with no or negligible retinopathy and with three grades of retinopathy. The intent was to assess its usefulness as an indicator for the presence and severity of diabetic retinopathy and to evaluate its efficacy as an ocular diagnostic tool for the screening of retinopathy in a diabetic population.

Materials and Methods

Subject Selection

Ninety-four IDDM patients aged 16–70 years and 46 healthy controls participated in this study (Table 1). The IDDM patients were selected from the Leiden University Hospital Outpatient Department of Ophthalmology, and the healthy controls were selected

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Table 1. Data of insulin-dependent diabetes mellitus patients and healthy controls

Group	Number of individuals	Age (yr) (mean ± SD)	Diabetes duration (yr) (mean ± SD)
Diabetic patients	94	39.5 ± 12.3	18.8 ± 10.5
Grade 1*	25	29.0 ± 10.1	7.8 ± 6.1
Grade 2†	12	37.4 ± 10.1	21.8 ± 10.1
Grade 3‡	23	42.7 ± 11.2	20.7 ± 9.0
Grade 4§	34	45.9 ± 9.9	24.7 ± 7.7
Healthy controls	46	30.9 ± 13.1	—

* Patients without retinopathy (see text for retinopathy classification).
† Patients with minimal background retinopathy.
‡ Patients with background retinopathy.
§ Patients with (pre-)proliferative retinopathy.

through advertisements in local papers. Individuals were selected on a normal aspect of all corneal layers on slitlamp biomicroscopy without fluorescein staining, to avoid interference with the fluorophotometric measurements. Excluded were individuals with contact lenses and topical ocular medication as well as IDDM patients with fundus photocoagulation performed in the previous three months.

Diabetic retinopathy was graded using the modified Airlie House classification,[7] by two retinal specialists using 30° fundus photographs covering seven standard fields and fluorescein angiography. Retinopathy was classified into four grades based on the findings of the most severely affected eye.

Grade 1. No or negligible retinopathy; at most two microaneurysms per field. This grade is identified as "without retinopathy" in the text.

Grade 2. Minimal background retinopathy; three or more microaneurysms per field only.

Grade 3. Background retinopathy; microaneurysms and one or more of the following items: retinal hemorrhages, hard or soft exudates and/or intraretinal microvascular abnormalities (IRMA), venous beading.

Grade 4. (Pre-) proliferative retinopathy; hemorrhages and microaneurysms and the following present in more than 2 fields: soft exudates, IRMA, venous beading, and/or new vessels and fibrous proliferations, and/or vitreous hemorrhages.

Age and diabetes duration in the four patient groups are presented in Table 1.

The study was approved by the Medical Ethical Committee of the Leiden University Hospital, and informed consent was obtained from each individual after the nature of the procedure had been explained fully.

Instrumentation

Corneal autofluorescence measurements were performed with a commercial fluorophotometer (Fluorotron Master; Coherent Radiation, Palo Alto, CA) fitted with the anterior segment adapter. The fluorophotometer had a 8.1 W tungsten halogen light source. The peak transmission of the excitation filters was 85%; for the fluorescence filters it was 90% (band pass width at half height: 430–490 nm and 530–630 nm, respectively); The transmission outside each bandwidth was effectively blocked (< 0.1%).[8] Scanning was restricted to about 1 mm before to 1 mm past the cornea by modifying the commercial software.[6] Each scan consisted of 40 steps of 0.06 mm each. At each step, the fluorescence was measured in a volume of 0.5 mm × 1.9 mm × 0.1 mm for 0.2 seconds. The total scan duration was 8 seconds.

Measurements

Corneal peak autofluorescence values were determined from four fluorophotometric scans of the cornea of each eye. The average value of both eyes was calculated because the values of right and left eyes were found to be correlated.[6] In the diabetic patients, a 5 ml sample of venous blood was taken to assess the momentaneous blood glucose level and percentage glycosylated hemoglobin (HbA1c).

In addition, the corneal autofluorescence before and after photocoagulation of the ocular fundus was determined in five patients with retinopathy to evaluate the effect of photocoagulation on corneal autofluorescence.

Finally, the diurnal variation in the corneal autofluorescence was determined in three patients by performing seven consecutive corneal fluorophotometric measurements at 9.00, 10.30, 11.00, 12.30, 13.30, 15.30, 16.00, and 17.00 hours, respectively.

Statistical Analysis

Retinopathy grading was performed without knowledge of the corneal autofluorescence data and vice versa. The normality of population distribution was assessed using D'Agostino's test for departure from normality.[9] Commercial statistical software, including multiple regression analysis, was used for evaluation of the data (Statgraphics; STSC, Inc., Rockville, MD). Differences in corneal autofluorescence values between retinopathy grades were analyzed with Student's t-test (two-tailed). Receiver operating characteristic curve analysis was performed to determine the decision level for optimal application of corneal autofluorescence as a diagnostic test for detection of retinopathy.[10]

Evaluation of Efficacy

The efficacy of corneal autofluorescence as a diagnostic tool for screening of retinopathy in diabetics was evaluated by determining sensitivity, specificity,

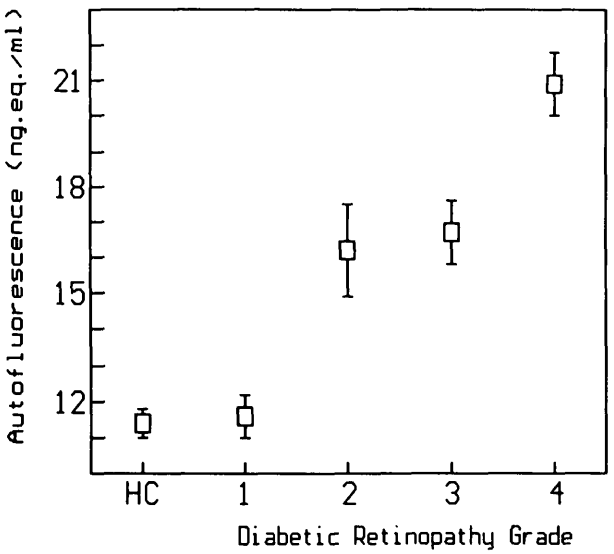

Fig. 1. Corneal autofluorescence expressed in ng equivalent fluorescein/ml in IDDM patients without retinopathy (grade 1), patients with retinopathy (grades 2, 3, and 4) and healthy controls (HC). The open squares and bars represent the mean value and the standard error of the mean in each group. Retinopathy is graded using the modified Airlie House classification (see text).

and predictive value of corneal autofluorescence for detection of diabetic retinopathy (grades 2, 3 and 4) and (pre-) proliferative retinopathy (grade 4), respectively.

The sensitivity of a diagnostic ocular test is defined as the percentage of positive diagnoses in all eyes that have the disease. The specificity is defined as the percentage of diagnoses of absence of disease in all eyes that do not have the disease.[11]

The positive predictive value of a diagnostic ocular test is defined as the percentage of true diagnoses in all eyes diagnosed as having the disease. The negative predictive value is the percentage of true diagnoses in all eyes diagnosed as not having the disease.[11] Both predictive values depend on the prevalence of the disease in a population.[1,11]

Results

The mean and standard deviation of the corneal autofluorescence values in each retinopathy grade and in the healthy controls was calculated because these values were found to be distributed normally in each group ($P < 0.01$).

The mean corneal autofluorescence values in diabetic retinopathy grades 1, 2, 3, 4 and in healthy controls are shown in Figure 1 (mean ± standard deviation in ngEq fluorescein/ml: 11.6 ± 3.0, 16.2 ± 4.4, 16.7 ± 4.3, 20.9 ± 5.4 and 11.4 ± 2.8, respectively). The mean values of patients with (minimal) background retinopathy (grades 2 and 3) and (pre-) proliferative retinopathy (grade 4) were significantly higher when compared to the mean value of patients without retinopathy (average increases: 40% and 80%, respectively; $P = 0.004$). The mean value of patients with (pre-) proliferative retinopathy was 30% higher than that of patients with (minimal) background retinopathy ($P < 0.01$). The mean of patients without retinopathy did not differ significantly from that of healthy controls ($P = 0.8$).

The corneal autofluorescence values correlated significantly with the severity of the retinopathy (linear correlation coefficient $r = 0.62$, $P < 0.0001$). The multiple correlation coefficient obtained by a regression procedure with severity of retinopathy and diabetes duration as variables for the corneal autofluorescence had a similar value ($r = 0.65$).

In the healthy controls, the corneal autofluorescence values were independent of age ($r = 0.5$, $P > 0.4$). In the patients, the corneal autofluorescence values correlated significantly with the diabetes duration ($r = 0.50$, $P < 0.001$; Figure 2), but not with the

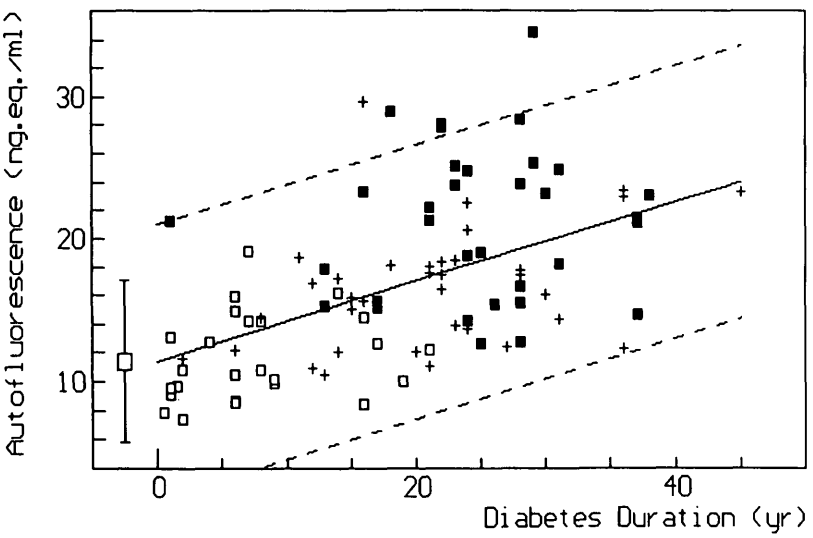

Fig. 2. Corneal autofluorescence versus diabetes duration in IDDM patients. Small open squares: patients without retinopathy; crosses: patients with (minimal) background retinopathy (grade 2 and 3); closed squares: patients with (pre-)proliferative retinopathy. The solid line was obtained by a linear regression procedure to the data points; the broken lines correspond with the 2 times standard deviation. Large square and bar corresponds with the mean and 95% probability interval of healthy controls.

Table 2. Effect of fundus photocoagulation on corneal autofluorescence in five diabetic patients

Age (yr)	Diabetes duration (yr)	Grade*	Eye (R/L)	N†	Corneal autofluorescence (ng eq/ml)			
					Before‡	1 Hour§	2 Weeks§	4 Weeks§
35	16	3	L	17	32.0	32.1	—	—
49	37	4	R	468	14.4	13.7	—	—
			L	0	15.0	15.3	—	—
56	29	4	R	0	25.6	25.0	—	—
			L	566	25.1	25.4	—	—
49	1	4	R	357	20.1	19.6	20.6	—
38	13	4	R	20	15.9	17.3	17.4	16.5

* As in Table 1.
† Number of photocoagulations in the ocular fundus.
‡ One hour before fundus photocoagulation.
§ Time after fundus photocoagulation.

blood glucose level (mean value: 11.5 mmol/L ± 6.1 SD, N = 84; r = 0.02, $P > 0.8$) or the HbA1c (mean value: 8.0% ± 1.4 SD, N = 85; r = 0.1, $P > 0.3$).

There were no significant differences in mean diabetes duration between grades 2, 3 and 4 (Table 1; $P > 0.08$). The mean diabetes duration in patients without retinopathy was on average 62% shorter than in those within the other retinopathy grades (Table 1; $P < 0.0001$).

The corneal autofluorescence values of right and left eyes were found to be correlated in the patient and control groups ($r > 0.9$; $P < 0.001$).

The distribution of the 25 grade 1 patients (classified as "without retinopathy") was as follows: 16 showed no signs of retinopathy, 5 showed at most one microaneurysm per field, and four showed at most two microaneurysms per field.

Effect of Photocoagulation

The mean corneal autofluorescence values of 5 diabetic patients before and after laser treatment of the ocular fundus and the number of photocoagulations are shown in Table 2. The difference in mean corneal autofluorescence before and after treatment ranged between −4.9% and +3.8% (mean difference: +2.9%).

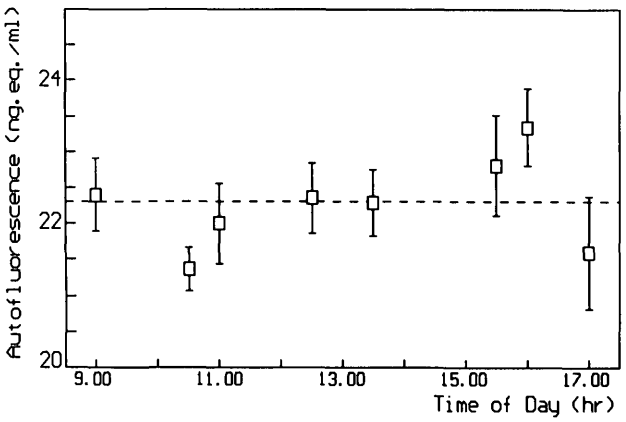

Fig. 3. Diurnal corneal autofluorescence in an IDDM patient. Each open square and bar corresponds with the mean and standard error of the mean of seven consecutive fluorophotometric measurements. The broken line represents the mean value over the day.

Diurnal Variation

An example of the diurnal variation of corneal autofluorescence in a diabetic patient is shown in Figure 3. The maximal deviations from the mean of the diurnal corneal autofluorescence values in the 3 patients were 4.5%, 10%, and 4.1%, respectively (mean: 6.2%).

Efficacy for Retinopathy Screening

The decision level for a positive diagnosis for the presence of diabetic retinopathy (grades 2, 3, and 4) was calculated to be 14.2 ngEq fluorescein/ml; for (pre-) proliferative retinopathy (grade 4) it was 17.0 ngEq fluorescein/ml.[10] These values were found to correspond to the mean corneal autofluorescence value of healthy controls +1 SD and +2 SD, respectively. The distributions of patients for evaluation for retinopathy and (pre-) proliferative retinopathy screening by corneal autofluorescence are presented in Table 3 and 4, respectively.

The sensitivity and specificity of corneal autofluorescence for detection of diabetic retinopathy in the patients were 80% and 76%, respectively. The prevalence of diabetic retinopathy in our study was 73% (69/94), resulting in positive and negative predictive

Table 3. Distribution of diabetic patients without or with retinopathy

Corneal autofluorescence (ng eq fluorescein/ml)	Number of patients	
	Without retinopathy*	With retinopathy†
<14.2	19	14
>14.2	6	55

* Grade 1, as in Table 1.
† Grades 2, 3 and 4, as in Table 1.

Table 4. Distribution of diabetic patients without or with (pre-) proliferative retinopathy

	Number of patients	
Corneal autofluorescence (ng eq fluorescein/ml)	*Without (pre-)proliferative retinopathy**	*With (pre-)proliferative retinopathy†*
<17.0	43	11
>17.0	17	23

* Grade 1, 2, and 3, as in Table 1.
† Grade 4, as in Table 1.

values of 90% and 58%, respectively. Assuming a 52% prevalence of diabetic retinopathy in the diabetic population,[1] the positive and negative predictive values were calculated to be 78% and 78%, respectively.

The sensitivity and specificity of corneal fluorophotometry for the detection of (pre-) proliferative diabetic retinopathy were 68% and 72%, respectively. The prevalence of (pre-) proliferative retinopathy in our study was 36% (34/94), resulting in positive and negative predictive values of 58% and 80%, respectively. Assuming a 10% prevalence of (pre-) proliferative retinopathy in diabetic patients,[1] the positive and negative predictive values were calculated to be 21% and 95%, respectively.

Discussion

Corneal autofluorescence was significantly increased in diabetic patients with retinopathy when compared to patients without retinopathy (average increases: 40% in the (minimal) background retinopathy group and 80% in the (pre-) proliferative retinopathy group, respectively). Corneal autofluorescence in patients with (pre-) proliferative retinopathy was significantly higher than in those with (minimal) background retinopathy (average increase: 30%). Corneal autofluorescence values showed a minimal diurnal variation in diabetic patients (6%).

An accumulation of fluorophores in the diabetic cornea related to duration of diabetes cannot adequately explain the increased autofluorescence found in patients with retinopathy because the corneal autofluorescence is 30% higher in patients with (pre-) proliferative retinopathy than in patients with (minimal) background retinopathy, while diabetes duration did not differ significantly between these groups ($P > 0.08$). These findings are confirmed by the better correlation of severity of retinopathy with corneal autofluorescence than with diabetes duration ($r = 0.62$ and $r = 0.50$, respectively).

For the fluorophotometer used in this study, at least 88% of the corneal fluorescence signal in diabetic patients was proved to originate from corneal autofluorescence, not from excitation light scattered at the corneal surface and leaking through the fluorophotometer filters.[6] In that study, changes in the metabolism of mitochondrial flavoproteins were held responsible for the increased corneal autofluorescence in diabetics because: (1) the autofluorescence of these proteins depends on metabolic changes in glucose levels, oxygen availability, and the mitochondrial respiratory state;[12,13] and (2) the fluorescence excitation and emission wavelengths ($\lambda = 460$ nm and $\lambda = 540$ nm, respectively) correspond with the excitation and detection wavelengths of the fluorophotometer used.[14,15]

The 30% increased corneal autofluorescence value in (pre-) proliferative retinopathy patients when compared to (minimal) background retinopathy patients cannot be attributed to photocoagulation treatment, because in 5 patients the values before and after photocoagulation were about the same (mean difference: 2.9%).

The unexpected correlation between the severity of retinopathy and corneal autofluorescence suggests a common pathogenesis, but explanations concerning this correlation remain speculative. As a first explanation, the vascular component of diabetes mellitus causing specific microangiopathy and consequently progressive retinopathy is probably interrelated with the metabolic disorder (inappropriate elevation of blood glucose level associated with alterations in lipid and protein metabolism).[16] This metabolic disorder also affects the cornea, which might result in a progressive metabolic impairment with a concomitant increase of corneal autofluorescence. Hyperglycemia did not seem to account for the increased corneal autofluorescence found in our patients with retinopathy because corneal autofluorescence was found to be independent of the blood glucose level and long-term diabetic control (HbA1c). As a second explanation, neovascular mediating substances are produced in the retinas of patients with diabetic retinopathy.[17] In these patients, neovascularization of the iris (rubeosis iridis) can occur, indicating the presence of these substances in the anterior chamber. These substances have been shown to induce changes in corneal metabolism with corneal epithelial proliferation.[17] This altered corneal metabolism might result in an increase of corneal autofluorescence.

This study shows corneal autofluorescence to be more efficient than diabetes duration at predicting onset and progression of retinopathy in diabetic patients. Until now, diabetes duration has been considered the most powerful predictor of diabetic retinopathy.[5] We found a sensitivity of 80% and a specificity of 76% for the detection of diabetic retinopathy through

corneal autofluorescence. The predictive value of corneal autofluorescence for detection of retinopathy is closely related to the frequency of retinopathy in the diabetic population.[10] In our study, 73% (69/94) of the patients had diabetic retinopathy based on grading by the retinal specialists, which is a higher percentage than the average prevalence (52%) reported in the diabetic population.[1] This 52% prevalence would result in a positive predictive value of 78% and a negative predictive value of 78%. Therefore, corneal autofluorescence can be a useful diagnostic tool for the screening of retinopathy in a diabetic population.

Corneal fluorophotometry also was found to be useful for screening of (pre-) proliferative retinopathy in a diabetic population (sensitivity 68% and specificity 72%, respectively). The percentage of missed (pre-) proliferative retinopathy diagnoses using corneal autofluorescence was 32% (11/34). This is an improvement over direct ophthalmoscopy performed under optimal conditions by internists (missed diagnoses, 52%), senior medical residents (missed diagnoses, 50%) and diabetologists (missed diagnoses, 33%).[11] The positive predictive value and negative predictive value were calculated to be 21% and 95%, respectively, assuming a (pre-) proliferative retinopathy prevalence of 10% in the diabetic population.[1] The low positive predictive value is a result of the low prevalence of (pre-) proliferative retinopathy in the diabetic population.

Measurement of corneal autofluorescence is performed by a simple noninvasive technique. The measurements cause no discomfort to the patient and can be performed by nonspecialized personnel in a few seconds. Fluorophotometric equipment for determination of corneal autofluorescence can be less sophisticated and thus less expensive than the commercial fluorophotometer used in this study because the instrument originally was developed for scanning of fluorescein in the vitreous.[18] This technique could be especially useful for general practitioners and diabetologists because the results show that measurement of corneal autofluorescence can be applied efficiently as a screening test for diagnosing retinopathy in a diabetic population. This may help reduce the large number of diabetic patients routinely referred to ophthalmologists.

Key words: diabetic retinopathy, fluorophotometry, corneal autofluorescence, diabetes mellitus, screening

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