Corneal Structure and Sensitivity in Type 1 Diabetes Mellitus

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PURPOSE. Corneal wound healing is impaired in diabetic cornea. The purpose of this study was to examine patients with type 1 diabetes mellitus for changes in corneal morphology and to correlate corneal sensitivity, subbasal nerve morphology, and degree of polyneuropathy with each other.

METHODS. Forty-four eyes of 23 patients with diabetes and nine control eyes were included. Corneal sensitivity was tested with a Cochet–Bonnet esthesiometer (Luneau, Paris, France), and corneal morphology and epithelial and corneal thickness were determined by in vivo confocal microscopy. The density of subbasal nerves was evaluated by calculating the number of long subbasal nerve fiber bundles per confocal microscopic field. The degree of polyneuropathy was evaluated using the clinical part of the Michigan Neuropathy Screening Instrument (MNSI) classification, and retinopathy was evaluated using fundus photographs.

RESULTS. A reduction of long nerve fiber bundles per image was noted to have occurred already in patients with mild to moderate neuropathy, but corneal mechanical sensitivity was reduced only in patients with severe neuropathy. Compared with control subjects the corneal thickness was increased in patients with diabetes without neuropathy. The epithelium of patients with diabetes with severe neuropathy was significantly thinner than that of patients with diabetes without neuropathy.

CONCLUSIONS. Confocal microscopy appears to allow early detection of beginning neuropathy, because decreases in nerve fiber bundle counts precede impairment of corneal sensitivity. Apparently, the cornea becomes thicker in a relatively early stage of diabetes but does not further change with the degree of neuropathy. A reduction in neurotrophic stimuli in severe neuropathy may induce a thin epithelium that may lead to recurrent erosions. (Invest Ophthalmol Vis Sci. 2000;41: 2915–2921)

Insulin-dependent diabetes mellitus (IDDM, type 1) is common in Finland, with the highest incidence in the world (35/100,000 per year) in children less than 16 years of age. Retinopathy, nephropathy, and neuropathy are the most recognized complications caused by this disease. Diabetic keratopathy has also been thought to represent a form of corneal neuropathy.1 Because patients with diabetes have decreased corneal sensitivity,2–4 they are more vulnerable to corneal trauma. The morphology of corneal nerves has been found to be altered in diabetic rats5 and humans (Linda Müller, unpublished observations, 1998) by light and electron microscopy. In addition to the observation of polymorphism in epithelium and endothelium,6,7 an increase in corneal thickness in patients with diabetes also has been reported.8,9

Confocal microscopy has provided a new in vivo method for corneal examination.10,11 With this method, only two studies on diabetic corneas have been published. Frueh et al.12 examined the corneas of 10 patients with type 1 diabetes, 10 patients with type 2 diabetes, and 10 patients without diabetes, by confocal microscopy. The abnormal morphologic findings included polymorphism of the epithelium and endothelium, and abnormal stromal nerves in only two patients with type 1 diabetes. No specific observations on the subbasal nerves or corneal sensitivity were reported. Morishige et al.13 found a correlation between corneal light-scattering index and stages of diabetic retinopathy. Nerve morphology was not reported. Evaluation of skin biopsy specimens using confocal microscopy has revealed that the number of epidermal nerve fibers per unit surface area in patients with diabetic polyneuropathy is reduced.14

The present study was conducted to provide a comparison between corneal nerve fiber density in healthy control individuals versus patients with diabetes, by in vivo confocal microscopy. Furthermore, an attempt was made to find a correlation between changes in corneal thickness, corneal sensitivity, and nerve fiber density and the degree of diabetic polyneuropathy.
METHODS

Patients and Examinations

Forty-four eyes of 23 patients with type 1 diabetes (7 women, 16 men; mean age, 45.2 ± 10.0 years) from Helsinki University Central Hospital were included in this study. Informed consent was obtained from all patients, and the study protocol was reviewed by the Ethics Committee of the Helsinki University Eye and Ear Hospital according to the Declaration of Helsinki. One patient had a prosthetic eye, and another’s eye was excluded due to a history of ocular surgery. The mean duration of diabetes was 25.9 ± 8.1 years. All patients were treated with insulin, and 14 of them received antihypertension treatment. None of the patients used topical ocular medication. The patients were examined by biomicroscopy to exclude manifest anterior segment disease. In the past 2 years one patient had had occasional bilateral dry-eye–like symptoms, one patient had experienced a mild unilateral corneal trauma, and one patient had used contact lenses. These patients were not excluded, because their corneas appeared unaffected on slit lamp examination. In all cases the intraocular pressures were normal. Initially, patients with diabetes were selected for the study based on the severity of diabetic complications. The presence (n = 12) or absence (n = 11) of diabetic nephropathy was used as a criterion. Of the patients with nephropathy, six were treated with peritoneal dialysis and six with hemodialysis. Two patients with occasional microalbuminuria were not to have nephropathy.

The degree of polyneuropathy was evaluated using the clinical part of the Michigan Neuropathy Screening Instrument (MNSI) classification. Briefly, the feet were examined for deformities, dryness of skin, callus, infections, fissuruses, and ulcerations. The vibration sensation of the large toes was tested, and the ankle reflex was evaluated. The maximum score was 8 points. In our study the first group (0–2 points) included patients without neuropathy (n = 11), the second group (2.5–4.5 points) patients with mild to moderate neuropathy (n = 7), and the third group (5–8 points) patients with severe neuropathy (n = 5). Corneal sensitivity of the central cornea and four quadrants was tested using a Cochet–Bonnet esthesiometer. The monofilament had a diameter of 0.08 mm. Each area was tested with each filament length, which was sequentially reduced in 5-mm steps starting from 60 mm. A positive answer was regarded as a positive result. The longest filament length resulting in a positive response was considered the corneal sensitivity threshold. Average sensitivity values of all five areas were used for statistical analyses. Corneal subbasal nerve density was evaluated by calculating the highest number of long subbasal nerve fiber bundles per confocal microscope image. Three researchers, one of whom was not aware of the diagnosis of the patients, analyzed the nerve images and agreed on the findings. The severity of retinopathy was graded by a retinal specialist (JJI) from fundus photographs, by using the classification developed for the EURODIAB IDDM study. Patients with level 10 to 20 changes according to this classification were considered to have no or mild retinopathy (n = 10), whereas patients with level 30 to 60 changes were considered to have severe retinopathy (n = 13). Nine eyes of healthy volunteers (4 women, 5 men; mean age, 39 ± 8.5 years) served as control eyes. The control subjects did not have diabetes and had no history of ocular disease. Biomicroscopy and confocal microscopy were performed. Morphology of corneal cells and subbasal nerves, as well as epithelial and corneal thickness, were evaluated, but corneal sensitivity was not tested. Because these subjects did not have diabetes, the MNSI score was not determined, nor were fundus photographs taken.

In Vivo Confocal Microscopy

The central area of each cornea was examined using a tandem scanning confocal microscope (TSCM, Model 165A; Tandem Scanning, Reston, VA.). The setup and operation of the confocal microscope has been described previously. Briefly, a X24, 0.6 numeric aperture (NA) variable working-distance objective lens was used. The field-of-view with this lens is 450 × 360 μm, and the z-axis resolution is 9 μm. Images were detected, by using a low-light-level camera (model VEi1000; Dage–MTI, Michigan City, IN), and recorded on S-VHS tape.

### Table 1. Statistics According to Grade of Neuropathy

<table>
<thead>
<tr>
<th>Grade of Neuropathy</th>
<th>Age (y)</th>
<th>Diabetes Duration (y)</th>
<th>Corneal Thickness (μm)</th>
<th>Epithelial Thickness (μm)</th>
<th>Corneal Sensitivity (mm)</th>
<th>Number of Long NFBs per Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>No diabetes (n = 9)</td>
<td>39.0 ± 8.5</td>
<td>0</td>
<td>526.8 ± 24.7</td>
<td>50.7 ± 3.5</td>
<td>ND</td>
<td>4.9 ± 1.1</td>
</tr>
<tr>
<td>No neuropathy (n = 11)</td>
<td>42.9 ± 7.7</td>
<td>21.5 ± 6.9</td>
<td>576.9 ± 48.0</td>
<td>51.9 ± 4.6</td>
<td>57.8 ± 4.9</td>
<td>4.0 ± 1.2</td>
</tr>
<tr>
<td>Mild to moderate neuropathy (n = 7)</td>
<td>46.4 ± 15.5</td>
<td>29.0 ± 6.1</td>
<td>558.5 ± 46.3</td>
<td>53.2 ± 7.4</td>
<td>56.9 ± 7.5</td>
<td>2.6 ± 1.4</td>
</tr>
<tr>
<td>Severe neuropathy (n = 5)</td>
<td>48.6 ± 1.8</td>
<td>31.0 ± 9.4</td>
<td>625.8 ± 83.4</td>
<td>45.3 ± 3.2</td>
<td>26.0 ± 27.9</td>
<td>1.8 ± 0.8</td>
</tr>
</tbody>
</table>

Data are shown according to MNSI score as mean ± SD. n, number of patients; NFBs, nerve fiber bundles; ND, no data; M–W, Mann–Whitney test.

* Compared with no diabetes group.
† Compared with patients with diabetes and no neuropathy.
‡ Compared with patients with diabetes and mild to moderate neuropathy.
Special attention was paid to subbasal nerve morphology. The subbasal plexus was viewed in the beginning and again at the end of the examination, which altogether lasted for 4 to 10 minutes per eye. There was no difficulty in getting the nerve fiber bundles into sharp focus. The number of long nerve fiber bundles in the image with most nerve bundles was calculated. In addition, confocal microscopy through-focusing scans (CMTF) were obtained, as previously described. Video images of interest were digitized using a PC-based imaging system with custom software (University of Texas, Southwestern Medical Center at Dallas), and printed using a color printer (Stylus Color 800; Seiko Epson, Nagano, Japan). The CMTF data were digitized into the computer by custom software, and intensity profile curves were calculated. From each scan, the epithelial and total corneal thicknesses were measured. An average of three CMTF scans of each eye were performed. In 12 eyes, no acceptable CMTF-profile could be produced because of the patients' inability to fixate steadily; the results of these scans were not included in the analysis. The average values of the measurements were used for all statistical calculations.

**Statistical Analyses**

Statistical analyses were performed by computer (SPSS for Windows, ver. 7.0; SPSS, Chicago, IL). Normality was tested using the Kolmogorov–Smirnov test, and a t-test, Mann–Whitney test, Wilcoxon signed rank test or χ² test were performed for comparison of the groups. Pearson’s or Spearman’s correlation coefficients were used for evaluation of parametric or nonparametric correlations, respectively. Data are expressed as mean ± SD. Differences were considered statistically significant when P < 0.05.

**RESULTS**

First, the right and the left eyes of each patient with diabetes were compared with each other pairwise. The corneal parameters estimated, such as epithelial thickness (one-sample t-test, P = 0.982), total corneal thickness (one-sample t-test, P = 0.106), the number of long nerve fiber bundles (one-sample t-test, P = 0.403), corneal sensitivity (Wilcoxon signed rank test, P = 0.088), or the degree of retinopathy (χ² test, P = 0.521) did not show any statistically significant differences between the two eyes. To avoid intraindividual bias in the results, only the right eyes were thus included in all further statistical analyses.

The patients with diabetes were divided into three groups based on the MNSI neuropathy classification: no neuropathy, mild to moderate neuropathy, and severe neuropathy. The MNSI score and duration of diabetes were positively correlated (Pearson’s correlation coefficient, r = 0.563; P = 0.005). The corneal innervation was evaluated by calculating the highest number of long nerve fiber bundles in the digitized confocal microscopic nerve images. The number of long nerve fiber bundles was inversely correlated with the MNSI score (Pearson’s correlation coefficient r = −0.661; P = 0.001) and positively correlated with corneal sensitivity (Pearson’s correlation coefficient r = 0.417; P = 0.048). Corneal sensitivity, on the contrary, was inversely correlated with the duration of diabetes (Spearman’s correlation coefficient r = −0.630; P = 0.001) and number of MNSI score (r = −0.631, P = 0.001).

When the results of the patients with different degrees of neuropathy were analyzed, the following findings were recorded (Table 1). The epithelial thickness and corneal sensitivity were significantly decreased in patients with severe neu-
TABLE 2. Statistics According to Nephropathy Status

<table>
<thead>
<tr>
<th></th>
<th>Age (y)</th>
<th>Diabetes Duration (y)</th>
<th>Corneal Thickness (μm)</th>
<th>Epithelial Thickness (μm)</th>
<th>Corneal Sensitivity (mm)</th>
<th>MNSI Score</th>
<th>Number of Long NFBS per Image</th>
</tr>
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<tr>
<td>No diabetes (n = 9)</td>
<td>39.0 ± 8.5</td>
<td>0</td>
<td>526.8 ± 24.7</td>
<td>50.7 ± 3.5</td>
<td>ND</td>
<td>ND</td>
<td>4.9 ± 1.1</td>
</tr>
<tr>
<td>Diabetes, no nephropathy (n = 11)</td>
<td>43.4 ± 12.5</td>
<td>21.2 ± 6.0</td>
<td>562.6 ± 52.3</td>
<td>51.9 ± 5.9</td>
<td>60.0 ± 0</td>
<td>1.2 ± 1.2</td>
<td>3.9 ± 1.5</td>
</tr>
<tr>
<td>Diabetes with nephropathy (n = 12)</td>
<td>46.9 ± 7.0</td>
<td>30.2 ± 7.6</td>
<td>600.7 ± 64.0</td>
<td>49.5 ± 5.9</td>
<td>42.0 ± 22.8</td>
<td>4.0 ± 1.7</td>
<td>2.3 ± 1.0</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD. Abbreviations: See Table 1.
* Compared with no diabetes group.
† Compared with patients with diabetes without nephropathy.

Confocal microscopic examination of 40 corneas of 23 patients with diabetes revealed the following morphologic characteristics. In four patients we had data on only one eye, because patients were uncooperative. The surface epithelium was normal in 36 of 40 eyes (Fig. 2A). We could not produce a sharp image for the evaluation of the surface epithelium of both eyes of one patient, and, in addition, one cornea showed focal areas of damaged epithelial cells (Fig. 2B). Another patient was the only contact lens wearer in our study and showed altered surface epithelial cells in one eye (Fig. 2C), which could have been related to contact lens wear. However, the surface epithelium of the fellow eye appeared normal. The basal epithelial cells were considered to be normal in 35 of 40 eyes. No data were obtained in two eyes. Two patients (the only eye of a patient with nephropathy and severe neuropathy and both eyes of a patient with mild to moderate neuropathy without nephropathy), however, had deposits in the basal epithelial cells similar to that described in corneal dystrophies (Fig. 2D).21 No corresponding abnormalities were observed by biomicroscopy. Although the subbasal nerve morphology ap-

TABLE 3. Statistics According to Grade of Retinopathy

<table>
<thead>
<tr>
<th></th>
<th>Age (y)</th>
<th>Diabetes Duration (y)</th>
<th>Corneal Thickness (μm)</th>
<th>Epithelial Thickness (μm)</th>
<th>Corneal Sensitivity (mm)</th>
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<td>526.8 ± 24.7</td>
<td>50.7 ± 3.5</td>
<td>ND</td>
<td>ND</td>
<td>4.9 ± 1.1</td>
</tr>
<tr>
<td>No to mild retinopathy (n = 10)</td>
<td>43.4 ± 12.7</td>
<td>22.4 ± 6.3</td>
<td>565.0 ± 56.6</td>
<td>53.0 ± 4.0</td>
<td>60.0 ± 0</td>
<td>1.4 ± 1.5</td>
<td>3.9 ± 1.6</td>
</tr>
<tr>
<td>Severe retinopathy (n = 13)</td>
<td>46.6 ± 7.4</td>
<td>28.5 ± 8.6</td>
<td>596.0 ± 62.5</td>
<td>49.1 ± 6.4</td>
<td>43.4 ± 22.4</td>
<td>3.7 ± 1.8</td>
<td>2.5 ± 1.1</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD. Abbreviations: See Table 1.
* Compared with no diabetes group.
† Compared with patients with diabetes and no to mild retinopathy.
peared normal in 34 of 40 corneas, patients with diabetes had less nerve fiber bundles (Fig. 2E) than the healthy control subjects (Fig. 2F). One patient had unusual curved nerve fiber bundles in the subbasal nerve plexus in both corneas (Fig. 3A). Two patients had small particles between the nerve fiber bundles in both eyes (Fig. 3B). We have proposed that these particles represent Langerhans’ cells.21 In 4 of 40 corneas, microfolds in Bowman’s layer were observed. These microfolds were all in different patients. The stromal keratocytes were normal in 34 corneas. Activated, highly reflective anterior keratocytes and increased extracellular matrix were noted in both corneas of two patients and one cornea of two patients (Fig. 3C). Accumulation of highly reflective small extracellular deposits was noted in one cornea. The stromal nerves seemed normal in all patients in whom nerves were visualized (Fig. 3D). However, in three patients no stromal nerves were observed in either cornea. Confocal microscopy was not the proper method for evaluation of corneal endothelial cells, because analysis of detailed morphology was difficult in most corneas. Consequently, we did not evaluate the exact morphology of the endothelial cells. In two eyes of different patients; however, small endothelial pits were observed (Fig. 3E) and folds in the endothelial layer (or Descemet’s membrane) were seen in three eyes (Fig. 3F).

DISCUSSION

Complications in patients with diabetes have been well documented, and numerous studies have been performed that associate complications with each other.1,2,22–26 In our study all patients with severe neuropathy had nephropathy and severe retinopathy. We have also been successful in our attempt to correlate corneal morphology with neuropathy of different severity, and corneal sensitivity with nerve morphology in patients with diabetes by using in vivo confocal microscopy.

Our results are in accordance with earlier data indicating that impairment of corneal sensitivity increases, with the duration of diabetes being in direct correlation with the degree of polyneuropathy.1,2,26 A new finding was that patients with severe neuropathy, measured by the MNSI score, showed decreased corneal sensitivity together with a decreased number of long nerve fiber bundles in the subbasal nerve plexus per
confocal microscopic image. An interesting question was whether the corneal nerve density in patients with diabetes without signs of polyneuropathy differs from that of healthy control subjects. We could not find any statistically significant difference in the nerve densities, although on average the patients with diabetes had one nerve fiber bundle less per image. Therefore, it is possible that the cornea is affected relatively early in the course of polyneuropathy. In all patients with diabetes with neuropathy the subbasal nerve densities were significantly reduced. The sensitivity, however, remained quite normal in patients with diabetes with no or mild to moderate neuropathy and showed reduction only provided that the patient had severe neuropathy. It thus appears that confocal microscopy may allow detection of beginning neuropathy earlier than measurement of corneal mechanical sensitivity. The clinical use of confocal microscopy is compromised by the fact that only a small central area of the cornea can be evaluated easily. The results may also have been affected by the fact that the control subjects were somewhat younger than the patients with severe neuropathy. There were also relatively more men among the patients with diabetes than among the control subjects. In addition, one patient with the most severe diabetic complications (amputation of one leg, prosthetic eye) had experienced a recent superficial corneal trauma that could have affected the nerve density and the corneal sensitivity.

The morphology of human corneal nerves has been carefully described by Müller et al.27 The nerve fibers terminate as free nerve endings in the corneal tissue and are derived from trigeminal sensory afferents.27–29 Although most patients with diabetes had nerve fiber bundles with a normal morphology, we observed some abnormally curved nerve fiber bundles in the subbasal nerve plexus of one patient who was undergoing dialysis and who had mild to moderate neuropathy. The stromal nerve density was not evaluated, because most of these nerves run obliquely to the surface and cannot be visualized in every confocal microscopic examination. No abnormal stromal nerves, as described by Fruch et al.,12 were observed in our study eyes.

The clinical part of the Michigan Neuropathy Screening Instrument was used as an indicator of diabetic neuropathy.15 According to Feldman et al.,15 the sensitivity of the MNSI score as a predictor of diabetic neuropathy is 80% and the specificity 95%. We found an inverse correlation between the MNSI score and the corneal sensitivity. Therefore, it appears that the MNSI score also reflects loss of corneal sensitivity. A disadvantage of the Cochet–Bonnet method is that it detects neurons sensitive to mechanical stimulation only. It would be interesting to examine the sensitivity of diabetic corneas using noncontact gas esthesiometry, which discriminates between the three known classes of nociceptors in the cornea: mechanosensory, polymodal, and cold-sensory neurons.30

Altered surface epithelium was observed in the only contact lens wearer in this study. Mechanical trauma or hypoxic damage could have contributed to this finding. However, the patient had normal corneal sensitivity. The epithelial damage in another patient could have been caused by manifest diabetic keratopathy or microtrauma during confocal microscopy. Deposits in the basal epithelial cells were noted in two of our patients. Similar-looking deposits have been observed in corneal dystrophies.21 Friend et al.31 also found accumulation of material, presumably calcium, beneath the basal epithelial cells in diabetic rats. Occasionally, microfolds were observed in Bowman’s layer. We hypothesize that the pathologic findings in the epithelial and Bowman’s layers are specific to some patients with diabetes. Problems affecting the epithelial basement membrane of diabetic corneas have been acknowledged earlier. Corneal abrasion in diabetic eyes leads to deeper damage than in healthy eyes, including detachment of the basement membrane.52 The pathologic nature of the adhesive structures in the diabetic basement membrane has not been completely unraveled. Recently, Morishige et al.13 showed that the light-scattering index of the basement membrane area correlates with the severity of retinopathy. Although the anterior stromal keratocytes were normal in most corneas, our findings indicate that the anterior stroma could be altered in some patients with diabetes as well. Three of the four patients with signs of keratocyte activation and matrix opacification had been treated with argon laser for proliferative retinopathy. The cornea with a recent trauma, however, had a normal corneal stroma. Endothelial folds were seen in three eyes. Earlier Busted et al.8 also reported the presence of endothelial folds by specular microscopy in patients with diabetes.

Our study shows a significant increase in corneal thickness when comparing patients with diabetes without neuropathy with control subjects, whereas no significant difference was found between severe neuropathy and absence of neuropathy. The change in thickness must have been caused by diabetes, whereas neuropathy itself apparently did not have a further effect on the increase in corneal thickness. Abnormally thick corneas have previously been reported in patients with diabetes.8,9 This has been thought to be due to insufficient endothelial cell function, leading to stromal edema.8 In contrast, some investigators have observed no differences in corneal thickness between patients with diabetes and control subjects.7,35 To our knowledge this is the first study of corneal epithelial thickness in patients with diabetes. The epithelium in patients with severe neuropathy was significantly thinner than that in patients with diabetes and no neuropathy. This is interesting, because corneal nerves may have a neurotrophic effect on epithelial cells.54 Decreased corneal sensitivity and improper neural regulation in the diabetic cornea apparently leads to problems in epithelial wound healing and occurrence of recurrent erosions.35

In conclusion, the present study showed several important findings and relationships between the different parameters measured. Corneal sensitivity decreases with the duration of type 1 diabetes and is inversely correlated with the degree of neuropathy (MNSI score). A decrease in the number of long nerve fiber bundles imaged by in vivo confocal microscopy corresponds well with reduced corneal sensitivity. An increase in corneal thickness occurs in the early stage of the disease. Epithelial thickness decreases only in cases of severe neuropathy. Confocal microscopy seems to detect early phases of ocular neuropathy, since a reduction of the number of long nerve fiber bundles is detected earlier than a decrease in corneal mechanical sensitivity.

References


