Corneal Diabetic Neuropathy: A Confocal Microscopy Study

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ABSTRACT

PURPOSE: To evaluate the role of corneal confocal microscopy in the diagnosis of morphologic damage of the corneal sub-basal nerve plexus in diabetic patients and to correlate corneal confocal microscopy findings with peripheral diabetic neuropathy.

METHODS: Corneal sub-basal nerve plexus parameters were quantified by corneal confocal microscopy in 42 diabetic patients and 27 age-matched controls. The parameters quantified were the number of fibers, the tortuosity of fibers, the number of beadings, and the branching pattern of the fibers. Peripheral neuropathy was also quantified using the Michigan Neuropathy Screening Instrument.

RESULTS: The number of fibers, number of beadings, and branching pattern of fibers significantly decreases in diabetic patients versus control subjects (P<.0001; P<.0001; P=.0006, respectively), whereas nerve tortuosity significantly increases (P<.0001). The same corneal sub-basal nerve plexus parameters show a statistical trend, suggesting progression of corneal neuropathy with peripheral diabetic neuropathy.

CONCLUSIONS: Corneal confocal microscopy represents a new tool in the diagnosis, clinical evaluation, and follow-up of peripheral diabetic neuropathy. This study found that diabetes damages corneal nerves, particularly the corneal sub-basal nerve plexus. This damage may be easily and accurately documented using corneal confocal microscopy. [J Refract Surg. 2006;22: S1047-S1052.]

The prevalence of diabetes mellitus is dramatically increasing worldwide and consequently, the prevalence of chronic complications due to diabetes will increase in the near future.¹ The most common cause of chronic disability in diabetic patients is diabetic neuropathy, particularly peripheral diabetic neuropathy. Peripheral diabetic neuropathy affects 50% of diabetic patients within 25 years of diagnosis.² Long-term effects of untreated peripheral diabetic neuropathy can lead to foot infections that do not heal, as well as foot ulcers. Patients may require subsequent amputation of the foot or digits, which can lead to a decreased quality of life and increased mortality.³

The early and accurate diagnosis and quantification of peripheral diabetic neuropathy are important in defining at-risk patients, decreasing patient morbidity, and assessing new therapies.⁴ The clinical diagnosis of peripheral diabetic neuropathy is often missed or diagnosed only in later stages, likely because a simple, noninvasive method for the early detection of peripheral diabetic neuropathy is not yet available,⁵ and clinical diagnosis is made only when patients with peripheral diabetic neuropathy become symptomatic. Early diagnosis is currently based on electrophysiological tests⁷ or more recently, on skin biopsy.⁸-¹⁰ Electrophysiological tests cannot detect the minute nerve fiber damage that is the most relevant component of nerve damage in patients with diabetes. Although skin biopsy can detect this minute damage, the disadvantage of this technique is its invasiveness.

The cornea is the most innervated tissue of the human body.¹¹-¹³ Corneal epitheliopathy is clinically well known in diabetic patients and is likely multifactorial in origin.¹⁴-¹⁷ The cornea may be examined with noninvasive procedures, such as in vivo corneal confocal microscopy, which allows

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the visualization and recording of each corneal layer, including corneal nerves. Qualitative and quantitative analysis of the corneal nerves, particularly the corneal sub-basal nerve plexus, within images taken by corneal confocal microscopy may be an ideal method to evaluate nerve fiber pathology in patients with diabetes mellitus.

The goal of this study was to evaluate the role of corneal confocal microscopy in the diagnosis of morphologic damage of the corneal sub-basal nerve plexus in diabetic patients and to correlate corneal confocal microscopy findings with peripheral diabetic neuropathy.

**PATIENTS AND METHODS**

**PATIENTS AND CLINICAL EXAMINATION**

Forty-two consecutive patients with diabetes mellitus (type I and type II) were evaluated using corneal confocal microscopy. Twenty-seven healthy subjects served as controls. Informed consent was obtained from all subjects, and all subjects underwent a complete ophthalmologic examination including anterior segment slit-lamp microscopy, fundus biomicroscopy, and intraocular pressure measurement. Patients with central neuropathy, peripheral neuropathy, or ocular diseases not related to diabetes were excluded.

Peripheral diabetic neuropathy was documented using the Michigan Neuropathy Screening Instrument (MNSI), which is a simple, reproducible, and noninvasive peripheral diabetic neuropathy screening test. An MNSI score >2 suggests the presence of peripheral diabetic neuropathy. Patients were classified into groups according to MNSI score based on the clinical examination of feet as proposed by Rosenberg et al. Subjects without diabetic neuropathy had an MNSI score ≤ 2; patients with mild to moderate diabetic neuropathy had an MNSI score > 2 and ≤ 4.5; patients with moderate to severe diabetic neuropathy had an MNSI score of 4.6 to ≤ 4.9; and patients with severe diabetic neuropathy had an MNSI score > 5.

**CONFOCAL MICROSCOPY**

Corneal confocal microscopy was performed using the NIDEK Confoscan 4.0 (CS4; NIDEK Co Ltd, Gamagori, Japan) scanning slit confocal microscope fitted with a z-ring adapter and an Achromplan (Carl Zeiss-Meditec, Jena, Germany) nonapplanating 40× immersion objective lens designed for full-thickness examination of the cornea. The working distance was 1.98 mm, and a motorized focusing device was used to image the area of interest properly. The z-ring adapter system uses an optomagnetic sensor, which aligns the eye with the tip of the confocal microscope during examination. Thus, with the z-ring sensor, the confocal microscope tip position is fixed with regard to the examined cornea, compensating for eye movements along the z-axis.

The center of the cornea was examined. Prior to examination, a drop of topical anesthetic 0.4% oxybuprocaine hydrochloride (Novartis AG, Basel, Switzerland) was instilled in the lower conjunctival fornix of the eye to reduce blinking. The patient was seated in front of the microscope while fixing the examined eye on a bright blue target on the instrument. One drop of 0.2% polyacrylic gel (Viscotears; Ciba Vision, Atlanta, Ga) was applied to the tip of the objective lens as an immersion fluid. Coarse alignment with the corneal apex was carried out manually by the operator by moving the z-ring adapter to the apex of the cornea. Fine adjustment was completed automatically by a motor-driven system within the CS4. Subsequently, the focal plane was automatically adjusted to recognize the anterior chamber, and corneal scanning and recording began. Images were always taken from the endothelium to the corneal epithelium. Image intensity depth profiles were generated from confocal microscope videorecordings by averaging the pixel intensity in the center of each consecutive video frame image and plotting data as a function of z-depth in the z-scan curve. Due to the z-ring adapter, all points on the z-scan curve directly correlate to high-resolution images, and the exact z-axis position of specific tissue landmarks (e.g., surface epithelium, sub-basal nerve plexus, endothelium, etc) can be used to calculate the distance between individual corneal layers. Each examination lasted approximately 14 seconds. The highest quality image of the corneal sub-basal nerve plexus was identified by an experienced clinician. Four corneal sub-basal nerve plexus parameters were evaluated, including the number of fibers, the number of fiber beadings, the degree of fiber branching, and the degree of fiber tortuosity.

The number of fibers was defined as the sum of the nerve fibers seen in the selected frame of the corneal sub-basal nerve plexus. The number of beadings was defined as the number of hyper-reflective points per unit of length (100 µm) in a single nerve fiber, randomly selected from all the nerve fibers seen in the corneal sub-basal nerve plexus image.

Grading systems were used to classify fiber branches and fiber tortuosity. For fiber tortuosity, the grading system proposed by Oliveira-Soto and Efron, based on frequency and amplitude of changes in nerve fiber direction, was used. Values ranged from 0 to 4, whereby grade 0 nerve fibers appear almost straight; grade 1 nerve fibers appear slightly tortuous; grade 2 nerve fibers appear moderately tortuous, with frequent, small amplitude changes in the direction of fibers; grade 3
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nerve fibers appear tortuous, with the amplitude of changes in fiber direction quite severe; and grade 4 nerve fibers appear very tortuous, showing abrupt and frequent changes in direction (Fig 1).

For fiber branching, the grading system values ranged from 0 to 3, whereby grade 0 shows no corneal sub-basal nerve plexus fibers branching; grade 1 shows one fiber presenting one or more direct branchings from the major nerve trunk; grade 2 shows one fiber presenting one branching originating from a principal nerve trunk branching; and grade 3 shows one fiber presenting one branching originating from a grade 2 fiber branching (Fig 2).

STATISTICAL ANALYSIS

All statistics were calculated using SAS Software (SAS Institute Inc, Cary, NC). Pearson index correlation, Spearman index correlation, Fisher test, analysis of covariance, multiple linear regression model, analysis of variance, post hoc test for multiple comparisons

RESULTS

Forty-two patients with diabetes (18 women and 24 men) were evaluated. Mean age was 55.4 ± 13 years (range: 30 to 73 years). Twenty-nine patients (7 women and 22 men) had type II diabetes, and 13 patients (11 women and 2 men) had type I diabetes. Twenty-seven healthy subjects (18 women and 9 men) with a mean age of 49.3 ± 19.9 years (range: 24 to 85 years) acted as controls. No statistical correlation between corneal sub-basal nerve plexus parameters and age was found when glycemic control and duration of disease were considered. No statistical difference between type I and type II diabetic patients was found when corneal sub-basal nerve plexus parameters and glycemic control and duration of diabetes were considered. No statistical difference was found in average MNSI score between type I and type II diabetic patients. Given these findings, results are classified as a single pathologic group.

Corneal sub-basal nerve plexus parameters were statistically significantly different between diabetic patients and control subjects. The mean number of fibers was 2.2 ± 0.3 in diabetic patients versus 4.4 ± 0.3 in control subjects ($P<.0001$). The number of beadings was 5.6 ± 0.4 for diabetic patients versus 12.3 ± 0.4 for control subjects ($P<.0001$). The degree of fiber branching was 0.8 ± 0.1 for diabetic patients and 1.3 ± 0.1 for control subjects ($P=.0006$). The tortuosity grading was −2.6 ± 0.2 for diabetic patients compared with 1.3 ± 0.1 for control subjects ($P<.0001$) (Fig 3).

Using the MNSI clinical examination score, 12 patients were classified as group A (absence of diabetic neuropathy), 12 patients as group M (mild to moderate diabetic neuropathy), and 18 patients as group S (severe diabetic neuropathy). Corneal sub-basal nerve plexus parameters were not statistically different among the three different peripheral diabetic
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neuropathy groups (number of fibers, \( P = .11 \); number of beadings \( P = .55 \); branching degree, \( P = .33 \); tortuosity degree, \( P = .19 \)). However, three parameters (number of fibers, number of beadings, and branching grade) showed a decreasing trend among A, M, and S groups (Fig 4).

Further analysis revealed that the number of fibers was statistically significantly lower in the M and S groups compared with the control group (\( P < .0001 \)) (Fig 5). The number of beadings was statistically higher in subjects in the control group compared with patients in all peripheral diabetic neuropathy groups (\( P < .0001 \)). The degree of branching was statistically different between control subjects and diabetic patients, with the S group having the least branching compared with the control group (\( P = .004 \)). Nerve fiber tortuosity was significantly lower in the control group compared with the A and S groups (\( P = .0002 \)).

**DISCUSSION**

Peripheral diabetic neuropathy is a major cause of disability among diabetic patients and can lead to chronic foot ulceration and amputation.1-4 Clinical evaluation of peripheral diabetic neuropathy is performed using screening tests such as the MNSI or more invasive techniques such as electrophysiological tests or skin biopsy. More invasive techniques are limited to selected patients or populations and cannot be used as standardized follow-up tests.5-10 The major limitations are related to the strict follow-up that is necessary when using an invasive test in diabetic patients whose healing problems and high rate of infection are well known. Therefore, peripheral diabetic neuropathy is more frequently screened using a simple, reliable test such as the MNSI, which does not indicate fine changes of small nerve fiber morphology or function.18-20 These observations emphasize the importance of developing new tests to quantify peripheral diabetic neuropathy.

Corneal confocal microscopy enables the clinician to examine corneal nerves, and specifically the corneal sub-basal nerve plexus in vivo with high repeatability. The corneal sub-basal nerve plexus is a monolayer of unmyelinated nerve fibers with a diameter of 4 \( \mu \)m. The corneal sub-basal nerve plexus may be easily documented using confocal microscopy, as previously reported.22-27 Morphology of the corneal sub-basal nerve plexus is not influenced by age, as reported by Erie et al.24 This allows the exclusion of a relevant biologic parameter when evaluating changes in the corneal sub-basal nerve plexus. Corneal confocal microscopy has been previously reported in the evaluation of the corneal sub-basal nerve plexus in diabetics.21,25-27 Rosenberg et al21 found that the number of nerve fibers of the corneal sub-basal nerve plexus is significantly lower in diabetic patients with mild to moderate and severe peripheral neuropathy versus control subjects, suggesting enhanced nerve degeneration. In addition, the decrease in nerve fiber numbers preceded impairment of corneal sensitivity assessed with a mechanical esthesiometer.21 Malik et al25 also found a statistically significantly reduced number of fibers in the corneal sub-basal nerve plexus of diabetic patients compared with control subjects, with a tendency for greater reduction in fiber number with increasing severity of peripheral diabetic neuropathy. They also reported a significant reduction of fiber branch density in the corneal sub-basal nerve plexus of diabetic patients, which suggests a reduction in regenerative capacity with progression of neuropathy severity.25 Popper et al26 demonstrated that the number of fibers in the corneal sub-basal nerve plexus of diabetic patients, even with short diabetes duration, was significantly lower than that in...
healthy control subjects. Kallinikos et al. used corneal confocal microscopy to quantify corneal sub-basal nerve plexus tortuosity in diabetic and healthy subjects. They demonstrated increased corneal sub-basal nerve plexus fiber tortuosity (which was independent of age, duration of diabetes, and glycemic control) in diabetic patients with increasing severity of peripheral diabetic neuropathy. They hypothesized that the increased tortuosity may represent a morphologic marker of nerve regeneration.

Previous studies have not considered all corneal sub-basal nerve plexus parameters in the same group of diabetic patients versus control subjects. Data from the current researchers show that diabetic patients have significant corneal sub-basal nerve plexus parameter changes compared with control subjects. Corneal sub-basal nerve plexus alterations do not depend on glycemic control, age, or duration of diabetes. In diabetic eyes, the number of corneal sub-basal nerve fibers was significantly reduced, and the remaining fibers were altered. The increase of tortuosity of visible fibers is a sign of degeneration and an attempt at fiber repair. Even lower corneal sub-basal nerve plexus branching may signal reduced nerve regeneration. The metabolic activity of the corneal sub-basal nerve plexus is documented by nerve beadings, which represent accumulation of mitochondria along the nerve. The significant decrease of nerve beadings represents pathologic metabolic activity of diabetic small nerve fibers (Fig 6). Peripheral diabetic neuropathy “classified” with the MNSI shows contrasting results when compared with confocal microscopy corneal sub-basal nerve plexus data. Data from the current researchers showed that not all corneal sub-basal nerve plexus parameters were significantly different between controls and all MNSI score groups. However, this probably reflects the “screening” value of the MNSI, which cannot be used as a specific grading score of peripheral diabetic neuropathy.

These clinical findings may also be useful when interpreting refractive surgery results, particularly in diabetic patients. In eyes treated with photorefractive keratectomy (PRK), the recovery of subepithelial corneal nerves starts from the borders of the ablated zone in a centripetal way. Corneal nerve regeneration is considered complete in 12 months, although the original nerve structure is not fully restored, and corneal sub-basal nerve plexus is similar (abnormal branching and increased nerve tortuosity) to that observed in the diabetic patients we examined. After LASIK, corneal nerve regeneration is slower, approximately 2 years, because larger nerve trunks have been severed by the microkeratome. The relationship between corneal nerves and keratocytes is disrupted after refractive surgery. Mueller et al. have shown that there is a direct innervation of individual keratocytes, whereas Vesaluoma et al. suggested that corneal denervation may play a role in reducing the density of keratocytes in central ablated cornea. The long-term consequences of corneal denervation and regeneration after refractive surgery are relatively unknown, and the impact of diabetes on this process is poorly documented. Future controlled trials evaluating the efficacy and safety of refractive surgery (LASIK and PRK) in diabetic patients should consider baseline corneal nerve status as a relevant parameter to stratify tested groups. Even dry eye syndrome before and after refractive surgery should be reconsidered according to the status of corneal nerves, particularly in diabetic patients.
The present investigators are confident that corneal sub-basal nerve plexus morphologic parameters directly documented using in vivo corneal confocal microscopy is the clinical tool of choice for evaluating small nerve fiber damage in diabetic patients. The correlation observed between corneal nerve alterations and peripheral diabetic neuropathy, even using a screening test, confirms that the in vivo evaluation of sub-basal corneal nerve plexus allows an improved diagnosis of peripheral diabetic neuropathy. The investigators aim to compare corneal sub-basal nerve plexus corneal confocal microscopy results with peripheral diabetic neuropathy classification using skin biopsy, which is an invasive morphologic test.  

Corneal confocal microscopy in vivo is a noninvasive test for peripheral diabetic neuropathy, which causes decreased corneal sensitivity and neurotrophic damage to the ocular surface. In the future, corneal diabetic neuropathy should be routinely documented with corneal confocal microscopy using a specific, universal grading system.

REFERENCES