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Differential reduction in corneal nerve fiber length in patients with type 1 or type 2 diabetes mellitus

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Abstract

Aim—To examine the relationship between corneal nerve fiber length (CNFL) and diabetic neuropathy (DN) status in patients with type 1 or type 2 diabetes mellitus (DM)

Methods—In this cross-sectional study, we examined 25 diabetic patients without DN, 10 patients with mild DN, 8 patients with severe DN, and 9 controls without diabetes. DN status was assigned based on a combination of clinical symptoms, signs, and electrophysiological testing. Patients underwent corneal confocal microscopy (CCM) of the sub-basal nerve plexus. Post-hoc analysis of the CCM images was performed to quantify the average CNFL, and ANOVA was used to assess for differences in CNFL.

Results—All 25 subjects without DN had type 1 DM, and subjects with DN had type 2 DM. Participants with severe DN had significantly lower CNFL (12.5 ± 6.1 mm/mm²) compared to controls (20.7 ± 2.2 mm/mm²) ($p=0.009$). However, lower CNFL was also found in participants with type 1 DM who did not have DN (15.1 ± 4.7 mm/mm²) relative to controls ($p=0.033$).

Conclusions—CCM of the sub-basal nerve plexus may be an indicator of early peripheral nerve degeneration in type 1 DM. Type of diabetes, in addition to degree of neuropathy, may influence the extent of corneal nerve damage.

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Keywords

Corneal confocal microscopy; diabetes mellitus; neuropathy; corneal nerves

INTRODUCTION

Diabetic neuropathy (DN) is a leading form of neuropathy in the western world, and affects up to 50% of patients with diabetes.¹ In spite of continuous efforts, multiple agents targeting pathogenic mechanisms to reverse DN have failed in human trials. One explanation for the failure of most DN clinical trials is related to the complex anatomy of the peripheral nervous system and heterogeneous spectrum of clinical manifestations of DN. Numerous diagnostic and outcome measures have been used to assess therapeutic efficacy or monitor for disease progression in clinical trials, contributing to poor reproducibility and inconclusive findings.² In addition, nerve conduction studies (NCS) considered for many years as “gold standard” diagnostic criteria provide information solely on large fiber dysfunction. The more recent use of intraepidermal nerve fiber density (IENFD) to assess small fibers³ is limited by its invasiveness.⁴ Given these limitations, there is a need for a more optimal measure of neuropathy severity that combines the best attributes from these other tests into a single metric.

Several groups of investigators have reported that corneal confocal microscopy (CCM) of the sub-basal nerve plexus provides a non-invasive, repeatable⁵ method of quantifying small fiber nerve damage from diabetes,^{3,6–12} and that it can discriminate between controls, patients with diabetes and no neuropathy, and diabetic patients with neuropathy.¹³ Furthermore, it was suggested that CCM may detect increased corneal nerve density¹⁴ after simultaneous pancreas and kidney transplantation before improvements can be detected by traditional measures of peripheral neuropathy severity.¹⁵ Taken together, these findings suggest that CCM may be a useful indicator of neuropathy severity that can be used to assess therapeutic efficacy in clinical trials. Indeed, examination of the corneal sub-basal nerve plexus has already been used to assess therapeutic efficacy of a vasopeptidase inhibitor in treating DN in an animal model of diabetes.¹⁶

Despite the promising role that CCM can play in stratifying patients with DN according to the stage of their disease, it remains unclear whether type of diabetes, in addition to stage of neuropathy, influences corneal nerve density. In the present study, we used CCM to examine the relationship between CNFL and DN status in patients with type 1 or type 2 diabetes mellitus (DM).

MATERIALS AND METHODS

Patients

Four groups of participants were recruited for this study: healthy controls, diabetic patients without DN, patients with mild DN, and those with severe DN. The main inclusion criteria were age ≥ 18 years and presence of diabetes as defined by the American Diabetes Association. Exclusion criteria for all participants were: 1) any systemic neuropathy other

than DN; 2) history of corneal disease or any eye surgery; 3) any neurodegenerative condition, such as Parkinson's disease or multiple sclerosis; 4) history of stroke or cancer; 5) history of spinal stenosis or previous back surgery; 6) not willing/able to provide consent.

This study was approved by the University of Michigan Institutional Review Board and complied with the tenets of the Declaration of Helsinki. Written informed consent was obtained from each subject prior to testing.

Diabetic neuropathy severity

Mild DN was defined as: the presence of 1 abnormal attribute (of amplitude, latency, F-wave, or nerve conduction velocity) in 2 separate nerves among the median, peroneal, and sural nerves in NCS studies, and the presence of small fiber dysfunction as evaluated by neurological examination performed by a board certified neurologist and by abnormal quantitative sensory testing (QST).^{17,18}

Severe DN was defined as: a combination of symptoms and signs of distal sensorimotor polyneuropathy with two or more of the following: neuropathic symptoms, decreased distal sensation (as assessed by neurological examination performed by a board certified neurologist and by abnormal QST), and unequivocally decreased or absent ankle reflexes plus abnormalities in NCS as described above and absence of a recordable sural nerve amplitude.

Corneal Confocal Microscopy

All subjects underwent CCM imaging of the central corneal sub-basal nerve plexus using the Heidelberg Retina Tomograph-2 (HRT-2) Rostock cornea module (Heidelberg Engineering, Heidelberg, Germany). After instilling one drop of topical proparacaine hydrochloride 0.5% into the right eye, the patient placed his/her head into the headrest of the confocal microscope. A small amount of Genteal eye gel (Novartis Pharmaceuticals, East Hanover, NJ) was applied to the lens of the microscope, and a sterile plastic cap (Tomocap, Heidelberg Engineering, Heidelberg, Germany) was placed over the lens. The patient was instructed to look straight ahead as the plastic-covered lens contacted the patient's cornea. "Sequence" mode was used to record consecutive $400 \times 400 \mu\text{m}$ images of the sub-basal nerve layer at 8 frames per second.

Analysis of the corneal nerve images began with selection of five representative, focused, non-overlapping images of the sub-basal nerve plexus from the central cornea from each participant. A masked grader (NR) used NeuronJ software (publicly available at <http://www.imagescience.org/meijering/software/neuronj/>) to calculate the sum length of all nerve fibers within each image. From this number, the CNFL was derived by dividing the sum length of the nerve fibers in each image by the area of the image ($1600 \mu\text{m}^2$). The five CNFL measurements from each individual were averaged to determine the final CNFL per patient.

Statistical Analysis

Means and standard deviations were calculated for continuous variables, and frequencies and percentages were used for categorical variables. We used analysis of variance (ANOVA) to compare the average CNFL measurements obtained from each group.

RESULTS

Patient Characteristics

This study included 25 diabetic patients without DN, 10 patients with mild DN, 8 patients with severe DN, and 9 controls without diabetes. Participant characteristics are listed in Table 1. All of the patients without DN had type 1 DM, and patients with mild or severe DN had type 2 DM. On average, individuals without DN were younger than those with mild ($p < 0.001$) or severe ($p = 0.019$) DN. 72% of patients without DN were female, and 20% of those with mild DN and 12.5% of individuals with severe DN were female ($p = 0.002$). There were no statistically significant differences in duration of diabetes or hemoglobin A1c (HbA_{1c}) values among the three groups, but those with type 1 DM tended to have a longer duration of DM compared to patients with type 2 DM and either mild or severe DN ($p = 0.058$).

Corneal Nerve Fiber Length

Figure 1 depicts representative CCM images from a participant within each group. Patients with type 2 DM and severe DN had fewer corneal nerves (CNFL $\pm$ 95% confidence interval ¹⁹ = 12.5 ± 5.1 mm/mm²) compared to controls (20.7 ± 1.7 mm/mm²) ($p=0.009$), and those with type 1 DM without DN also had lower CNFL (15.1 ± 1.9 mm/mm²) relative to controls ($p=0.033$) (Figure 2). However, individuals with mild DN did not exhibit significantly lower CNFL compared to controls ($p=1.000$).

DISCUSSION

The goal of this cross-sectional study was to determine whether type of diabetes influences the level of corneal nerve loss across the spectrum of diabetic neuropathy. Using CCM, we found that patients with type 1 DM and no DN had 27% lower CNFL relative to controls, but patients with type 2 DM and mild DN did not have lower CNFL compared to controls. Patients with type 2 DM and severe DN however, exhibited 40% lower CNFL relative to the control group.

Our finding that patients with severe DN have lower levels of corneal nerves compared to controls is consistent with several other reports of corneal nerve loss in DN.^{6,20} However, unlike recent studies which showed a progressive loss of CNFL across the disease spectrum,^{13,21} we found that patients with type 1 DM who did not meet any conventional criteria for DN exhibited lower CNFL relative to patients with type 2 DM who had mild DN, in spite of equivalent HbA_{1c} levels. Importantly, the tools that we used to stratify the participants into different neuropathy subgroups are the same tools other investigators have used to categorize patients according to neuropathy severity,^{13,21} so it seems unlikely that neuropathy group assignment error could account for our different results. Furthermore,

gender does not appear to influence corneal nerve density, so it is unlikely that differences in gender distribution between the groups would account for our observations.²² Studies evaluating the effects of age on corneal innervation have yielded mixed results, with one study showing no effect of age on corneal nerve density²³ and another finding that increasing age is correlated with reduced corneal innervation.²² In either case, it is unlikely that differences in age among the neuropathy subgroups would account for our results because the patients with DM but no neuropathy were younger, on average, than patients with mild DN or severe DN. Diabetes duration, however, is inversely correlated with CNFL,¹³ and in our study, patients with type 1 DM tended to have a longer duration of DM compared to patients with type 2 DM and mild DN. It is possible that the effect on CNFL that was observed in the type 1DM patients is rather due to their longer exposure to hyperglycemia than to their specific type of DM. However, given that type 2 DM patients may be completely asymptomatic for many years prior to a formal diabetes diagnosis it is likely that the duration is grossly underestimated in patients with type 2 DM.²⁴ Thus, it seems reasonable to attribute the differences in CNFL between the no DN and mild DN subgroups to differences in type of diabetes rather than differences in DM duration.

There are several potential explanations for our finding that type of DM appeared to influence the extent of corneal nerve damage among the study participants. The fundamental difference in pathophysiology between type 1 and type 2 DM could theoretically account for the discrepancy. That is, in type 1 DM there is a systemic lack of insulin while in type 2 DM insulin abounds but its target cells are resistant to its effects. As insulin has been shown to be important for neuronal growth and survival,²⁵ the differences in insulin production/utilization between type 1 and type 2 DM could influence corneal nerve degeneration in disparate ways.

This study, while small in scope, had several strengths. First, subjects were extensively characterized with neurophysiologic testing, clinical exams, and patient history in a standardized fashion, which ensured that all participants were placed into appropriate neuropathy categories based on disease severity. Second, measurements of CNFL using NeuronJ were done in a masked fashion by the same person in all of the study participants, which eliminates potential bias in image assessment.

Despite the strengths of this study, several limitations need to be acknowledged. This study involved a small number of patients, so definitive conclusions about the effect of type of diabetes on corneal nerve destruction cannot be drawn. Also, we did not enroll any patients with type 2 DM without DN or any individuals who had type 1 DM with neuropathy. Lastly, we were unable to control for potential confounding factors in this study, such as duration of diabetes.

In conclusion, the results from this cross-sectional study suggest that type of diabetes may influence the extent of corneal nerve loss in patients with DN. Specifically, CCM measurements of CNFL may be a more sensitive indicator of early nerve damage in patients with type 1 DM compared to those with type 2 DM. The results of this study, if confirmed in subsequent investigations, have important implications for the use of CCM in the detection of DN. For example, the diagnostic threshold of CNFL used to detect DN might be different

for type 1 DM compared to type 2 DM (lower for type 1, and higher for type 2). While CCM shows promise as a technique for assessing DN severity and progression, it will need to be more rigorously tested in larger cohorts of patients with both types of diabetes, and across multiple centers to more fully understand the implications of this outcome measure.

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Figure 1.

Representative images of the corneal sub-basal nerve plexus from patients with varying degrees of diabetic neuropathy. The tortuous white lines in each picture are the corneal nerves, and the small white dots are Langerhans cells. Participants with diabetes and no neuropathy and those with diabetes and severe neuropathy appear to have fewer corneal nerves compared to controls. Abbreviations: T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; DN, diabetic neuropathy.

Figure 2.

Average corneal nerve fiber length (CNFL) for each group $\pm$ 95% confidence interval. CNFL is defined as the sum length of all corneal nerves within the image divided by the area of the image (0.16 mm^2). Patients with type 1 diabetes and no neuropathy ($n=25$) and those with type 2 diabetes and severe neuropathy ($n=8$) had, on average, fewer nerves per area of cornea relative to controls ($n=9$). Patients with type 2 diabetes and mild DN ($n=10$) had a similar CNFL compared to controls. The maximum/minimum CNFL values were as follows: Control ($24.4/17.6$), No DN ($24.5/6.5$), Mild DN ($29.1/12.1$), and Severe DN ($19.4/1.2 \text{ mm}$). Abbreviations: DN, Diabetic Neuropathy; mm, millimeters. Symbols: *, $p < 0.05$ vs. Control; #, $p < 0.05$ vs. Mild DN.

Participant characteristics according to severity of diabetic neuropathy. Abbreviations: DN, Diabetic Neuropathy; DM, Diabetes Mellitus; HbA1c, Hemoglobin A1c; SD, Standard Deviation; T1DM, Type 1 Diabetes Mellitus; T2DM, Type 2 Diabetes Mellitus.

Table 1

	CONTROLS	NO DN (T1DM)	MILD DN (T2DM)	SEVERE DN (T2DM)	P-value
Total participants, n (%)	9 (17.3)	25 (48.1)	10 (19.2)	8 (15.4)	
Age, mean (SD)	43.9 (10.2)	38.7 (14.2)	57.7 (8.1) [†]	53.4 (8.0) [†]	p < 0.001 [‡]
HbA1c, mean % (SD) [mmol/mol]	5.4 (0.2) [35.5]	8.1 (1.0) [*] [65.0]	7.9 (1.1) [*] [62.8]	8.3 (1.5) [*] [67.2]	p < 0.001 [‡]
Duration of DM in years, mean (SD)	-	13.5 (6.7)	7.6 (3.8)	10.6 (3.6)	p = 0.058 [‡]
Sex, n (%)					p = 0.002 [§]
Males	3 (33.3)	7 (28.0)	8 (80.0)	7 (87.5)	
Females	6 (66.7)	18 (72.0)	2 (20.0)	1 (12.5)	
Type of Diabetes, n (%)					p < 0.001 [§]
Type 1	-	25 (100)	0 (0)	0 (0)	
Type 2	-	0 (0)	10 (100)	8 (100)	

^{*} p < 0.05 vs. Control

[†] p < 0.05 vs. No DN

[‡] ANOVA

[§] Chi-square test