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Chronic Migraine Is Associated With Reduced Corneal Nerve Fiber Density and Symptoms of Dry Eye

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Abstract

Background—We used *in vivo* corneal confocal microscopy to investigate structural differences in the sub-basal corneal nerve plexus in chronic migraine patients and a normal population. We used a validated questionnaire and tests of lacrimal function to determine the prevalence of dry eye in the same group of chronic migraine patients. Activation of the trigeminal system is involved in migraine. Corneal nociceptive sensation is mediated by trigeminal axons that synapse in the gasserian ganglion and the brainstem, and serve nociceptive, protective, and trophic functions.

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Noninvasive imaging of the corneal sub-basal nerve plexus is possible with *in vivo* corneal confocal microscopy.

Methods—For this case–control study, we recruited chronic migraine patients and compared them with a sex- and age-similar group of control subjects. Patients with peripheral neuropathy, a disease known to be associated with a peripheral neuropathy, or prior corneal or intraocular surgery were excluded. Participants underwent *in vivo* corneal confocal microscopy using a Heidelberg Retinal Tomography III confocal microscope with a Rostock Cornea Module. Nerve fiber length, nerve branch density, nerve fiber density, and tortuosity coefficient were measured using established methodologies. Migraine participants underwent testing of basal tear production with proparacaine, corneal sensitivity assessment with a cotton-tip applicator, measurement of tear break-up time, and completion of a validated dry eye questionnaire.

Results—A total of 19 chronic migraine patients and 30 control participants completed the study. There were no significant differences in age or sex. Nerve fiber density was significantly lower in migraine patients compared with controls (48.4 ± 23.5 vs 71.0 ± 15.0 fibers/mm^2, $P < .001$). Nerve fiber length was decreased in the chronic migraine group compared with the control group, but this difference was not statistically significant (21.5 ± 11.8 vs 26.8 ± 5.9 mm/mm^2, $P < .084$). Nerve branch density was similar in the two groups (114.0 ± 92.4 vs 118.1 ± 55.9 branches/mm^2, $P < .864$). Tortuosity coefficient and log tortuosity coefficient also were similar in the chronic migraine and control groups. All migraine subjects had symptoms consistent with a diagnosis of dry eye syndrome.

Conclusions—We found that in the sample used in this study, the presence of structural changes in nociceptive corneal axons lends further support to the hypothesis that the trigeminal system plays a critical role in the pathogenesis of migraine. *In vivo* corneal confocal microscopy holds promise as a biomarker for future migraine research as well as for studies examining alterations of corneal innervation. Dry eye symptoms appear to be extremely prevalent in this population. The interrelationships between migraine, corneal nerve architecture, and dry eye will be the subject of future investigations.

Keywords

migraine; corneal nerves; *in vivo* corneal confocal microscopy; dry eye syndrome

Migraine pain is thought to arise from activation of the trigeminovascular network, whose primary sensors are cranial nerve V afferents in the dura, cranium, face, and eye, modulated by vascular and autonomic perturbations as well as feedback from higher structures in the network.[1] Abnormalities in trigeminal afferent nerves may contribute to the experience of eye pain in migraine sufferers. Symptoms of dry eye also are thought to be due to activation of the sensory trigeminal nerves of the cornea.[2]

The nerves that comprise the sub-basal corneal nerve plexus from the first division of the trigeminal nerve run parallel to the corneal surface. Their axons synapse in the brainstem and subserve nociceptive, protective, and trophic functions.[3,4] The sub-basal corneal nerve plexus is a dynamic structure[5] that is similar between the right and left eyes.[6]

In vivo corneal confocal microscopy (ICCM) noninvasively images the corneal sub-basal nerve plexus, which consists of nociceptive afferents of the first division of the trigeminal nerve. ICCM is a reproducible and noninvasive method for viewing the cornea.[4,5,7,8]

Alteration of corneal innervation could precipitate or contribute to migraine, especially in patients with concurrent dry eye, as the sensation of dry eye could activate the trigeminal system. Alternatively, abnormal corneal nerves in chronic migraineurs could lead to a sensation of dry eye. The objective of this study was to determine if there are structural differences in the sub-basal corneal nerve plexus between chronic migraine patients and a normal population. We also investigated the prevalence of dry eye symptoms and signs in the same group of patients with chronic migraine.

METHODS

The University of Utah Institutional Review Board approved this case–control study, and all participants provided written informed consent. Chronic migraine patients were prospectively recruited from the clinics of the investigators between July 1, 2013 and December 31, 2013. Previous studies of the sub-basal corneal nerves in our laboratory have indicated that a study size of 15–20 participants is typically required to power a study for comparisons of nerve fiber length (NFL), nerve fiber density (NFD), nerve branch density (NBD), and tortuosity coefficient (TC). All authors had full access to all study data.

Eligibility criteria included presence of chronic migraine (at least 15 headache days per month), which was defined based on the International Headache Society guidelines for chronic daily migraine.[9] Patients and control participants with symptoms or signs of peripheral neuropathy, use of medications known to cause peripheral neuropathy, corneal disease, or prior corneal or intraocular surgery were excluded. We did not exclude migraine subjects using medications that may be associated with dry eye (eg, tricyclic antidepressants, onabotulinum toxin).

Study procedures took place at the Department of Ophthalmology and Visual Sciences, John A. Moran Eye Center and the Department of Neurology, University of Utah Health Sciences Center, Salt Lake City, Utah. Medical and ocular histories, body mass index, and current medications were obtained from all participants. All participants underwent ICCM. NFL, NFD, NBD, and TC were measured using an established methodology.[5] Each participant with migraine also underwent measurement of basal tear secretion using Schirmer's test after instillation of proparacaine, corneal sensitivity assessment with a cotton-tip applicator (CTA), and measurement of tear break-up time (TBUT). Subjects also completed the Dry Eye Questionnaire (DEQ) 5, a validated symptom questionnaire for dry eye.[10]

ICCM was performed using a Heidelberg Retinal Tomography III confocal microscope with a Rostock Cornea Module (HRT III RCM) (Heidelberg Engineering GmbH, Heidelberg, Germany). The HRT III RCM utilizes a 670 nm class 1, red wavelength diode laser. A 63× objective lens with a numerical aperture of 0.9 also was used. The dimensions of each image were 300 × 300 μm, and the manufacturer-quoted transverse resolution and optical section

thickness were 2 and 4 μm, respectively.[5] Masked observers confirmed the quality of images prior to image analysis.

Imaged eyes were anesthetized with one drop of 0.5% proparacaine, and Systane gel (Novartis Ophthalmics, New York, NY, USA) was used as a coupling agent between the applanating lens cap and the laser. After the Systane gel was placed in the superior and inferior fornix, the participant blinked in order to spread the gel evenly over the corneal surface. Both the microscope and fixation light were adjusted so the participant could fixate on the white light.[5] Images were collected from four standardized locations on the central cornea of the left eye, and the central cornea of the right eye. Whenever possible, ICCM values were based on the mean of the four images from the left eye. When there were insufficient high-quality images from the left eye, the mean of the central image from the two eyes were used. This analysis paradigm has been validated and found to have acceptable reliability.[5,8]

Semi-automated analytical software (CCMetrics Image Analysis tool version 1.1, CCMetrics, Manchester, UK) was used to obtain measurements of NFD, NBD, NFL, and TC. All nerve fibers in each image were manually traced and branching points marked using a tracing pad (Bamboo Pen and Touch CTH460, Wacom, Saitama, Japan; the Figure). These four parameters were measured using the following assessment methods:NFL (mm/mm^2): measured by tracing the nerve fibers and multiplying by a conversion factor (0.00075 mm) to account for the pixel height and width and then by dividing by the area of the scan; NBD ($branches/mm^2$): measured by counting the branches in the scan field and then dividing by the area of the scan;NFD ($fibers/mm^2$): calculated by the software by detecting nerve branches marked on the manual tracings;TC (unit-less measure): another metric that was calculated by the software and that roughly reflects the degree of curvature of the nerve branches.

Controls

ICCM data collected from the migraine subjects were compared with data collected from a sex- and age-similar control participants selected from an ICCM normative dataset that has been collected over the period 2010–2013. A total of 30 control participants of similar age and sex were selected from the dataset by constraining the extremes of age and confirming similar age and sex distribution to the migraine cohort. This normative dataset has been established by the authors to be used in a number of case–control investigations of the sub-basal corneal nerve plexus. Data collected from some of these control subjects have been used in previously published work from our laboratory and may be used in future investigations.[5,8,11] Control subjects did not undergo measurement of basal tear secretion, corneal sensitivity, or TBUT. Control subjects did not complete the DEQ5.

Statistical Analyses

SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) was used for analyses of quantitative variables. We used unpooled two-sample *t*-tests for comparisons of NFD, NFL, and NBD. The Wilcoxon-signed rank sum test was used for comparisons of TC and the log

transformation of TC. All comparisons were performed without formal adjustment for multiple testing. A *P* value less than .05 was considered statistically significant.

RESULTS

All enrolled participants completed the study procedures and were included in the analyses. There were 19 chronic migraine patients enrolled, and their data were compared with 30 sex- and age-similar control participants. The mean age of the 14 female and 5 male participants with migraine was 38.6, and the mean age of the 18 female and 12 male control participants was 44.7. These differences in age and gender were not statistically significant.

Results are summarized in the Table. NFD was significantly lower in migraine patients compared with controls (48.4 ± 23.5 vs 71.0 ± 15.0 fibers/mm^2, $P < .001$). NFL was decreased in the chronic migraine group compared with the control group, but this difference did not meet statistical significance (21.5 ± 11.8 vs 26.8 ± 5.9 mm/mm^2, $P < .084$). NBD was similar in the migraine group compared with controls (114.0 ± 92.4 vs 118.1 ± 55.9 branches/mm^2, $P < .864$). TC and log transformation of TC were also similar in the chronic migraine group and control group.

The mean DEQ5 score in the migraine group was 8.3 (±3.6) and all 19 migraine subjects had DEQ5 scores greater than 6. Basal tear secretion, corneal sensitivity, and TBUT were normal in all 19 migraine subjects.

DISCUSSION

ICCM studies have been helpful in understanding several eye conditions. Quantitative ICCM studies in normal patients have shown a possible decline in nerve density with age, but results of these studies are quite variable.[3] Dry eye patients appear to have structural alterations in sub-basal corneal nerves including lower density and increased tortuosity.[12] ICCM studies have also shown changes of the corneal nerve plexus in patients with systemic diseases. The best-studied patient population so far has been patients with diabetes. Diabetic neuropathy patients show decreased nerve density and increased tortuosity as well as a correlative decrease in corneal sensitivity.[3] Zhivov et al found a decrease in corneal sensitivity that correlated with a decrease in sub-basal nerve plexus density and abnormal morphology.[13] Two groups have shown that ICCM may be used as a noninvasive means of detection early peripheral neuropathy in patients with diabetes.[14_16]

In this present study, we found that NFD was statistically reduced in our chronic migraine patients compared with nonmigraine control participants. We observed decreased NFL in our chronic migraine patients, but this observation was not statistically significant. Our results are similar to those in patients with diabetic peripheral neuropathy.[13] The presence of structural changes in nociceptive corneal axons supports the role of the trigeminal system in migraine. These results raise the possibility that structural changes in the trigeminal nerve system may play a role in the pathophysiology of migraine. At this time, it is not possible to tell if migraine causes these structural changes or if these structural changes lead to migraine by chronic stimulation of the first division of the trigeminal nerve.

Because the trigeminal system is intimately involved in the lacrimal function unit,[2–4] we simultaneously looked for signs and symptoms of dry eye in our migraine subjects. Estimates of the prevalence of dry eye are highly variable due to differences in methodology. The best data available indicate that the prevalence of dry eye syndrome lies somewhere between 5% and 30% in patients aged 50 years and over.[17] We assessed dry eye symptoms with the DEQ5. The first two questions of the DEQ5 ask subjects about ocular discomfort ("How often do your eyes feel discomfort?" and "When your eyes felt discomfort, how intense is this feeling of discomfort at the end of the day?"). Because some migraine patients may experience headache pain in the eyes or orbits, our migraine subjects were asked to try to interpret these questions as asking about ocular irritation, rather than ocular pain. Nevertheless, these two questions may be a source of confounding in a cohort of migraine patients. The final question of the DEQ5 asks about excess tearing ("How often did your eyes look or feel excessively watery?"). Although tearing may occur as a trigeminal autonomic symptom, the authors believe that this symptom is infrequently encountered in migraine patients and should therefore not be a source of confounding in a cohort of migraine patients.

In a control population, the DEQ5 score is approximately 2.7; a DEQ5 score greater than 6 is considered to be consistent with a diagnosis of dry eye syndrome; a score greater than 12 is suspicious for Sjogren's syndrome.[10] All 19 of the migraine subjects had DEQ5 scores greater than 6, and the mean DEQ5 score in the migraine group was 8.3. These subjects had normal tear secretion testing and TBUT, indicating that they are unlikely to have severe dry eye syndrome or Sjogren's syndrome. This result indicates that the prevalence of dry eye symptoms in chronic migraine may be more than three times than that in a normal population. Because of the overwhelming prevalence of dry eye in this population, consideration should be given to treating dry eye in symptomatic patients. Patients with dry eye often report burning, tearing, ocular discomfort, foreign body sensation, and visual blurring. Typical treatments include topical over-the-counter artificial tears, topical cyclosporine, oral flaxseed oil supplementation, and punctual occlusion. Although treatment of dry eye is relatively safe and easy, it is unknown whether addressing dry eye symptoms will improve migraine symptoms and it is not possible for us to determine if the dry eye symptoms reported by migraine patients could be a result of abnormal trigeminal nerve function.

The corneal nerve findings and dry eye symptoms we have described may either be a result of the migraine process, or the symptoms may lead to chronic migraine by chronic stimulation of the first division of the trigeminal nerve. Additional studies will be needed to further evaluate these possibilities.

CONCLUSION

The presence of structural changes in nociceptive corneal axons lends further support to the hypothesis that the trigeminal system plays a critical role in the pathogenesis of migraine. Based on our observations of this study population, the authors propose that ICCM has the potential to serve as a biomarker for future migraine research as well as for studies examining alterations of corneal innervation. Furthermore, dry eye symptoms appeared to be

extremely prevalent in this population. The interrelationships between migraine, corneal nerve architecture, and dry eye will be the subject of future investigations.

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Semi-automated analytical software (CCMetrics Image Analysis Tools version 1.1) was provided courtesy of Dr. Mohammad A. Dabbah (University of Manchester, UK). Ms. Susan Schulman (University of Utah School of Medicine) assisted with manuscript preparation.

Abbreviations

ICCM	*in vivo* corneal confocal microscopy
NFL	nerve fiber length
NFD	nerve fiber density
NBD	nerve branch density
TC	tortuosity coefficient
CTA	cotton-tip applicator
TBUT	tear break-up time
DEQ	Dry Eye Questionnaire
HRT III RCM	Heidelberg Retinal Tomography IIIRostock Cornea Module

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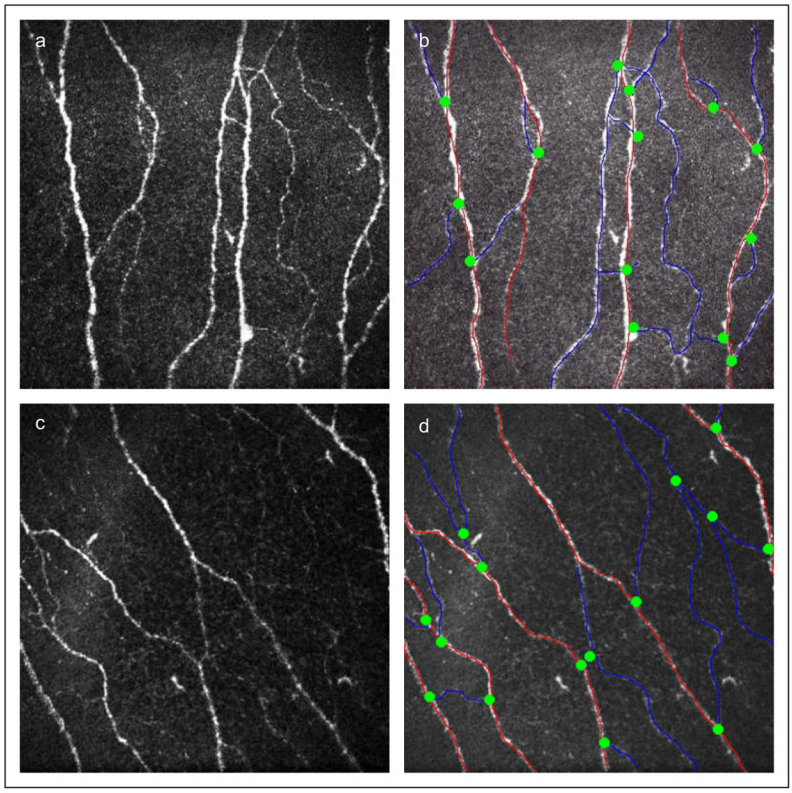

Figure.

Representative *in vivo* corneal confocal microscopy (ICCM) images obtained with the Heidelberg Retinal Tomography III, Rostock Cornea Module with 670 nm diode laser, 300 × 300 μm images and aperture of 0.9. (a) Image of the sub-basal nerve plexus in a control subject. (b) Analysis of the same image of the same control subject. Red = main nerve fibers; blue = branches; green = branch points. (c) Image of the sub-basal corneal nerve plexus in a chronic migraine subject. (d) Analysis of the same image from the same migraine subject.

Table

A Comparison of Sub-Basal Corneal Nerve Fiber Parameters Measured in 19 Chronic Migraine Patients and 30 Control Subjects

	Migraine (N = 19)				Control (N = 30)				
	Mean (SD)	**25th**	**Median**	**75th**	**Mean (SD)**	**25th**	**Median**	**75th**	***P* value***
Nerve fiber density (NFD)	48.4 (23.5)	27.8	44.5	66.7	71.0 (15.0)	61.1	72.2	83.3	<.001
Nerve fiber length (NFL)	21.5 (11.8)	11.7	23.2	30.6	26.8 (5.9)	22.7	27.3	30.0	<.084
Nerve branch density (NBD)	114.0 (92.4)	41.7	95.8	163.9	118.1 (55.9)	77.8	105.6	150.0	<.864
Tortuosity coefficient (TC)	0.14 (0.09)	0.03	0.16	0.23	0.16 (0.03)	0.14	0.17	0.18	<.798
Log tortuosity coefficient	−2.25 (0.91)	−3.10	−1.76	−1.49	−1.84 (0.20)	−1.97	−1.77	−1.71	<.975

*Two-sample *t*-test used for NFD, NFL, and NBD.

Wilcoxon rank sums test used for TC and the log transformation of TC.

SD, standard deviation.