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Effects of Corneal Nerve Density on the Response to Treatment in Dry Eye Disease

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Purpose: To evaluate whether levels of corneal subbasal nerve fiber length (SNFL) in dry eye disease (DED) could prognosticate the level of improvement in signs and symptoms after treatment.

Design: Phase IV, double-masked, randomized clinical trial.

Participants: Sixty patients with meibomian gland dysfunction–associated DED and 27 age-matched controls.

Methods: Patients with DED were randomized to receive topical artificial tears, loteprednol etabonate 0.5%, or loteprednol etabonate 0.5%/tobramycin 0.3% twice daily for 4 weeks. At baseline, in vivo confocal microscopy of central cornea was performed in both eyes. Patients with DED were divided into 2 subgroups: those with low baseline SNFL and those with near-normal baseline SNFL for this purpose (the cutoff point: the mean SNFL in controls minus 2 standard deviations). Clinical signs and symptoms at baseline and after 4 weeks of treatment were compared between the subgroups with low and near-normal SNFL for all therapeutic groups.

Main Outcome Measures: Symptom questionnaires, corneal fluorescein staining (CFS), conjunctival staining with lissamine green, tear break-up time, Schirmer's test, and SNFL.

Results: In patients with DED, baseline SNFL (17.06 ± 5.78 mm/mm^2) was significantly lower than in controls (23.68 ± 3.42 mm/mm^2, $P = 0.001$). In the artificial tear and loteprednol groups, although no significant improvement in any sign or symptom was noted in patients with low baseline SNFL (<16.84 mm/mm^2), subjects with near-normal baseline SNFL (≥16.84 mm/mm^2) showed significant improvement in both symptoms and CFS score (all $P < 0.05$). In the loteprednol/tobramycin group, no significant change was evident for any sign or symptom in either subgroup of low or near-normal baseline SNFL.

Conclusions: Significant improvements in CFS and patient symptomatology after DED treatment were evident only in the subgroup with near-normal corneal SNFL. Consideration of SNFL may assist in explaining the variability of patients' response to DED therapy. *Ophthalmology 2015;■:1–7 © 2015 by the American Academy of Ophthalmology.*

Dry eye disease (DED) is one of the most commonly encountered conditions in ophthalmic practice. It is estimated that more than 5% to 30% of the population aged 50 years or older have DED, with a higher prevalence in some regions of the world, such as Asia.[1–3] Although various subtypes of DED exist, such as aqueous tear deficient and evaporative subtypes, the common denominator of the disease is tear film instability and ocular surface inflammation.[4,5] This fact has clearly been reflected in the new definition of DED by the Dry Eye WorkShop in 2007.[6] Because of the role of inflammation in the pathogenesis of DED, anti-inflammatory agents are commonly used for treatment of DED,[7] although with variable degrees of success.[8,9] However, it remains unknown why not all patients with DED respond favorably to anti-inflammatory therapy.

The cornea is the most densely innervated tissue in the body with a nerve density of 300 to 600 times that of the skin.[10,11] A large number of studies have demonstrated that in addition to providing sensation, corneal nerves play a significant role in the maintenance of corneal epithelial health.[11] These nerves, which promote epithelial proliferation and viability,[12] have been shown to be reduced in a variety of ocular and systemic conditions, resulting in compromised ocular surface and reduced tear function.[13,14] Examples include corneal insults (e.g., infections, injuries, or surgeries), systemic disease (e.g., diabetes mellitus), and any damage to the trigeminal nerve.[11,13,14] In addition to these conditions, the density of corneal nerves has been shown to be reduced in DED,[15–22] which correlates with ocular surface staining.[22] However, it remains unclear whether the density of corneal nerves at the time of treatment initiation plays any role in the therapeutic response in patients with DED.

Because corneal nerves are required for the maintenance of ocular surface health,[11,12] their trophic function may be important not only in normal conditions but also in disease states. Therefore, in this study we hypothesized that the response to treatment in patients with DED is dependent on the presence of near-normal corneal nerve density, and thus variable responses to DED therapy stem from different levels of corneal nerve density in each individual.

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Methods

Study Design and Participants

This prospective, double-masked, phase IV randomized clinical trial included 60 patients with DED associated with meibomian gland dysfunction (MGD) who received 1 of the following medications for the treatment of ocular surface inflammation: loteprednol etabonate 0.5% ophthalmic suspension (Lotemax, Bausch & Lomb Inc, Rochester, NY), a combination of loteprednol etabonate 0.5% and tobramycin 0.3% ophthalmic suspension (Zylet, Bausch & Lomb Inc), or artificial tears (Advanced Eye Relief Dry Eye Environmental Lubricant Eye Drops, Bausch & Lomb Inc). The study protocol was approved by the Human Studies Committee of the Massachusetts Eye and Ear Infirmary (Boston, MA), and the research was conducted in accord with the requirements of the Health Insurance Portability and Accountability Act and the tenets of the Declaration of Helsinki. This study was registered on clinicaltrials.gov (identifier NCT01456780).

This study included adult patients with MGD-associated DED. All patients had symptoms of DED with an Ocular Surface Disease Index (OSDI) score greater than 22, corneal fluorescein staining (CFS) of 4 or more (National Eye Institute [NEI] grading scale, 0−15), and a diagnosis of MGD. The latter was diagnosed on the basis of a systematic evaluation of the lid margin for obstruction of meibomian glands. Exclusion criteria consisted of the following: the use of steroids, antibiotics, or optical soft contact lenses within 2 weeks before enrollment; any change in the dosage of topical anti-inflammatory medications, other than steroids, or oral tetracyclines within 2 weeks before enrollment; and the use of isotretinoin within the past 6 months. Additional exclusion criteria included history of Stevens−Johnson syndrome or mucous membrane pemphigoid, history of herpetic keratitis, active ocular allergies, and allergy to aminoglycosides, steroids, or benzalkonium chloride. Potential participants were also excluded if they had a known history of glaucoma, an intraocular pressure >22 mmHg in either eye, or a known family history of glaucoma in a first-degree relative. The details of the study and the potential benefits and harms were thoroughly explained to the patients, and all patients signed an informed consent form before participating in the study.

Study Treatment

These 60 patients were randomized to receive loteprednol etabonate 0.5% ophthalmic suspension (Lotemax, n = 20), a combination of loteprednol etabonate 0.5% and tobramycin 0.3% ophthalmic suspension (Zylet, n = 20), or artificial tears (Advanced Eye Relief Dry Eye Environmental Lubricant Eye Drops, n = 20). All these medications were used twice daily for both eyes for 4 weeks, after which the study medication was discontinued.

Clinical Evaluation

Each participant had 3 clinic visits: before starting the study medication (baseline), after 4 weeks (range, ±7 days) of treatment, and 4 weeks (range, ±10 days) after discontinuation of the treatment (8 weeks after enrollment into the study). During each visit, all participants had a complete masked ophthalmic evaluation, which included the following: assessment of symptoms using the OSDI and Symptom Assessment in Dry Eye (SANDE) questionnaires; measurement of the best-corrected visual acuity and intraocular pressure; slit-lamp biomicroscopy to assess CFS (NEI scale, 0−15); conjunctival staining with lissamine green (NEI scale, 0−18); tear break-up time; and Schirmer's test with anesthesia.

In Vivo Confocal Microscopy

In this study, in vivo confocal microscopy (IVCM) was used to measure the corneal subbasal nerve fiber length (SNFL) before starting the treatment and after 4 weeks of therapy. All participants underwent laser IVCM of the central cornea in both eyes using Heidelberg Retina Tomograph 3 with the Rostock Cornea Module (Heidelberg Engineering, Heidelberg, Germany), as described previously.[23] This IVCM machine, which uses a 670-nm red wavelength diode laser, provides a magnification of 800 times and a lateral resolution of 1 μm. It obtains digital images at a rate of 3 frames per second, with 100 images per sequence. Each image represents a coronal section of 384×384 pixels, which is equivalent to 400×400 μm of the cornea. A total of 3 to 5 sequence scans were obtained from the full-thickness of the central cornea, with at least 2 sequence scans focused on the subepithelial area and the subbasal nerve plexus, usually at a depth of 50 to 80 μm. The images from subepithelial layer of the cornea were used to measure SNFL. For each eye, 3 images most representative of the subepithelial layer were chosen for the analysis.

To measure SNFL, the subbasal nerve fibers were traced using NeuronJ (http://www.imagescience.org/meijering/software/neuronj/), which is a semiautomated nerve analysis plug-in of ImageJ (National Institutes of Health, Bethesda, MD). The SNFL was defined as the total length of all nerve fibers traced per a 0.16 mm^2 image, which was then expressed as millimeters/millimeters squared. To avoid subjective bias, 2 masked observers measured SNFL independently. The mean value of both observers was calculated, and for each patient the average of the SNFL values of both eyes was used for further analysis.

To compare the results of IVCM in patients with DED with a normal group, the data were used from 27 age-matched normal controls who did not have any sign or symptoms of DED. The SNFL measurement was done in the central cornea of both eyes of these individuals using IVCM, as described earlier. The SNFL measurements in the control group were used to define a cutoff point for having a low or near-normal SNFL. For this purpose, the cutoff point was considered as the mean value of SNFL in controls minus 2 standard deviations. On the basis of this cutoff point, patients with DED in each of 3 treatment groups were divided into 2 subgroups: those with low baseline SNFL and those with near-normal baseline SNFL.

Statistical Analysis

Statistical analysis was performed with SPSS for Windows version 21 (SPSS Inc, Chicago, IL). For each variable, except for the symptoms, patient's data were calculated by averaging the scores from both eyes. The differences in each variable among the 3 treatment groups were compared with chi-square for the qualitative variables and the analysis of variance (ANOVA) with Bonferroni correction for quantitative variables. Repeated measure ANOVA was also used to compare the changes in each quantitative variable during 8 weeks of the study within each group. The differences between the subgroups of near-normal or low baseline SNFL were analyzed with independent sample t test. Data normality was verified using the Shapiro−Wilk test. Two-sided P values <0.05 were considered statistically significant for all comparisons.

Results

This study included 60 patients with MGD-associated DED who were equally randomized into 3 treatment groups. Of these, 6 patients were withdrawn or lost to follow-up before the 4-week visit. Furthermore, 3 additional subjects, 1 in each group, did not complete the 8-week visit. Therefore, 54 patients completed the 4-week