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Patients with Dry Eye Disease and Low Subbasal Nerve Density are at High Risk for an Accelerated Corneal Endothelial Cell Loss

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Abstract

Purpose—To evaluate the changes in corneal endothelial cell density (CECD) over time in patients with dry eye disease (DED) and to correlate the endothelial cell loss with corneal subbasal nerve density.

Methods—This retrospective study included 40 eyes of 20 patients with DED. Laser in vivo confocal microscopy had been performed in the central cornea of both eyes at an initial visit and repeated after a mean follow-up of 33.2 ± 10.2 months. The densities of corneal endothelial cells and subbasal nerves were measured in both visits and compared with 13 eyes of 13 normal age-matched controls.

Results—At the initial visit, the DED group had lower densities of corneal endothelial cells (2620 ± 386 cells/mm²) and subbasal nerves (17.8 ± 7.5 mm/mm²) compared with the control group (2861 ± 292 cells/mm² and 22.8 ± 3.0 mm/mm², with $P=0.08$ and $P=0.01$, respectively). At the end of follow-up, although there was no significant change in subbasal nerve density (16.7 ± 7.2 mm/mm², $P=0.43$), the mean CECD significantly decreased to 2465 ± 391 cells/mm² ($P=0.01$), with a mean corneal endothelial cell loss of $2.1 \pm 3.6\%$ per year. The endothelial cell loss showed a statistically significant negative correlation with the initial subbasal nerve density ($R_s = -0.55$, $P=0.02$).

Conclusion—Patients with DED have an accelerated corneal endothelial cell loss which is more than what has been reported in the literature for normal aging. Those with lower subbasal nerve density, in particular, are at a higher risk for endothelial cell loss over time.

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Keywords

Corneal endothelium; corneal subbasal nerves; dry eye disease

INTRODUCTION

Dry eye disease (DED) is the most common reason for patients to see an ophthalmic care provider and affects 5–35% of the population.^{1–3} Although DED is traditionally considered as a disease of the tear film and ocular surface, recent studies have demonstrated a more extensive involvement of the conjunctiva and cornea in these patients. Using in vivo confocal microscopy (IVCM), various conjunctival and corneal changes have previously been reported, such as increased density of conjunctival inflammatory cells, reduced density of corneal superficial epithelium, increased density of corneal basal epithelium, increased number of subbasal immune dendritic cells, decreased density of subbasal nerves, increased density of stromal cells, and increased number of hyper-reflective activated stromal keratocytes.^{4–6}

Furthermore, we have recently observed that patients with moderate to severe DED have a significant reduction in central corneal endothelial cell density (CECD) which is accompanied with a decreased density of subbasal corneal nerves.⁷ The cornea has the highest nerve density in the body,^{8,9} and these nerves are known to provide a trophic support for the corneal epithelium.^{9,10} As simultaneous reduction of corneal nerves and endothelial cells has been observed in many local and systemic conditions,^{11–15} we previously suggested that these nerves may play a role in the maintenance of corneal endothelial cell function and survival.^{7,16}

In this study, we have hypothesized that due to the potential supportive role of nerve-derived molecules for corneal endothelial cells, patients with DED who have a reduced corneal nerve density suffer from an accelerated corneal endothelial loss, which is more than normal aging. Therefore, this study was designed to evaluate corneal endothelial cell changes over time in patients with DED and to correlate these changes with corneal subbasal nerve density.

METHODS

This retrospective, observational study included 40 eyes of 20 patients with DED (the DED group) and 13 eyes of 13 normal age-matched controls (the control group). To evaluate changes in densities of corneal endothelial cells and subbasal nerves over time, patients were included in the DED group who had two full-thickness IVCM scans of the central cornea at least 18 months apart as their routine evaluation for DED. In all these individuals, images obtained by IVCM were used to measure CECD and subbasal nerves, as detailed below. The patients had been seen at the Cornea and Refractive Surgery Service, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts. The protocol of the study was approved by the Institutional Review Board of Massachusetts Eye and Ear Infirmary, and the study was in compliance with the Health Insurance Portability and Accountability Act (HIPAA) and in adherence to the tenets of the Declaration of Helsinki.

The DED group included patients with typical symptoms of DED with Schirmer's test with anesthesia <10 millimeters and/or tear break-up time (TBUT) <10 seconds. Exclusion criteria consisted of any of the following before the initial visit or during the follow-up: history of increased intraocular pressure, history of any ocular surgery, contact lens wear, history of use of any medications known to have corneal toxicity, any corneal disease including herpetic keratitis, or presence of guttae in clinical examination or IVCN scans. Patients with diabetes mellitus were excluded from this study as well.

The control group consisted of healthy individuals with normal ocular examination without any previous ocular disease or surgery. Those who showed endothelial guttae in IVCN, history of contact lens wear, or those with diabetes mellitus were excluded for the control group.

All individuals had a complete ophthalmic evaluation including a detailed slit-lamp biomicroscopy with measurement of corneal fluorescein staining (Oxford scale, grades 0–5) and TBUT as well as Schirmer's test with anesthesia. Furthermore, all these individuals have a full-thickness IVCN imaging of the central cornea as described below.

In vivo confocal microscopy

All participants had undergone laser-scanning IVCN of central cornea using Heidelberg Retina Tomograph 3 with the Rostock Cornea Module (Heidelberg Engineering, Heidelberg, Germany). The microscope uses a 670-nm diode laser source and provides images from full thickness of the cornea. These images represent a coronal section of $400 \times 400 \mu\text{m}$ of the cornea.

The details of IVCN imaging of the cornea have been described before.¹⁷ Hypromellose 0.3% (GenTeal gel; Novartis Ophthalmics, East Hanover, NJ) was used to fill the disposable sterile polymethylmethacrylate cap (Tomo-cap; Heidelberg Engineering). The same gel was also used as a coupling medium between the cap and the cornea. The module was manually advanced until the gel touched the corneal surface. After visualization of the corneal epithelium, the focus was advanced to the corneal subbasal layer, the stroma, and the corneal endothelium. Multiple locations in the central cornea were imaged using Sequence Scan mode to obtain at least 1–2 sequence scans in each layer with each sequence consisting of 100 digital images (at least 100–200 images per layer).

Image Analysis

To measure the density of subbasal nerves, three representative images from various locations of the layer immediately posterior to the basal epithelial layer were selected. All visible nerve fibers in each frame were traced and their density was calculated using NeuronJ software (<http://www.imagescience.org/meijering/software/neuronj/>), which is a semi-automated plug-in program of ImageJ (National Institutes of Health, Bethesda, Maryland, <http://imagej.nih.gov/ij/>).¹⁷ The nerve density of three different IVCN images was averaged and expressed as mm/mm^2 .

To measure the CECD, three representative images of corneal endothelial layer were chosen for analysis. The CECD measurement was performed using variable frame-method.^{7,18} To

do this, IVCN images were exported and then analyzed using ImageJ. First, the boundary of the largest possible area in which all cell borders were clearly distinct was traced using Polygon Selection tool of ImageJ. Second, the area of the selected part was measured in μm^2 . Third, the number of the cells in the selected area was counted using the Cell Counter plug-in of the ImageJ. These were used to calculate CECD as cells/ mm^2 . The mean CECD of three IVCN images was then calculated.

All measurements in this study were performed by two independent, masked observers, and the average values were used for the analysis.

Statistical Analysis

SPSS for Windows version 21 (SPSS Inc., Chicago, IL) was employed for statistical analysis. The lack of normal distribution of the data was first confirmed using Shapiro-Wilk test. To compare the age and gender between DED and control groups, Mann-Whitney U test and Chi square test were utilized, respectively. To compare CECD and subbasal nerve density between DED and control groups as well as between initial and follow-up visits in the DED group, mixed model analysis was used to compensate for including both eyes of each individual. In this model, the potential confounding effect of age was also accounted for. To compare CECD and subbasal nerve density between both eyes at the initial and follow-up visits, Mann-Whitney U test was used. To compare the changes in CECD and subbasal nerve density over follow-up in right and left eyes separately Wilcoxon signed-rank test was used. Moreover, to correlate the changes of the CECD over time with age and subbasal nerve density, Spearman's rank-order correlation test was carried out. Two-sided P values of less than 0.05 were considered as statistically significant for all comparisons.

RESULTS

This study included 40 eyes of 20 patients (15 female and 5 male) with a mean age of 60.4 ± 12.3 years (range, 32–88 years) as the DED group and 13 eyes of 13 patients (8 female and 5 male) with a mean age of 56.8 ± 4.8 years (range, 50–64 years) as the control group. There were no significant differences in term of age or gender between the two groups ($P=0.22$ and $P=0.46$, respectively). In the DED group, all patients had chronic dry eye with a mean duration of 3.8 ± 3.3 years.

At the initial visit, the mean values for corneal fluorescein staining, TBUT, and Schirmer's test in the DED group were 1.3 ± 0.9 (range, 0–4), 4.3 ± 2.3 seconds (range, 0–8 seconds), and 9.4 ± 4.1 mm (range, 2–15 mm), respectively. The mean follow-up time in the DED group was 33.2 ± 10.2 months (range, 21–57 months). In this follow-up, patients had been treated with autologous serum drops in 15 (75%), topical anti-inflammatory medications in 15 (75%), omega-3 fatty acid supplements in 14 (70%), and doxycycline or azithromycin for the associated meibomian gland dysfunction in 15 (75%).

At the initial visit, central corneal subbasal nerve density was significantly lower in the DED group (17.8 ± 7.5 mm/ mm^2) than in the control group (22.8 ± 3.0 mm/ mm^2), with $P=0.02$ (Fig. 1). The CECD was 2620 ± 386 cells/ mm^2 and 2861 ± 292 cells/ mm^2 in DED and

control groups, respectively, with the difference being marginally significant ($P=0.08$) (Figures 1 and 2).

At the end of follow-up, although there was no significant change in central subbasal nerve density (16.7 ± 7.2 mm/mm², $P=0.53$), the mean CECD significantly decreased to 2465 ± 391 cells/mm² ($P=0.01$) (Figures 1 and 2). The mean corneal endothelial cell loss during the follow-up was $2.1 \pm 3.6\%$ per year.

The corneal endothelial cell loss during follow-up showed a statistically significant negative correlation with the initial subbasal corneal nerve density ($R_s = -0.55$, $P=0.02$) but not with the age at the initial visit ($P=0.33$) or initial CECD ($P=0.82$).

Comparisons of CECD and subbasal nerve density in both eyes at the initial and follow-up visits have been presented in Tables 1 and 2.

DISCUSSIONS

This study demonstrated reduced densities of corneal endothelium and subbasal nerves in patients with DED compared with an age-matched control group. In addition, we found that patients with DED had an accelerated corneal endothelial cell loss over time compared with the physiologic aging. The cell loss was more prominent in those who had an initially lower corneal subbasal nerve density. These findings provide additional evidence of a more extensive involvement of corneal structures in DED.

At the initial visit of this study, we noted a significantly lower density of corneal subbasal nerves in the central cornea of patients with DED compared with the control group, and the nerve density in the DED group did not show any significant change during the mean follow-up of 33 months. Although reduced subbasal nerve density in DED has previously been reported,^{19–22} its changes over time have not been evaluated before. Although the exact mechanisms of corneal nerve loss in DED are not clear, it may be speculated that the induced inflammation may be the causative factor for such nerve loss. Therefore, treatment of inflammation, as had been performed in majority of our patients, might have prevented further loss of corneal nerves, resulting in a stable nerve density. On the other hand, most of our patients were using autologous serum eye drops, which have some nerve growth factors;²³ this may also further increase the nerve density,^{24,25} or at least compensate for the DED-induced nerve loss.

In our study, we noted that at the initial visit patients with DED had a lower CECD than the control group with the difference being of marginally significant. This further confirms our previous study which demonstrated a significantly lower CECD in patients with DED.⁷ The lack of statistically significant difference in the CECD between the two groups may be due to the fact that DED was less severe in the current study compared with the previous one, or alternatively it may be due a smaller sample size in the current study.

Interestingly, repeat IVCN imaging after a mean follow-up of 33 months showed that the CECD decreased significantly in our patients with DED, with a mean corneal endothelial cell loss rate of 2.1% per year. This is higher than physiologic corneal endothelial cell loss

with normal aging which has rates of 0.22–0.6% per year.^{26–28} It is important to note that in studies on the effects of normal aging on corneal endothelial cell loss, although obvious causes of endothelial cell loss have been excluded, such as intraocular surgeries, corneal dystrophies, contact lens wear, or glaucoma, they have not excluded DED. Therefore, with excluding DED which is more common in elderly,¹ normal aging may be associated with less endothelial cell loss than reported before. In addition, higher standard deviations of corneal endothelial cell loss in older individuals, which have been reported before,^{29–31} may partly be due to the presence of DED in only a subgroup of these cases.

The mechanisms for this accelerated corneal endothelial cell loss in patients with DED are not clear. Accelerated endothelial cell loss compared with normal aging has previously been described in various conditions including Fuchs endothelial dystrophy, corneal infections, glaucoma, and intraocular surgeries such as cataract surgery and corneal transplantation.^{32–36} Although accelerated corneal endothelial cell loss over time may be easily explained by direct involvement of the endothelial cells in some of these conditions, the presence of such phenomenon after intraocular procedures is more complex. It has been shown that corneal endothelial cell loss after surgical procedures has two phases, a rapid early phase due to the surgical trauma and a slow chronic phase which persists for many years after surgery.³⁷ Although the mechanisms involved in the accelerated endothelial cell loss in this slow chronic phase have not been understood, chronic subclinical inflammation due to the breakdown of blood-ocular barrier has been proposed to play a role.³⁷ Therefore, it may be speculated that in patients with DED, chronic inflammation involving deep cornea and/or aqueous humor may result in accelerated corneal endothelial cell loss. Majority of our patients had been treated with anti-inflammatory medications during their follow-up in this study. Thus, presence of the observed accelerated cell loss may implicate that the treatment of subclinical inflammation in DED was not adequate to prevent corneal endothelial cell loss, or alternatively it may show the role of other contributing mechanisms such as corneal nerves.

Interestingly, our study demonstrated a significant correlation between initial corneal subbasal nerve density and the rate of corneal endothelial cell loss over time, with more cell loss noted in patients with low subbasal nerve density. Previously, a significantly lower corneal endothelial density compared with the controls has been reported in patients with neurosurgically-induced neurotrophic keratitis which showed correlation with the duration of the neurotrophic state.³⁸ Moreover, in addition to DED, a reduction of density and/or function of both corneal endothelium and subbasal nerves has been reported in other ocular conditions such as diabetes, contact lens wear, and herpes simplex keratitis.^{11–15} Thus, it may be speculated that nerve-derived molecules may directly or indirectly play a role in maintenance of corneal endothelial cells.

The potential effects of nerve-derived molecules on corneal endothelial cells may be expected as these cells originate from neural crest.³⁹ It has been shown that these cells express muscarinic acetylcholine receptors.^{40,41} Moreover, vasoactive intestinal polypeptide (VIP), which is a neuropeptide, has been demonstrated to enhance the survival of corneal endothelium.^{42,43} Therefore, reduced number of corneal nerves in DED may be associated with a decreased trophic support for corneal endothelial cells, resulting in an accelerated loss

of these cells. Further research is needed to evaluate the potential support of nerve-derived molecules on the corneal endothelium.

It should be noted although we observed a mean annual rate of 2.1% for corneal endothelial cell loss in patients with DED, it may be unlikely that this rate remains linear as DED is not a known cause for corneal endothelial decompensation. Thus, compensatory mechanisms, yet to be determined, probably play a role to mitigate the effect of DED on CECD. Furthermore, the DED characteristics, including its chronicity and severity, could have some effects on this cell loss.

Although we measured corneal endothelial cell density using variable-frame method, which has been shown to be accurate,⁴⁴ we did not evaluate the morphology of endothelial cells, the interim time points, or the effects of each individual treatment. On the other hand, only central CECD was measured in our study without evaluating peripheral endothelial cells. Furthermore, the use of different DED treatments in our patients, as usually seen in the practice, precludes us from investigating the natural course of corneal endothelial cell loss in patients with DED. The data from our retrospective study on small sample size of patients with DED can be used as basis for future large prospective studies to confirm corneal endothelial cell changes in this very common condition.

References

1. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop. *Ocul Surf.* 2007; 5:93–107. [PubMed: 17508117]
2. Lin PY, Tsai SY, Cheng CY, et al. Prevalence of dry eye among an elderly Chinese population in Taiwan: the Shihpai Eye Study. *Ophthalmology.* 2003; 110:1096–1101. [PubMed: 12799232]
3. McCarty CA, Bansal AK, Livingston PM, et al. The epidemiology of dry eye in Melbourne, Australia. *Ophthalmology.* 1998; 105:1114–1119. [PubMed: 9627665]
4. Alhatem A, Cavalcanti B, Hamrah P. In vivo confocal microscopy in dry eye disease and related conditions. *Semin Ophthalmol.* 2012; 27:138–148. [PubMed: 23163268]
5. Villani E, Mantelli F, Nucci P. In-vivo confocal microscopy of the ocular surface: ocular allergy and dry eye. *Curr Opin Allergy Clin Immunol.* 2013; 13:569–576. [PubMed: 23974688]
6. Villani E, Baudouin C, Efron N, et al. In vivo confocal microscopy of the ocular surface: from bench to bedside. *Current eye research.* 2014; 39:213–231. [PubMed: 24215436]
7. Kheirkhah A, Saboo US, Abud TB, et al. Reduced corneal endothelial cell density in patients with dry eye disease. *Am J Ophthalmol.* 2015; 159:1022–1026. [PubMed: 25782347]
8. Rozsa AJ, Beuerman RW. Density and organization of free nerve endings in the corneal epithelium of the rabbit. *Pain.* 1982; 14:105–120. [PubMed: 7177676]
9. Muller LJ, Marfurt CF, Kruse F, et al. Corneal nerves: structure, contents and function. *Exp Eye Res.* 2003; 76:521–542. [PubMed: 12697417]
10. Sigelman S, Friedenwald JS. Mitotic and wound-healing activities of the corneal epithelium; effect of sensory denervation. *AMA Arch Ophthalmol.* 1954; 52:46–57. [PubMed: 13170864]
11. Ishida N, Rao GN, del Cerro M, et al. Corneal nerve alterations in diabetes mellitus. *Arch Ophthalmol.* 1984; 102:1380–1384. [PubMed: 6236781]
12. Tavakoli M, Petropoulos IN, Malik RA. Corneal confocal microscopy to assess diabetic neuropathy: an eye on the foot. *J Diabetes Sci Technol.* 2013; 7:1179–1189. [PubMed: 24124944]
13. Efron N. Contact lens-induced changes in the anterior eye as observed in vivo with the confocal microscope. *Prog Retin Eye Res.* 2007; 26:398–436.
14. Patel SV, McLaren JW, Hodge DO, et al. Confocal microscopy in vivo in corneas of long-term contact lens wearers. *Invest Ophthalmol Vis Sci.* 2002; 43:995–1003. [PubMed: 11923239]

15. Nagy RM, McFall RC, Sery TW, et al. Scanning electron microscope study of herpes simplex virus experimental disciform keratitis. *Br J Ophthalmol.* 1978; 62:838–842. [PubMed: 737164]
16. Muller RT, Pourmirzaie R, Pavan-Langston D, et al. In vivo confocal microscopy demonstrates bilateral loss of endothelial cells in unilateral herpes simplex keratitis. *Invest Ophthalmol Vis Sci.* 2015; 56:4899–4906. [PubMed: 26225629]
17. Cruzat A, Witkin D, Baniasadi N, et al. Inflammation and the nervous system: the connection in the cornea in patients with infectious keratitis. *Invest Ophthalmol Vis Sci.* 2011; 52:5136–5143. [PubMed: 21460259]
18. Kheirkhah A, Saboo US, Marmalidou A, et al. Overestimation of corneal endothelial cell density in smaller frame sizes in in vivo confocal microscopy. *Cornea.* 2016; 35:363–369. [PubMed: 26606299]
19. Benitez del Castillo JM, Wasfy MA, Fernandez C, et al. An in vivo confocal masked study on corneal epithelium and subbasal nerves in patients with dry eye. *Invest Ophthalmol Vis Sci.* 2004; 45:3030–3035. [PubMed: 15326117]
20. Villani E, Magnani F, Viola F, et al. In vivo confocal evaluation of the ocular surface morpho-functional unit in dry eye. *Optom Vis Sci.* 2013; 90:576–586. [PubMed: 23670123]
21. Labbe A, Liang Q, Wang Z, et al. Corneal nerve structure and function in patients with non-sjogren dry eye: clinical correlations. *Invest Ophthalmol Vis Sci.* 2013; 54:5144–5150. [PubMed: 23833066]
22. Erdelyi B, Kraak R, Zhivov A, et al. In vivo confocal laser scanning microscopy of the cornea in dry eye. *Graefes Arch Clin Exp Ophthalmol.* 2007; 245(1):39–44. [PubMed: 16874525]
23. Matsumoto Y, Dogru M, Goto E, et al. Autologous serum application in the treatment of neurotrophic keratopathy. *Ophthalmology.* 2004; 111:1115–1120. [PubMed: 15177961]
24. Aggarwal S, Kheirkhah A, Cavalcanti BM, et al. Autologous serum tears for treatment of photoallodynia in patients with corneal neuropathy: efficacy and evaluation with in vivo confocal microscopy. *Ocul Surf.* 2015; 13:250–262. [PubMed: 26045233]
25. Rao K, Leveque C, Pflugfelder SC. Corneal nerve regeneration in neurotrophic keratopathy following autologous plasma therapy. *Br J Ophthalmol.* 2010; 94:584–591. [PubMed: 19965821]
26. Bourne WM, Nelson LR, Hodge DO. Central corneal endothelial cell changes over a ten-year period. *Invest Ophthalmol Vis Sci.* 1997; 38:779–782. [PubMed: 9071233]
27. Moller-Pedersen T. A comparative study of human corneal keratocyte and endothelial cell density during aging. *Cornea.* 1997; 16:333–338. [PubMed: 9143808]
28. Laing RA, Sanstrom MM, Berrospi AR, et al. Changes in the corneal endothelium as a function of age. *Exp Eye Res.* 1976; 22:587–594. [PubMed: 776638]
29. Wilson RS, Roper-Hall MJ. Effect of age on the endothelial cell count in the normal eye. *Br J Ophthalmol.* 1982; 66:513–515. [PubMed: 7104267]
30. Laule A, Cable MK, Hoffman CE, et al. Endothelial cell population changes of human cornea during life. *Arch Ophthalmol.* 1978; 96:2031–2035. [PubMed: 718491]
31. Abib FC, Barreto J Junior. Behavior of corneal endothelial density over a lifetime. *J Cataract Refract Surg.* 2001; 27:1574–1578. [PubMed: 11687354]
32. Liesegang TJ. The response of the corneal endothelium to intraocular surgery. *Refract Corneal Surg.* 1991; 7:81–86. [PubMed: 2043553]
33. Nanavaty MA, Wang X, Shortt AJ. Endothelial keratoplasty versus penetrating keratoplasty for Fuchs endothelial dystrophy. *Cochrane Database Syst Rev.* 2014; 2:CD008420.
34. Hillenaar T, Weenen C, Wubbels RJ, et al. Endothelial involvement in herpes simplex virus keratitis: an in vivo confocal microscopy study. *Ophthalmology.* 2009; 116:2077–2086. [PubMed: 19744733]
35. Hau S, Barton K. Corneal complications of glaucoma surgery. *Curr Opin Ophthalmol.* 2009; 20:131–136. [PubMed: 19240546]
36. Zhang J, Patel DV. The pathophysiology of Fuchs' endothelial dystrophy--a review of molecular and cellular insights. *Exp Eye Res.* 2015; 130:97–105. [PubMed: 25446318]
37. Armitage WJ, Dick AD, Bourne WM. Predicting endothelial cell loss and long-term corneal graft survival. *Invest Ophthalmol Vis Sci.* 2003; 44:3326–3331. [PubMed: 12882777]

38. Lambiase A, Sacchetti M, Mastropasqua A, et al. Corneal changes in neurosurgically induced neurotrophic keratitis. *JAMA Ophthalmol.* 2013; 131:1547–1553. [PubMed: 24158681]
39. Waring GO 3rd, Bourne WM, Edelhauser HF, et al. The corneal endothelium. Normal and pathologic structure and function. *Ophthalmology.* 1982; 89:531–590. [PubMed: 7122038]
40. Walkenbach RJ, Ye GS, Boney F, et al. Muscarinic cholinceptors in native and cultured human corneal endothelium. *Curr Eye Res.* 1993; 12:155–162. [PubMed: 8449026]
41. Grueb M, Reinthal E, Rohrbach JM, et al. Muscarinic acetylcholine receptor subtypes in human corneal epithelium and endothelium. *Graefes Arch Clin Exp Ophthalmol.* 2006; 244:1191–1195. [PubMed: 16506072]
42. Koh SW, Waschek JA. Corneal endothelial cell survival in organ cultures under acute oxidative stress: effect of VIP. *Invest Ophthalmol Vis Sci.* 2000; 41:4085–4092. [PubMed: 11095600]
43. Koh SW. Corneal endothelial autocrine trophic factor VIP in a mechanism-based strategy to enhance human donor cornea preservation for transplantation. *Exp Eye Res.* 2012; 95:48–53. [PubMed: 22036689]
44. Jonuscheit S, Doughty MJ, Ramaesh K. Assessment of a variable frame (polygonal) method to estimate corneal endothelial cell counts after corneal transplantation. *Eye.* 2012; 26:803–809. [PubMed: 22441023]

Fig. 1. Corneal subbasal nerve density and corneal endothelial cell density in initial and follow-up visits of patients with dry eye disease as well as in the age-matched control group. The data presented as mean $\pm$ standard error of the mean.

Fig. 2.

Corneal subbasal nerves (A) and corneal endothelial cells, as their borders were marked by the variable-frame method, (B) in a normal control. A patient with dry eye disease shows reduced densities of corneal subbasal nerves (C) and corneal endothelial cells (D) compared with the controls. After a follow-up of more than 3 years, there was no substantial change in corneal subbasal nerve density (E). However, corneal endothelial cell density showed a significant reduction (F). Please note that not all cases demonstrated such a dramatic decrease in endothelial cell density.

Table 1

Comparison of corneal endothelial cell density (CECD) between both eyes at the initial and follow-up visits.

CECD	Initial Visit	Follow-up Visit	P value
Right Eye (cells/mm ²)	2627 ± 383	2460 ± 476	0.05
Left Eye (cells/mm ²)	2547 ± 455	2390 ± 443	0.04
P value	0.70	0.70	

Table 2

Comparison of subbasal nerve density between both eyes at the initial and follow-up visits.

Subbasal Nerve Density	Initial Visit	Follow-up Visit	P value
Right Eye (mm/mm ²)	17.8 ± 7.3	17.4 ± 7.5	0.84
Left Eye (mm/mm ²)	17.2 ± 7.4	16.4 ± 7.8	0.75
P value	0.78	0.71	