Corneal Changes Assessed Using Confocal Microscopy in Patients With Unilateral Buphthalmos

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Purpose. To compare corneal structures in buphthalmic eyes and healthy eyes in patients with unilateral congenital glaucoma using a corneal confocal microscope.

Methods. Ten patients with unilateral buphthalmos (mean ± SD age, 14.85 ± 5.12 years) were examined using corneal confocal microscopy. The cell density and cell area of endothelial cells and superficial and basal epithelial cells and the number of keratocytes were evaluated.

Results. There was no significant difference between the cell density of superficial epithelial cells in buphthalmic eyes relative to healthy eyes (P = 0.1944). The cell density of basal epithelial cells was significantly higher (P = 0.0234) and the cell area was significantly smaller (P = 0.0181) in buphthalmic eyes relative to healthy eyes. There was no difference between the number of keratocytes in buphthalmic eyes and healthy eyes in the anterior stroma (P = 0.273) or in the posterior stroma (P = 0.0799). The cell density of endothelial cells was significantly lower and the cell area was significantly larger in buphthalmic eyes relative to healthy eyes (P = 0.0009).

Conclusions. We demonstrated a lower cell density of endothelial cells in buphthalmic eyes. We found no differences in keratocyte density between the buphthalmic eyes and healthy eyes. The cell density of basal epithelial cells was higher in buphthalmic eyes. These differences could be due to buphthalmos or due to the previous surgical and medical therapies. Monitoring of these changes could help to contribute to accurate assessments regarding future ocular surgical procedures.

Keywords: corneal confocal microscopy, congenital glaucoma, corneal endothelial cells, buphthalmos, corneal epithelial cells

Congenital glaucoma remains a major sight-threatening condition in children. Uncontrolled congenital glaucoma leads to an increase in the corneal diameter and axial length of the affected eye. This often causes the shift of refraction to high myopia. The cornea becomes significantly thinner, and ruptures of the Descemet’s membrane (causing astigmatism) occur frequently in these cases.1–6 The condition is usually described as buphthalmos or hydrophthalmos.

Few studies7–10 have been published that describe changes in buphthalmic eyes and histologic changes in the particular corneal layers in patients with buphthalmos. Corneal confocal microscopy allows study of the human cornea in vivo at the microscopic level. Imaging of healthy corneas and many corneal diseases, including dystrophies, keratopathies, and others, have already been described.11–18 In this study, we recorded corneal structures in buphthalmic eyes and in healthy eyes of patients with unilateral glaucoma using a corneal confocal microscope.

Methods

Ten cooperative patients (7 male and 3 female) having unilateral congenital glaucoma with buphthalmos (horizontal corneal diameter ≥12.5 mm) were included in the study. Eight patients had unilateral primary congenital glaucoma (congenital or infantile onset), and two patients had glaucoma secondary to Sturge-Weber syndrome. The mean ± SD age of the patients was 14.85 ± 5.12 years (minimum age, 7.75 years; maximum age, 24.75 years). All the buphthalmic eyes had undergone glaucoma surgery. Nine of the patients underwent a trabeculectomy during infancy, which in one case was later followed by a trabeculectomy. One patient with glaucoma secondary to Sturge-Weber syndrome underwent a deep sclerectomy in the buphthalmic eye. Nine of the 10 patients still used topical medication at the time of the study to control the intraocular pressure in the buphthalmic eye. Drug preparations with preservative (benzalkonium chloride [BAC]) were used in all nine cases. One patient has been able to go without any topical medication since surgery (Table). None of the patients required medication for their healthy eyes. The changes in corneal tissue structures were studied using corneal confocal microscopy. All the methods used in this study complied with the provisions of the ethics review board of The Motol University Hospital, Prague, Czech Republic, and the Declaration of Helsinki guidelines for research involving human participants.

A complete ophthalmic examination, including perimetry and scanning laser polarimetry (GDx VCC; Carl Zeiss Meditec, Inc., Dublin, CA), was performed to confirm well-controlled
TABLE  Characteristics of the Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Glaucoma Type</th>
<th>Surgical Procedure</th>
<th>Horizontal Corneal Diameter in Healthy Eye, mm</th>
<th>Horizontal Corneal Diameter in Buphthalmic Eye, mm</th>
<th>Visual Acuity of the Buphthalmic Eye</th>
<th>Therapy at the Time of the Study*</th>
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<td>TT</td>
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<td>TT</td>
<td>12.25</td>
<td>14.0</td>
<td>1.0</td>
<td>Ganfort + Azoft + Pilogel</td>
</tr>
<tr>
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<td>0.6</td>
<td>Duo trav</td>
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<tr>
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<td>0.1</td>
<td>Cosopt + Travatan</td>
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<tr>
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<td>0.6</td>
<td>Combi gan + Xalatan</td>
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<td>13.0</td>
<td>1.0</td>
<td>Xalatan</td>
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<tr>
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<td>12.25</td>
<td>14.25</td>
<td>1.0</td>
<td>Azoft</td>
</tr>
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</table>

*All drugs were BAC preserved. The drug therapies at the time of the study included the following: Xalatan (latanoprost; Pfizer, Prague, Czech Republic), Xalacom (latanoprost-timolol; Pfizer), Ganfort (bimatoprost-timolol; Allergan Pharmaceuticals Ireland, Westport, Ireland), Combi gan (brimonidine-timolol; Allergan Pharmaceuticals Ireland), Travatan (travoprost; Alcon Laboratories, Hemel Hempstead, UK), Duo trav (travoprost-timolol; Alcon Laboratories), Azopt (brinzolamide; Alcon Laboratories), Pilogel (pilocarpine; Alcon Laboratories), and Cosopt (dorzolamide-timolol; Merck Sharp & Dohme, Haarlem, The Netherlands).

Assessed values are reported as mean ± SD. The values of the buphthalmic eye were compared with the values of the healthy eye in each patient. The paired t-test was used for statistical analysis of the results (StatView 5.0; SAS Institute, Inc., Cary, NC). P < 0.05 was considered to be statistically significant.

RESULTS

The mean horizontal corneal diameter of the buphthalmic eyes was 13.35 mm (range, 12.5–14.25 mm), and the mean horizontal corneal diameter of the healthy eyes was 11.83 mm (range, 11.5–12.25 mm). Haab’s striae were apparent in seven of 10 buphthalmic corneas examined using a slitlamp (Fig. 1). The best-corrected visual acuity of the buphthalmic eyes ranged from 0.1 to 1.0 (decimal scale) using Snellen optotypes (Table). The best-corrected visual acuity of the healthy eyes was 1.0 in all cases. The central corneal thickness was significantly lower in buphthalmic eyes (495 ± 56 μm) compared with healthy eyes (552 ± 50 μm) (P = 0.0005).

There was no significant difference between the cell density of superficial epithelial cells in buphthalmic eyes (1701 ± 552 cells/mm²) and healthy eyes (2000 ± 602 cells/mm²) (P = 0.1944). Therefore, there was no significant difference in the area of cells between the buphthalmic eyes (638 ± 175 μm²) and the healthy eyes (537 ± 139 μm²) (P = 0.1424).

The cell density of the basal epithelial cells was significantly higher in buphthalmic eyes (6882 ± 489 cells/mm²) compared with healthy eyes (6394 ± 591 cells/mm²) (P = 0.0254) (Fig. 2). As this finding would suggest, the mean cell area of the basal epithelial cells was significantly smaller in buphthalmic eyes (146 ± 10 μm²) compared with healthy eyes (158 ± 15 μm²) (P = 0.0181).

We did not find any differences in the distribution of keratocytes in the stroma between buphthalmic eyes and healthy eyes (Fig. 3). There was no significant difference between the number of keratocytes in buphthalmic eyes (962 ± 180 cells/mm²) and healthy eyes (1050 ± 88 cells/mm²) (P = 0.273) in the anterior stroma, and there was no significant difference between the number of keratocytes in buphthalmic eyes (706 ± 62 cells/mm²) and healthy eyes (757 ± 42 cells/mm²) (P = 0.0799) in the posterior stroma. The number of...
Endothelial cell density (in cells per millimeter squared). The density of endothelial cells was significantly lower in buphthalmic eyes than in healthy eyes ($P < 0.0001$). Haab's striae were apparent in patients 1, 4, 6, 7, 8, 9, and 10 on slitlamp examination; hyperreflective scar tissue was observed in patients 1, 2, 4, 6, 7, 8, 9, and 10 at the level of the Descemet's membrane during confocal microscopy in buphthalmic eyes.

Density of basal epithelial cells (in cells per millimeter squared). The density of basal epithelial cells was significantly higher in buphthalmic eyes compared with healthy eyes ($P = 0.0234$).

Distribution of keratocytes (in cells per millimeter squared). There was no significant difference between the number of keratocytes in buphthalmic eyes and healthy eyes ($P = 0.275$) in the anterior stroma (AS-buphthalmos and AS-healthy). There was also no significant difference between the number of keratocytes in buphthalmic eyes and healthy eyes ($P = 0.0799$) in the posterior stroma (PS-buphthalmos and PS-healthy). The number of keratocytes was significantly higher in the anterior stroma compared with the posterior stroma in both buphthalmic eyes ($P = 0.0007$) and healthy eyes ($P < 0.0001$).
keratocytes was significantly higher in the anterior stroma relative to the posterior stroma in both buphthalmic eyes ($P = 0.0007$) and healthy eyes ($P < 0.0001$). We detected coil-shaped stromal nerve morphologies in three of 10 buphthalmic eyes (Fig. 4).

At the level of the Descemet’s membrane, hyperreflective acellular scar tissue was observed in eight of 10 buphthalmic corneas. These were the same corneas where the Haab’s striae were also apparent, plus one cornea (in patient 2) in which no Haab’s striae were detected during slitlamp examination. Traction and distortions were found in four of 10 buphthalmic corneas (in patients 1, 4, 7, and 9) on the endothelial cells at the inner corneal surface of these regions, causing breaks in the endothelial layer (Figs. 5, 6). These findings were not observed in healthy eyes.

The cell density of endothelial cells was significantly lower in buphthalmic eyes (1893 ± 470 cells/mm$^2$) relative to healthy eyes (3135 ± 217 cells/mm$^2$) ($P < 0.0001$) (Fig. 1), and the cell area of endothelial cells was significantly larger in buphthalmic eyes (561 ± 151 µm$^2$) compared with healthy eyes (320 ± 22 µm$^2$) ($P = 0.0009$). There was no significant difference between the coefficient of variation in cell size and percentage of hexagonality values in buphthalmic eyes (26.9% ± 7.8% and 62.1% ± 15.0%, respectively) compared with healthy eyes (26.4% ± 5.5% and 61.3% ± 12.30%, respectively) ($P = 0.8744$ and $P = 0.8935$, respectively).

**DISCUSSION**

The increase in intraocular pressure in congenital or infantile glaucoma causes enlargement of the globe due to the softness and elasticity of the infant globe, which is particularly evident as a progressive increase in corneal diameter. Breaks in the Descemet’s membrane, termed Haab’s striae, are another typical finding. Only a few studies have analyzed the histopathological findings in buphthalmic corneas. Mastropasqua et al. described findings from two adult patients with congenital glaucoma with buphthalmos using an in vivo confocal microscope. Using this modern technology, we studied the corneal changes in a group of patients with unilateral buphthalmos.

In our study, we found that the number of endothelial cells in healthy eyes was similar to that reported in previous studies. In accord with earlier studies, we found abnormalities in the Descemet’s membrane and the endothelium in buphthalmic eyes. The density of endothelial cells was significantly lower in buphthalmic eyes, and the mean area of these cells was significantly larger compared with healthy eyes. However, we did not prove increased polymegathism and pleomorphism of the endothelial cells in buphthalmic eyes compared with the healthy eyes. The mean endothelial cell area in the buphthalmic eyes was smaller than that in the cases by Mastropasqua et al. Morphologic changes in the buphthalmic corneas were also clearly visible as Haab’s striae on the Descemet’s membranes. Very hyperreflective acellular scar tissue was also often observed at this tissue level. This is in agreement with the histologic description of Haab’s striae. It was also possible to observe that the inner surface of the above-described tissue revealed evident tractions and distortions of the endothelial cells, causing breaks in the endothelial layer in some cases. Similar findings were not observed in any of the healthy eyes. The mechanical expansion of the corneal tissue is assumed to be an important factor in the development of changes in buphthalmic corneas. These changes result in reduced density and compensatory hypertrophy of endothelial cells, which have very limited ability to proliferate, and lead to

**FIGURE 4.** Stromal nerve fibers. An abnormal morphology of stromal nerve fibers was occasionally present in the buphthalmic corneas.

**FIGURE 5.** Traction on endothelial cells. Traction and distortion were found on the endothelial cells in regions with hyperreflective tissue at the level of the Descemet’s membrane in buphthalmic corneas.

**FIGURE 6.** Breaks in the endothelial layer. In some of the buphthalmic corneas, a focal cellular lesion of the endothelial cell layer in the region of tractions was also found.
Corneal Changes Assessed Using Confocal Microscopy

The final density of the endothelial cells observed in this study much different ocular history than an age-matched control.

The number of keratocytes in the anterior and posterior stroma corresponded to findings in healthy eyes that have been previously reported. However, we observed abnormal stromal nerve fiber morphologies in some buphthalmic eyes. The altered stromal nerve morphology could be consistent with a possible mechanical force acting on the stroma at some point in the patient's past.

Compared with previous results, we found a slightly higher density of basal epithelial cells in healthy eyes. Although earlier studies failed to find a correlation between basal epithelial cell density and age, none of the studies focused on patients as young as the ones who participated in our study. So, the age variation could explain the observed differences in results. We also found a higher density of basal epithelial cells in buphthalmic corneas compared with healthy corneas.

Recent studies have documented the effects of topical antiglaucoma therapy (with preservative) on corneal morphology. The basal epithelial cell density of glaucomatous preservative therapy groups was significantly higher than that of control and preservative-free groups. The density of superficial epithelial cells was significantly reduced in glaucoma patients except for patients in the preservative-free group. The authors hypothesized that the increase in basal epithelial cell density could be attributable to a proliferative stimulus from the superficial layer. Although in our study the mean superficial epithelial cell density was lower in buphthalmic eyes, the difference was not statistically significant. Proliferative activity of different cells can be affected by the action of mechanical factors, basement membrane modifications, and effects of growth factors. Different growth factors have been identified that can modify corneal epithelial cell mitotic activity. Corneal epithelial cells are derived from mitotic activity in the limbal basal cells and basal epithelial cells continuously. The toxic action of preservative, especially BAC, on the eye surface has been widely demonstrated. BAC promotes activation of lipoxigenases and synthesis and secretion of eicosanoids, cytokines, and inflammatory mediators. Thus, chronic antiglaucoma medication as a cause of high basal epithelial cell density in buphthalmic eyes is possible. However, we also found a higher basal epithelial cell density in the buphthalmic eye in one patient who was not using topical medication. Mechanical forces acting at the time of buphthalmos development could also affect the proliferative capacity of basal and limbal cells, and the growth factors released during trauma and the subsequent healing process of corneal tissue may influence the basement membrane structure and rate of corneal epithelial cell proliferation. Thus, the effect of additional factors that could contribute to changes in buphthalmic eyes cannot be ruled out. Further studies are needed to fully clarify the process.

In the present study, we described corneal changes in buphthalmic eyes using a corneal confocal microscope. Unilateral glaucoma with buphthalmos is rare. We had a unique opportunity to compare findings from patients with unilateral impairment and, in so doing, clearly document the structural changes that appear in buphthalmic corneas. The buphthalmic eye in each of these patients has a much different ocular history than an age-matched control. However, using the same patient for his or her own control is a way to eliminate some of the confounding factors. Nevertheless, we cannot quite exclude the possibility of concurrence of more complex anterior segment dysgenesis and an inherent corneal pathology. Another limitation is the fact that the measurements represent small areas within the central cornea.

A possible weakness of the study is that the measurements were not performed in truly masked fashion because the basic data of a selected patient are always displayed on the screen. However, the observer who evaluated the scans did not know which eye was healthy and which eye was buphthalmic. Thus, we believe that the measurements performed were not biased.

Progress in the treatment of congenital glaucoma maintains usable vision of the affected eyes up to older age. Thus, we may be increasingly faced in the future with the problem of dealing with consequent potential pathologies such as cataracts or endothelium exhaustion. Also, refractive surgery could enable full correction of high anisometropia (in cases of unilateral buphthalmos) or high astigmatism in some cases. Monitoring of corneal changes and their evolution over time could help to contribute to accurate assessments regarding future ocular surgical procedures.

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References