Histopathologic and Immunohistochemical Studies of Keratoglobus

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Objective: To examine histopathologic and immunohistochemical features of human corneal buttons from patients who developed keratoglobus.

Methods: Nine corneal buttons were obtained during penetrating keratoplasty from patients with keratoglobus. Histologic features were examined using hematoxylin-eosin–stained sections. Immunohistochemical staining for α1-proteinase inhibitor, Sp1, and matrix metalloproteinases 1, 2, and 3 was performed, with 2 normal and 2 corneal sections with keratoconus as controls.

Results: Hematoxylin-eosin staining revealed diffuse stromal thinning and focal disruptions in Bowman’s layer in all keratoglobus specimens. Similar abnormal immunostaining results for α1-proteinase inhibitor and Sp1 were detected in keratoglobus and keratoconus at their respective active disease sites. Immunostaining for matrix metalloproteinases 1, 2, and 3 was significantly more intense in corneas with keratoglobus than in normal controls. Matrix metalloproteinase staining intensity was especially prominent in areas where the underlying Bowman’s layer was disrupted.

Conclusions: Histological features in our keratoglobus specimens are consistent with previous reports. The similarities in immunohistochemical labeling between keratoglobus and keratoconus suggest that these entities may share common mechanisms that are involved in stromal thinning.

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Keratoglobus is a rare corneal disease characterized by limbus-to-limbus corneal thinning, often greatest in the periphery, with globular protrusion of the cornea.1,2 Patients with this condition have severe visual impairment due to extreme myopia, irregular astigmatism, corneal scarring, and the occasional corneal rupture.3 Keratoglobus has been described as both an acquired and a congenital disease. Acquired keratoglobus has been associated with dysthyroid ophthalmopathy, vernal keratoconjunctivitis, and chronic marginal blepharitis.4,5 Eye rubbing has been proposed to be the major contributing factor in the latter 2 entities.2 The congenital form of the disease has been associated with Leber congenital amaurosis and the blue sclera syndrome.6 Keratoglobus has been associated with other connective tissue disorders, including Ehlers-Danlos syndrome type VI,7 Marfan syndrome,8 and Rubinstein-Taybi syndrome.9

Studies of the histologic changes in keratoglobus have been rare. Reported findings include marked stromal thinning with scarring, frequent disruptions, or complete absence of Bowman’s layer; breaks in Descemet’s membrane; and thickening of Descemet’s membrane.2,14 Keratoglobus and keratoconus are both noninflammatory ectatic disorders of the cornea. The distinction between the 2 similar conditions was first made by Cavara in 1950.1 The cornea in keratoglobus is diffusely thinned, often more markedly in the peripheral cornea, whereas in keratoconus the thinning is most prominent in the central cornea. The histopathologic changes seen in keratoglobus, including disruption of Bowman’s layer and Descemet’s membrane breaks, are very similar to those seen in advanced keratoconus.15 In fact, the histologic similarities have led to the speculation that keratoglobus may be an end-stage manifestation of advanced keratoconus.14 The biochemical abnormalities found in keratoconus have not been studied in.
keratoglobus. These biochemical alterations include decreased expression of the protease inhibitor α1-antiprotease inhibitor (α1-PI, formerly also known as α1-antitrypsin) and upregulation of transcription factor Sp1.16-18 In addition, matrix metalloproteinases (MMPs), a group of enzymes that are involved in tissue remodeling, have also been examined. Among them, MMP-1 and membrane type (MT)–MMP-1 have been reported to be upregulated in corneas with keratoconus.19-22 In this study, we examined the histopathologic characteristics of keratoglobus using light microscopy and performed immunohistochemistry to determine if biochemical alterations in keratoglobus shared features with those documented in keratoconus.

METHODS

Nine corneal buttons were obtained at the time of penetrating keratoplasty from 9 patients who had clinically diagnosed keratoglobus at the University of Illinois at Chicago or the L.V. Prasad Eye Institute, Hyderabad, India. Two normal human corneal buttons from donors (ages 25 and 69 years) were obtained from the Illinois Eye Bank, Chicago, within 24 hours of death. None of the donors had known ocular diseases, and their corneas were clear and unremarkable. Two corneal buttons from patients with typical features of keratoconus (ages 40 and 48 years) were obtained following transplantation from the cornea service at the University of Illinois at Chicago as another set of controls. The corneas that were excised from normal human eyes and keratoglobus and keratoconus buttons were fixed in 10% buffered formalin, processed, and embedded in paraffin.

The thickness of the stroma in the central and mid-peripheral cornea was measured in hematoxylin-eosin–stained sections using AxioVision software, version 4.4.1.0 (Carl Zeiss Microlmaging Inc, Gottingen, Germany) after being photographed under a Zeiss Axioskop 2 Plus microscope using a Zeiss AxioCam camera (Carl Zeiss Microlmaging Inc). Statistical analysis was performed using the Wilcoxon signed-rank test to compare stromal thickness in the central and mid-peripheral cornea. P < .05 was considered statistically significant.

Immunohistochemical studies were also conducted. The sections were incubated at 4°C with primary antibodies for 16 hours. The primary antibodies used in the study included (1) a polyclonal rabbit anti-α1-PI antibody (1:100; MP Biomedicals, Aurora, Ohio), (2) a polyclonal rabbit antibody to Sp1 (PEP 2, 1:100; Santa Cruz Biotechnology, Santa Cruz, California), (3) a polyclonal rabbit anti–MMP-1 antibody (1:100; Laboratory Vision, Fremont, California), (4) a monoclonal mouse antibody specific to MMP-2 (A-Gel VC2, 1:50; Laboratory Vision), and (5) a monoclonal mouse antibody to MMP-3 (SL-1 IID4, 1:20; Laboratory Vision). Before primary antibody incubation for MMPs, an antigen retrieval method was applied on each section with 8M buffered urea (pH 3.5) at room temperature for 2 hours. Biotinylated donkey anti–goat IgG (1:2,500), donkey anti–rabbit IgG (1:2,500), and donkey anti–mouse IgG (1:2,500; all from Jackson ImmunoResearch, West Grove, Pennsylvania) were used as secondary antibodies at room temperature for 45 minutes. The color reaction for the anti-Sp1 was carried out with Fast Red TR/Naphthol AS-MX phosphate (Sigma, St Louis, Missouri) after sections were incubated with alkaline phosphatase–conjugated ExtrAvidin (1:50; Sigma). For α1-PI, MMP-1, MMP-2, and MMP-3, sections were incubated with avidin-biotin complex (Vectorstain; Vector Laboratories, Burlingame, California) for 45 minutes followed by incubation with 3,3-diaminobenzidine tetrahydrochloride (Sigma). After mounting in Permoun (Thermo Fisher Scientific, Waltham, Massachusetts), for α1-PI, MMP-1, MMP-2, and MMP-3 or aqueous mounting fluid (Vectashield; Vector Laboratories, for Sp1), the staining intensity in each experiment was scored by 3 masked observers on a scale from 0 to 3, with 0 indicating no staining and 3 the most intense staining. Each experiment was repeated twice. Statistical analysis was performed using the Mann-Whitney U test to compare the staining intensity of keratoglobus and keratoconus corneas with those from normal controls. Within the corneas with a keratoglobus, immunostaining intensity was compared between the central and mid-peripheral areas. P < .05 was considered statistically significant.

RESULTS

CLINICAL HISTORY

Nine patients with the diagnosis of keratoglobus (6 female, 3 male) had penetrating keratoplasty at a mean age of 27 years (range, 3-49 years). Clinical histories were available for 7 of the 9 patients, all of whom presented with varying complaints of bilateral visual loss and had bilateral keratoglobus clinically diagnosed. Preoperative best-corrected visual acuity ranged from 20/60 to light perception. Penetrating keratoplasty was performed on 1 eye in all 9 patients. The salient ophthalmic findings are summarized in Table 1 and

<table>
<thead>
<tr>
<th>Patient (Eye)/Sex/Age, y</th>
<th>Laterality</th>
<th>OD</th>
<th>OS</th>
<th>BCVA</th>
<th>Stiltlamp Examination Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Right)/F/37</td>
<td>Both</td>
<td>20/60</td>
<td>20/30</td>
<td>Thinning with scarring in both eyes</td>
<td></td>
</tr>
<tr>
<td>2 (Left)/F/3</td>
<td>Both</td>
<td>LP</td>
<td>LP</td>
<td>Full thickness corneal tear following minimal trauma in the left eye</td>
<td></td>
</tr>
<tr>
<td>3 (Left)/M/66</td>
<td>Both</td>
<td>20/50</td>
<td>20/150</td>
<td>Stromal thinning with subepithelial scarring/corneal diameter in each eye</td>
<td></td>
</tr>
<tr>
<td>4 (Right)/F/49</td>
<td>Both</td>
<td>20/150</td>
<td>CF at 0.5 m</td>
<td>Thinning with protrusion in the right eye; descemetocele in the left eye</td>
<td></td>
</tr>
<tr>
<td>5 (Left)/F/18</td>
<td>Both</td>
<td>20/200</td>
<td>20/200</td>
<td>Thinning with scarring and vascularization in each eye</td>
<td></td>
</tr>
<tr>
<td>6 (Right)/F/39</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Adherent leukoma with vascularization in the right eye; thinning in each eye</td>
<td></td>
</tr>
<tr>
<td>7 (Left)/M/46</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Thinning with scarring, vascularization, and protrusion in each eye</td>
<td></td>
</tr>
<tr>
<td>8 (Left)/F/10</td>
<td>Both</td>
<td>20/400</td>
<td>20/400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 (Left)/M/35</td>
<td>Both</td>
<td>CF at 2 m</td>
<td>CF at 0.5 m</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Clinical Ophthalmic Findings

Abbreviations: BCVA, best-corrected visual acuity; CF, counting fingers; LP, light perception; NA, not available.
None of the patients had prior findings suggestive of keratoconus documented at any point during the clinical course. Cases 2 and 3 were unusual, as these patients had visual loss following trivial trauma to the left eye at a young age. Corneal diameters in patient 2 were 12.0 mm (horizontal) × 11.5 mm (vertical) in the right eye and 12.0 mm (horizontal) × 12.5 mm (vertical) in the left eye. Intraocular pressures under anesthesia were 12 mm Hg in both eyes. Fundus examination did not show optic nerve cupping, which would suggest congenital glaucoma. Patient 3 was aged 6 years. His horizontal corneal diameters were 12 mm and his intraocular pressure was 16 mm Hg in both eyes. Optic nerve examination results were unremarkable.

Two patients had systemic findings. Patient 3 had hyperextensible joints without other features suggestive of a connective tissue disorder. Patient 8 had a high arched palate with some Marfan-like features in her extremities. Cardiac workup results in this patient were normal.

**Figure 1.** None of the patients had prior findings suggestive of keratoconus documented at any point during the clinical course. Cases 2 and 3 were unusual, as these patients had visual loss following trivial trauma to the left eye at a young age. Corneal diameters in patient 2 were 12.0 mm (horizontal) × 11.5 mm (vertical) in the right eye and 12.0 mm (horizontal) × 12.5 mm (vertical) in the left eye. Intraocular pressures under anesthesia were 12 mm Hg in both eyes. Fundus examination did not show optic nerve cupping, which would suggest congenital glaucoma. Patient 3 was aged 6 years. His horizontal corneal diameters were 12 mm and his intraocular pressure was 16 mm Hg in both eyes. Optic nerve examination results were unremarkable.

Two patients had systemic findings. Patient 3 had hyperextensible joints without other features suggestive of a connective tissue disorder. Patient 8 had a high arched palate with some Marfan-like features in her extremities. Cardiac workup results in this patient were normal.

**HISTOLOGIC ANALYSIS**

Measurements of corneal stromal thickness were performed on histologic specimens of all 9 keratoglobus corneas. Five measurements taken in each of the following areas of the cornea were then averaged: center, right mid-periphery, and left mid-periphery (mid-peripheral cornea refers to the peripheral portions of the excised corneal button; this region would vary depending on the size of the corneal button and size of the cornea). The mean mid-peripheral stromal thickness (212 µm [standard deviation (SD), 89 µm]) was significantly thinner than that in the central cornea (244 µm [98 µm], P = .05). For comparison, normal control stromal thickness was 399 µm (SD, 5.7 µm) centrally and 430 µm (SD, 7.1 µm) peripherally. Details of thickness measurements are reported in Table 2.

The corneas with a keratoglobus had a range of findings on histology (Table 3). These included local Bowman’s layer disruption and diffuse stromal thinning that was especially evident in the peripheral cornea in all keratoglobus buttons. Epithelial hyperplasia in the central cornea was observed in 3 buttons, stromal scarring in 5, stromal neovascularization in 4, focal disruptions of Descemet’s membrane in 5, and diffuse Descemet’s membrane thickening in 2. Inflammatory infiltrates were not seen in any of the keratoglobus buttons. The cases in Figure 2 and Figure 3 are illustrative of the spectrum of histologic changes observed in our series of keratoglobus cases.

**IMMUNOHISTOCHEMICAL ANALYSIS**

Immunohistochemical experiments demonstrated decreased immunostaining for α1-PI in the epithelia of corneas with keratoglobus or keratoconus that was statistically significant when compared with normal controls (P = .01) (Figure 4). Immunolabeling for Sp1 in the nuclei of epithelial cells in both keratoglobus and keratoconus corneas was significantly increased compared with normal controls (P = .01) (Figure 4). The keratoglobus and keratoconus showed similar immunostaining intensity. However, unlike in keratoconus corneas, the abnormal staining patterns for both α1-PI and Sp1 in keratoglobus were more prominently observed in the mid-peripheral than in the central cornea. Each of these differences were statistically significant (α1-PI, P = .04; Sp1, P = .049). The corneas with keratoglobus had significantly higher epithelial stain-
ing intensities for MMP-1, MMP-2, and MMP-3 than corneas from normal control, in which staining was nearly absent ($P=.01$). The staining intensity of MMP-1 in the epithelia of corneas with keratoconus was also higher than in normal controls, whereas MMP-2 and MMP-3 immunolabeling in the keratoconus cases was absent or at the background level. Although positive epithelial staining for each MMP in keratoglobus was globally observed, it was more notable in the basal epithelium and in areas where the underlying Bowman’s layer was disrupted (Figure 5). Semiquantitative immunostaining results for all antibodies are displayed in Figure 6 (α1-PI and Sp1) and Figure 7 (MMP-1, MMP-2, and MMP-3). Measurement of immunohistochemical staining intensity within keratocytes was difficult given the appearance of the nuclear counterstain (hematoxylin). No positive staining above background levels was detected within the stromal extracellular matrix, including in areas where stromal scarring was observed.

Table 3. Summary of Histopathologic Findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>Epithelium</th>
<th>Bowman’s Layer</th>
<th>Stroma$^a$</th>
<th>Descemet’s Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unremarkable</td>
<td>Focal disruptions</td>
<td>Scarring</td>
<td>Unremarkable</td>
</tr>
<tr>
<td>2</td>
<td>Areas of hyperplasia</td>
<td>Focal disruptions</td>
<td>Scarring, central vascularization</td>
<td>Focal disruptions</td>
</tr>
<tr>
<td>3</td>
<td>Areas of hyperplasia</td>
<td>Focal disruptions</td>
<td>Scarring, central vascularization</td>
<td>Focal disruptions, thickened</td>
</tr>
<tr>
<td>4</td>
<td>Unremarkable</td>
<td>Focal disruptions</td>
<td>Peripheral vascularization</td>
<td>Unremarkable</td>
</tr>
<tr>
<td>5</td>
<td>Not present</td>
<td>Focal disruptions</td>
<td>Scarring, central vascularization</td>
<td>Focal disruptions</td>
</tr>
<tr>
<td>6</td>
<td>Unremarkable</td>
<td>Focal disruptions</td>
<td>Unremarkable</td>
<td>Focal disruptions</td>
</tr>
<tr>
<td>7</td>
<td>Unremarkable</td>
<td>Focal disruptions, thin</td>
<td>Scarring, central vascularization</td>
<td>Unremarkable</td>
</tr>
<tr>
<td>8</td>
<td>Mild hyperplasia</td>
<td>Focal disruptions</td>
<td>Scarring</td>
<td>Focal disruptions, thickened</td>
</tr>
<tr>
<td>9</td>
<td>Unremarkable</td>
<td>Focal disruptions</td>
<td></td>
<td>Unremarkable</td>
</tr>
</tbody>
</table>

$^a$Stromal thinning was observed in all cases.

This study examined the histologic and immunohistochemical features of corneas obtained from 9 patients with a keratoglobus. The histologic findings in these keratoglobus buttons were consistent with those reported in the literature, including marked stromal thinning with scarring, frequent disruptions, and complete absence of Bowman’s layer; breaks in Descemet’s membrane; and thickening of Descemet’s membrane. Additionally, stromal neovascularization was noted in 4 of our specimens. Stromal vascularization was associated with clinically documented corneal perforation (case 2) or breaks in Descemet’s membrane with stromal edema.

Immunohistochemistry performed on corneas with keratoglobus and keratoconus and in normal controls revealed that the expression of α1-PI and Sp1 in keratoglobus was similar to that of keratoconus. Previous reports have described a decrease in α1-PI expression and upregulation of transcription factor Sp1 in corneas with keratoconus. Additionally it was found that Sp1 overexpression suppresses the promoter activity of the α1-PI gene, which conceivably alters tissue degradation processes in corneas with keratoconus. We observed similar biochemical changes in our keratoglobus specimens. Such a finding suggests that the mechanisms by which corneal stromal thinning develops in keratoglobus may bear similarities to those in keratoconus. Interestingly, the abnormal staining patterns in keratoglobus were more prominently observed in the mid-peripheral cornea than in the central cornea for both α1-PI and Sp1. This is consistent with clinical and histological observations for keratoglobus that corneal thinning is more pronounced in the peripheral cornea both in our group of patients and elsewhere.
An increase in the expression of MMP-1 and MT–MMP-1 has been described in areas of central thinning in corneas with keratoconus. Matrix metalloproteinases have also been implicated in various other corneal conditions, including wound repair and healing, ulcerations, and diabetic corneas. Our results showed that the MMP-1, MMP-2, and MMP-3 immunostaining intensity in keratoglobus was significantly higher than that seen in normal controls. The MMP-1 immunolabeling was also somewhat elevated in keratoconus corneas, whereas the MMP-2 and MMP-3 staining was comparable with normal controls. The MMP immunostaining was observed diffusely throughout the entire keratoglobus cornea, though it was more notable.

Figure 3. Histopathologic analysis of keratoglobus cases at high magnification. Peripheral cornea of patient 2 (A) and central cornea of patient 3 (B) showing disruption of Bowman’s layer and subepithelial scars (arrows). C, Peripheral cornea of patient 4 showing disruption of Descemet’s membrane (double arrows). D, Central cornea of patient 9 showing a disrupted and thickened Descemet’s membrane (double arrows). E, Peripheral cornea of patient 3 showing absence of Bowman’s layer. F, Central cornea of patient 2 showing epithelial hyperplasia, with Bowman’s layer replaced by a collagenous scar (arrowheads encircling scar) and neovascularization (asterisk) (hematoxylin-eosin). Scale bars equal 50 µm for all.
in the basal epithelium in areas where the underlying Bowman’s layer was disrupted. In comparison, the MMP-1 immunoreactivity in keratoglobus and the cornea with keratoconus is markedly reduced (chromogen 3,3-diaminobenzidine tetrahydrochloride). The intensity of Sp1 nuclear staining is much lower in the epithelium of the normal control compared with that in keratoglobus and keratoconus (chromogen Fast Red TR/Naphthol AS-MX phosphate staining). The abnormal staining patterns in the keratoglobus corneas were more prominently observed in the mid-peripheral than the central cornea for both α1-PI and Sp1. Scale bars equal 50 µm for all.

**Figure 4.** Immunostaining results for α1-proteinase inhibitor (α1-PI) and Sp1. There is intense staining of α1-PI in the epithelium of the normal control. By comparison, epithelial staining in both corneas with keratoglobus and the cornea with keratoconus is markedly reduced (chromogen 3,3-diaminobenzidine tetrahydrochloride). The intensity of Sp1 nuclear staining is much lower in the epithelium of the normal control compared with that in keratoglobus and keratoconus (chromogen Fast Red TR/Naphthol AS-MX phosphate staining). The abnormal staining patterns in the keratoglobus corneas were more prominently observed in the mid-peripheral than the central cornea for both α1-PI and Sp1. Scale bars equal 50 µm for all.

in the basal epithelium in areas where the underlying Bowman’s layer was disrupted. In comparison, the MMP-1 immunoreactivity in keratoconus was localized to the central portion of the cornea. It appears thus that the MMP-1–mediated protein degradation may be occurring at the active sites of both conditions, diffusely in keratoglobus and centrally in keratoconus. By comparison, epithelial staining in the normal controls is nearly absent. Staining within the corneas with keratoglobus is the strongest where the Bowman’s membrane is disrupted (chromogen 3,3-diaminobenzidine tetrahydrochloride). Scale bars equal 50 µm for all.

**Figure 5.** Immunostaining results for the matrix metalloproteinases (MMPs). The underlying Bowman’s layer in keratoglobus, while present, was markedly attenuated compared with that in normal controls. Note the increased staining intensity for all MMPs in the corneas with keratoglobus and MMP-1 in the cornea with keratoconus. By comparison, epithelial staining in the normal controls is nearly absent. Staining within the corneas with keratoglobus is the strongest where the Bowman’s membrane is disrupted (chromogen 3,3-diaminobenzidine tetrahydrochloride). Scale bars equal 50 µm for all.
genomic pathways, though extra degradative events may be occurring additionally in keratoglobus. A similar theory was suggested previously by Pouliquen et al\(^4\) based on histological findings alone.

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REFERENCES


