Atypical Corneal Phenotype in Patients With Trachoma and Secondary Amyloidosis

Noopur Gupta, MS, PhD,* Saumya Yadav, MD,* Anthony W. Solomon, PhD, FRCP,† Shubhi Jain, BSc,* Seema Kashyap, MD,‡ Murugesan Vanathi, MD,* and Radhika Tandon, MD*

**Purpose:** To report clinical presentation, in vivo confocal microscopic features, and corneal phenotype in patients with trachomatous keratopathy (TK) and secondary amyloidosis.

**Methods:** Histopathological records of all patients undergoing keratoplasty at the Dr. Rajendra Prasad Centre for Ophthalmic Sciences over a 3-year period were scanned retrospectively for a diagnosis of TK and amyloidosis. Demographic profile and details of preoperative comprehensive ophthalmic assessment were extracted. The histopathology was freshly reviewed.

**Results:** Fifteen patients (29 eyes) with TK and atypical corneal involvement due to amyloid deposition were identified. Herbert’s pits and upper palpebral conjunctival scarring were present in all cases. Central or total diffuse corneal scarring was present involving the anterior stroma in 5 (31%) and the full thickness of the cornea in 11 (69%) of the eyes. Eight (73%) of 11 patients with deep stromal amyloid deposits revealed bilateral, discrete, blue-white opacities at the level of deep stroma and Descemet membrane (DM). Endothelial cells were atrophic and flattened with gutta formation. Confoscans revealed hyperreflective, needle-shaped crystalline deposits of extracellular amyloid at various depths of the corneal stroma up to DM. All host corneal buttons demonstrated Congo red-positive amyloid deposits on histopathological examination.

**Conclusions:** We describe a distinct form of TK unlike the usual presentation of dense, leucomatous, vascularized corneal scarring in trachoma. We believe that amyloid deposits in DM and the corneal endothelium have not previously been reported in patients with trachoma.

**Key Words:** trachoma, secondary amyloidosis, corneal phenotype, pseudoguttae, confocal microscopy, histopathology

**Cornea** 2022;41:609–615

Trachomatous keratopathy (TK), a long-term outcome of chronic keratoconjunctivitis caused by particular strains of *Chlamydia trachomatis*, is the leading infectious cause of blindness globally.¹ ² Trachoma is characterized by recurrent or persistent inflammation of the conjunctivae, leading, after many episodes of infection, to conjunctival scarring.³ TK usually occurs when conjunctival scarring precipitates trichiasis (with or without entropion), with eyelash–corneal contact encouraging corneal abrasions that heal with the formation of vascularized corneal opacities. Conjunctival scarring–induced tear film changes facilitate secondary corneal infections that further damage the cornea.⁴ ⁵ ⁶ Corneal disease contributing to vision loss in patients with chronic sequelae of trachoma can also occur because of the deposition of amyloid in the corneal stroma.⁷ Secondary corneal amyloidosis is a form of corneal degeneration that occurs as a feature of several chronic ocular disorders such as trachoma, keratoconus, trichiasis, spheroideal degeneration, uveitis, and viral keratitis.⁸ ⁹ ¹⁰ Data on the clinical and confocal microscopic features of TK with secondary amyloidosis are scarce in the literature.¹¹ This study reports the atypical clinical presentation, in vivo confocal microscopic features, and corneal phenotype of patients with TK and secondary amyloidosis.

**MATERIALS AND METHODS**

The study was approved by the Institutional Ethics Committee, All India Institute of Medical Sciences, New Delhi, India (IEC-741/04.10.2019). We undertook a retrospective review of records of patients presenting to the Dr. Rajendra Prasad Centre for Ophthalmic Sciences between January 1, 2017, and December 31, 2019, identifying those who underwent penetrating or lamellar keratoplasty for TK with visual impairment. The histopathology request-and-result forms for those patients were scanned for 1) a presumptive clinical diagnosis of atypical corneal involvement in the setting of TK mimicking endothelial dystrophy or atypical corneal dystrophy and 2) a histopathological diagnosis of TK plus amyloidosis. All patients fulfilling both criteria were included in the study.
Demographic profile; medical history; duration of disease; previous, concomitant, and subsequent ophthalmic surgical procedures; and family history were noted. Examiners recorded visual acuity, laterality, clinical signs of trachoma, including corneal opacity and its size, location, and depth, corneal vascularization, eyelid and eyelash abnormalities, upper pole pannus, Herbert’s pits, and upper tarsal conjunctival scarring. Findings on anterior segment optical coherence tomography (ASOCT; RTVue, Optovue Inc, Fremont, CA) and confocal microscopy (Confoscan 4.0 confocal microscope, Nidek Technologies, Italy) were documented.

For the purposes of this study, histopathological slides of patients with atypical corneal phenotype were freshly reviewed under the microscope by 2 observers, including an ophthalmic pathologist (S.K.) and an ophthalmologist (N.G.). A detailed layer-by-layer study was undertaken of the epithelial morphology, stromal details, amyloid deposits, involvement of posterior corneal layers including Descemet membrane (DM), and any secondary endothelial changes.

RESULTS

On review of histopathological records of host corneal buttons, we identified 163 patients with a presumed preoperative clinical diagnosis of TK. Of these, 15 patients (29 eyes) were found to have TK with atypical corneal involvement, distinct from the common presentation of scarred, vascularized corneal opacities occurring because of in-turned eyelashes and secondary infective keratitis with concomitant ocular surface disease (Fig. 1). Patient 10 had left phthisis bulbi secondary to trauma; hence, only his right eye was included in the study (Table 1). The median patient age was 66 years (range 51–75 years). Seven (47%) were male (Table 1). No patient had a documented previous history of nontrabomatous infectious keratitis or any known systemic disease predisposing to generalized amyloid deposition.

Preoperative Clinical Characteristics

The median duration of symptomatic visual impairment/corneal opacity was 48 months (range 8–120 months). The median preoperative best-corrected visual acuity by eye was 1.8 logarithm of the minimum angle of resolution (range, 1.2–2.6 logarithm of the minimum angle of resolution); all eyes had a best-corrected visual acuity of ≤6/36 at presentation (Table 1). Mild upper and lower eyelid entropion (posterior migration of meibomian glands) was present in 13 (87%) of 15 patients. Eight (53%) of 15 patients had 1 to 2 peripheral trichiatric eyelashes that were treated with epilation, but none were in contact with the cornea at the time of presentation. Two patients had previous histories of eyelid surgery for entropion correction.

On slit-lamp examination, regressed corneal pannus extending 1 to 4 mm at the superior corneal limbus was seen in 8 (53%) and superficial corneal vascularization (1–4 quadrants) in 13 (87%) of 15 patients. Herbert’s pits (Fig. 2A) and upper palpebral conjunctival scarring (Fig. 2B), representing signs of chronic trachoma, were present in all cases. There were no signs of active trachoma or conjunctival amyloid deposits. Corneal sensations were intact in all patients. Coexisting climatic droplet keratopathy or spheroidal degeneration, appearing as small yellow-golden globules on the corneal surface (Fig. 2C), was present in 9 (60%) and Salzmann nodular degeneration in 1 (75%) of 15 patients. Diffuse corneal haze was present in the anterior stroma (Fig. 2D) in 4 patients (27%) and full-thickness corneal involvement in 11 patients (73%). Of the 11 patients with full-thickness involvement, 8 (73%) revealed bilateral, numerous, circular to oval discrete blue-white opacities at the level of deep stroma and fine, gray and linear opacities at the level of DM and the endothelium, concentrated at the center, but some located in the inferior peripheral and paracentral region of the cornea (Fig. 2E). Central endothelial guttae were also seen in these 8 patients with endothelial involvement (Fig. 2F). The mean corneal thickness of patients was 506 μm (range 349–602 μm), and the depth of corneal opacification seen on ASOCT corresponded to clinical findings in all patients. The thickness of DM in 8 eyes with posterior stromal amyloid deposits ranged from 17 to 22 μm (Fig. 3).

Confocal Microscopy

Features seen on confocal imaging were similar in all eyes. Epithelial morphology was normal (Fig. 4A) except where spheroidal degeneration was present. Specific forms of extracellular amyloid deposition were seen at various depths of the corneal stroma up to DM. Amorphous deposits of amyloid were first to appear at a depth of 120 μm (Fig. 4B) and persisted at all greater depths but were more noticeable anterior to the mid stroma (160–260 μm) in most cases (Fig. 4C). Short, needle-shaped, crystalline deposits (Fig. 4D) alongside amorphous deposits were most frequently found. Longer, hyperreflective, crystalline, linear, needle-like deposits (Fig. 4E) were more prevalent in the deeper stroma (498–520 μm) and in some cases touched the endothelial layer. Endothelial cells displayed polymorphism and polymegathism in most eyes, whereas dropout areas were seen in 16 (55%) of 29 eyes (Fig. 4F).

Surgical Procedure

Eleven patients (11 eyes) underwent penetrating keratoplasty, whereas 4 patients (5 eyes) underwent lamellar keratoplasty. All host corneal buttons were processed for histopathological evaluation.

Histopathological Findings

Light microscopic examination of excised host corneal buttons fixed in formaldehyde–glutaraldehyde solution and subsequently stained with hematoxylin–eosin revealed scattered, fusiform deposits located in the corneal stroma that were oriented parallel to the stromal lamellae with an increase in their waviness (Fig. 5A). These deposits were periodic acid–Schiff positive and stained red-brown with Masson trichrome. Eyes with posterior stromal amyloid deposits showed evidence of mildly thickened DM and reduced endothelial cells (8 of 11 eyes that underwent penetrating keratoplasty). The endothelial cells in these corneal specimens were vacuolated, atrophic and...
| Patient Number | Age (yr)/Sex | Systemic Disease | BCVA at Presentation | Duration of CO (mo) | Type of Keratoplasty | Amyloid Deposits on HPE* | Endothelial Involvement on HPE* |
|---------------|-------------|------------------|----------------------|-------------------|---------------------|------------------------|-------------------------------|
| 1             | 66/F        | Nil              | 6/36 FCF†            | 18                | PK triple           | No subepithelial deposits | DM thickened Creased cell count No gutta |
| 2             | 72/M        | Nil              | 6/36 4/60†           | 24                | PK triple           | Mainly in the posterior stroma Amyloid on DM and the endothelium | Vacuolated cells Decreased cell count; PG+ |
| 3             | 68/F        | Nil              | 1/60† PL negative    | 60                | PK triple           | Mainly in the mid stroma | DM thickened Decrease in cell count Vacuolated endothelial cells; PG+ |
| 4             | 69/F        | Rheumatoid arthritis | 1/60 HMF†           | 120               | PK triple           | Sparse deposits in the mid and deep stroma | DM absent in the specimen |
| 5             | 65/M        | Depression       | FCCF                | 36                | PK                 | Few deposits in the mid stroma | DM not thickened Reduced cell count; PG+ DM thickened Vacuolated endothelial cells with sparse cytoplasm Reduced cell count; PG+ DM thickened Few vacuolated cells Markedly reduced cell count; PG+ |
| 6             | 75/M        | Chronic obstructive pulmonary disease, coronary artery disease, and hypertension | 2/60† 2/60          | 8                 | PK                 | Deposits in the anterior and posterior stroma Amyloid deposits on DM | — |
| 7             | 73/F        | Hypertension     | 1/60† 1/60          | 24                | PK                 | Deposits more in the mid and posterior stroma Deposits on DM | — |
| 8             | 70/M        | Type 2 diabetes mellitus | 1/60† 1/60†         | 60                | R-SALK L-ALTK      | Epithelium normal to slightly thickened Deposits in the anterior stroma | — |
| 9             | 64/F        | Nil              | 6//36 HMF†          | 24                | PK triple           | Mid and posterior stromal deposits | DM markedly thickened Endothelial cells absent; PG+ |
| 10            | 60/M        | Nil              | 1/60† 1/60          | 48                | SALK triple        | Few deposits in the anterior stroma | — |
| 11            | 66/M        | Nil              | 2/60 2/60†          | 60                | PK triple           | Mainly in the posterior stroma Amyloid on DM and the endothelium | Vacuolated cells Decreased cell count; PG+ |
| 12            | 73/F        | Hypertension     | FCF† 2/60           | 56                | ALTK               | Epithelium normal to slightly thickened Few deposits in the anterior stroma | — |
| 13            | 51/F        | Nil              | FCF† 6/60           | 48                | PK triple           | Deposits in all layers Deposits more in the mid and posterior stroma | Slightly thickened DM Normal endothelial cells |
| 14            | 65/M        | Nil              | 4/60† 6/60          | 59                | ALTK triple        | Deposits present in the anterior stroma | — |
| 15            | 64/F        | Type 2 diabetes mellitus | 1/60 PL+†          | 36                | PK triple           | Deposits in the central stroma | DM thickened Decrease in cell count Vacuolated endothelial cells; PG+ |

*HPE findings of the host cornea retrieved from 15 eyes after keratoplasty.
†Eyes underwent corneal transplantation for visual rehabilitation.
ALTK, automated lamellar therapeutic keratoplasty; BCVA, best-corrected visual acuity; B/L, bilateral; FCF, finger counting close to face; HMF, hand movements close to face; HPE, histopathological examination; PG, pseudoguttae; PK, penetrating keratoplasty; PL, perception of light; SALK, superficial anterior lamellar keratoplasty; Triple, keratoplasty + cataract extraction + intraocular lens implantation.
demonstrated decrease in cytoplasm. These cells were flattened and, in some places, appeared to be degenerating (Fig. 5B) with gutta formation (Fig. 5C). Congo red stains of the fusiform stromal deposits confirmed the diagnosis of amyloidosis by exhibiting birefringence and dichroism under polarized light. The amyloid deposits were distributed across all layers of the cornea from the subepithelial layer to various depths of the stroma reaching up to DM in some cases (Fig. 5B, D).

Cases with climatic droplet keratopathy demonstrated hyaline deposits in the subepithelial layer, the Bowman layer, and the superficial layer of the cornea. There was conspicuous absence of blood vessels or inflammatory cells in all but 1 corneal host tissue specimen; in that single case, there were dilated capillaries observed in the mid and deep stromal layers.

In patients with typical TK, host corneas had a thickened epithelium with stromal scarring and the presence of subepithelial cystic spaces in some cases (Fig. 6).

DISCUSSION

Trachoma is a neglected tropical disease and a leading cause of preventable blindness caused by an infectious agent. In endemic populations, ocular C. trachomatis infections are seen most frequently in preschool-age children, while the chronic sequelae leading to blindness become apparent years later. TK is the eventual result of the cicatrizing process that follows recurrent bouts of conjunctival inflammation and fibrosis. We present herein atypical corneal stromal features highlighted through confocal microscopy and histopathological examination in patients with TK associated with secondary amyloidosis.

We identified 15 patients with TK and secondary amyloidosis with corneal guttae and deep blue-white dot degeneration. Trachoma has previously been associated with localized secondary amyloidosis of the eyelids, conjunctiva, and cornea. Hidayat and Risco, in their clinicopathologic study of 62 corneal buttons with TK, reported amyloid deposition throughout the stroma and larger deposits in the posterior stroma; they inferred trachoma as the cause. DM and the endothelium were normal in all of their specimens. The findings of their study could not be corroborated with confocal microscopy because this novel technology was introduced after 1990. In all eyes of our study, preoperative confocal microscopy revealed hyperreflective needle-shaped crystalline deposits at the level of anterior, mid, and deep stroma including DM in some cases, and postoperative histopathological examination of corneal buttons demonstrated Congo red-positive amyloid deposition involving both superficial and deep stromal layers. This finding corroborates the work of Hidayat and Risco. In addition, we found amyloid deposits between the posterior stroma and DM, consistent with deep blue-white dot degeneration. We also noticed decreased endothelial cell counts, flattened and atrophic endothelial cells, and endothelial cell dropout areas on confocal microscopy and histopathological assessment, manifesting as guttae. The concept of pseudoguttae, although novel, has been discussed by Moshirfar et al. The distinguishing confocal features of pseudoguttae, described
as hyporeflective dark areas without hyperreflective centers, match the confocal findings in our patients.\(^{16}\)

The pathogenesis of secondary amyloidosis in trachoma is not clear. Previous reports have suggested tears, inflammatory cells, blood, stromal fibrocytes, degenerated collagen, and the corneal epithelial cells as possible precursors of amyloid in the cornea, but none have provided conclusive evidence.\(^{11,15,17,18}\) In preoperative examination of our patients, only superficial vascularization, extending less than 2 mm into the cornea, was present. Only 1 host corneal button revealed intracorneal blood vessels on histopathological examination. There was a conspicuous absence of inflammatory cells in all 15 corneal buttons. Previously, Lin et al\(^ {19}\) described 4 cases in which the anatomical location of trichiasis corresponded to nodular amyloid deposits, presumably related to mechanical trauma. In our series, 8 patients had trichiasis at presentation; it was adequately managed before keratoplasty, and no patient had contact between the cornea and trichiatic cilia by the time of surgery. Moreover, the amyloid deposits in our patients were observed within the corneal stromal layers with no increase in corneal thickness, as evidenced on ASOCT, confocal imaging, and histopathological examination. These were not superficial amyloid deposits occurring secondary to long-standing friction between trichiatic cilia and the corneal surface, leading to thickened and irregular corneal epithelium.\(^ {20}\) We hypothesize that corneal amyloidosis in our patients was not secondary to trichiasis, distichiasis, or epiblepharon as has been reported previously.\(^ {17,19}\)

**FIGURE 3.** ASOCT scan of a patient with TK and secondary amyloidosis showing normal corneal thickness (524 \(\mu\)m), hyperreflective endothelial deposits (red arrow), and mildly thickened DM (22 \(\mu\)m).
Matta et al.\textsuperscript{18} in their study of 22 patients with climactic droplet keratopathy and coexisting inactive trachoma, observed lattice lines extending up to DM. They proposed that TK could have caused the presence of amyloid in their patients. However, they did not report any endothelial involvement in their patients with corneal amyloidosis, in contrast to the findings in our series.

FIGURE 4. Confocal features showing extracellular hyperreflective deposits at various levels: A, Normal epithelial morphology. B, At 123 μm, amorphous deposits appear. C, At 233 μm, an increased number of amorphous deposits along with few short needle crystalline deposits are evident. D, Multiple, short, needle-shaped crystalline deposits seen at 258 μm. A few long needle-shaped deposits also begin to appear at this depth. E, Numerous long, stellate, fine needle-shaped crystalline deposits seen in the deeper stroma. F, Endothelial cells displaying polymegathism and few dropout areas (guttae).

FIGURE 5. Histopathological characteristics of findings in host corneal buttons: A, Light microscopic image [100×, hematoxylin and eosin (H&E) stain] revealing extracellular amyloid throughout the stroma extending up to DM (black arrows). Note that DM is detached and coiled. Reduced endothelial count and vacuolated endothelial cells can also be appreciated (blue arrows). B, Mildly thickened DM (black arrow), and vacuolated (blue arrow) and degenerated endothelial cells (light blue arrow) (<200, H&E stain). C, Guttae seen as anvil-shaped thickening of DM (red arrows) (<200, H&E stain). (D) Polarized light photograph demonstrating apple-green birefringence, involving the deep stroma and pre-DM layer (white arrows), characteristic of amyloid after staining with Congo red. DM is detached showing secondary coiling (kindly ignore the artifacts on the glass slide arising due to light reflectance and noise due to polarized particles). (The full color version of this figure is available at www.corneajrnl.com.)
To the best of our knowledge, no previous study on TK has reported the presence of associated endothelial changes and formation of guttae. The endothelial guttae seen in our cases were central in location and were visible on histopathological slides and in vivo confocal scans. Amyloid deposits in the form of blue-white dots and excrescences were seen in 16 of 29 eyes.

Our work has limitations. We did not perform genetic testing for TGFBI because of limited resources. Considering the elderly age profile of our patients, negative family history, distinct clinical features of trachoma, and absence of corneal lattice lines, host genetic studies would not have affected patient care or likely prognosis. The retrospective nature of our work increases the risk of misclassification bias. Although the patients we included had features of cicatricial trachoma, including Herbert’s pits, and an absence of any other obvious cause for corneal scarring, trachoma cannot be proven to be the cause of their corneal opacification or their amyloidosis.

We propose here an unusual form of TK with a novel pathogenesis and structural pattern. Ophthalmologists working in trachoma-endemic regions should take note to avoid diagnostic confusion with corneal dystrophy. This manifestation of TK may also be of interest to public health specialists because it may reveal an alternative pathogenesis of corneal blindness in some patients who have trachoma.

REFERENCES

1. Hadfield J, Harris SR, Seth-Smith HMB, et al. Comprehensive global genome dynamics of Chlamydia trachomatis show ancient diversification followed by contemporary mixing and recent lineage expansion. Genome Res. 2017;27:1220–1229.
2. Bourne RR, Stevens GA, White RA, et al. Causes of vision loss worldwide, 1990–2010: a systematic analysis. Lancet Glob Health. 2013;1:e339–e349.
3. Gambhir M, Basanez MG, Burton MJ, et al. The development of an age-structured model for trachoma transmission dynamics, pathogenesis and control. Plos Negl Trop Dis. 2009;3:e462.
4. el-Rakhawy KE, Zayed AI, van Bijsterveld OP. Tear function in relation to the World Health Organization classification of cicatization in trachoma. Int Ophthalmonol. 1988;12:31–35.
5. Al-Fawaz A, Wagoner MD, King Khaled Eye Specialist Hospital Corneal Transplant Study Group. Penetrating keratoplasty for trachomatous corneal scarring. Cornea. 2008;27:129–132.
6. Chaudhry IA. Eradicating blinding trachoma: what is working? Saudi J Ophthalmonol. 2010;24:15–21.
7. Santo RM, Yamaguchi T, Kanai A. Spheroidal keratopathy associated with subepithelial corneal amyloidosis. A clinicopathologic case report and a proposed new classification for spheroidal keratopathy. Ophthalmology. 1993;100:1455–1461.
8. McPherson SD, Kiffney GT, Freed CC. Corneal amyloidosis. Am J Ophthalmol. 1966;62:1025–1033.
9. Tejwani D, Azuara-Blanco A, MacKenzie J. Localised corneal amyloidosis associated with herpetic keratitis. Br J Ophthalmonol. 2003;87:1049.
10. Stern GA, Knapp A, Hood CI. Corneal amyloidosis associated with keratoconus. Ophthalmology. 1988;95:52–55.
11. Hidayat AA, Risco JM. Amyloidosis of corneal stroma in patients with trachoma. A clinicopathologic study of 62 cases. Ophthalmonol. 1989;96:1203–1211.
12. Ending the Neglect to Attain the Sustainable Development Goals: A Road Map for Neglected Tropical Diseases 2021–2030. Geneva, Switzerland: World Health Organization; 2020. Available at: https://apps.who.int/iris/handle/10665/338565. Accessed January 28, 2021.
13. Gupta VP, Aggarwal R, Aggarwal S. Conjunctival amyloidosis: is it related to trachoma? Acta Ophthalmonol (Copenh). 1993;71:839–840.
14. Smith ME, Zimmerman LE. Amyloidosis of the eyelid and conjunctiva. Arch Ophthalmonol. 1966;75:42–50.
15. Selvan H, Singh A, Tandon R. Deep blue dot corneal degeneration: confocal characteristics. Int Ophthalmonol. 2019;39:667–669.
16. Moshirfar M, Liu Y, Vaidyanathan U, et al. Diagnosis and management of pseudogutta: a literature review. Med Hypothesis Discov Innov Ophthalmonol. 2019;8:156–162.
17. Aldave AJ, Principe AH, Lin DY, et al. Lattice dystrophy-like localized amyloidosis of the cornea secondary to trichiasis. Cornea. 2005;24:112–115.
18. safe.}

FIGURE 6. Histopathological features of host corneal tissue in patients with TK with no clinical evidence of amyloid. A, Light microscopic image reveals intraepithelial cystic spaces (blue arrow), thickened epithelium, absence of the Bowman layer, and scarring within the stroma along with mononuclear inflammatory cells [100x, hematoxylin and eosin (H&E) stain]. B, Corneal epithelium is thickened (red arrow) with hyperkeratosis. Note the dense scar replacing the corneal stromal layer (black arrow), barely thinner than the overlying epithelium with decrease in total corneal thickness due to replacement of normal collagenous stroma with fibrous tissue and consequent shrinkage that occurs in TK. The area on the left (blue arrow) with disorganized collagenous stromal tissue shows evidence of inflammatory cell infiltration [100x, H&E stain]. (The full color version of this figure is available at www.cornejrnl.com.)