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CASE REPORT

Surgical management and immunohistochemical study of corneal plaques in vernal keratoconjunctivitis

Hsiu-Yi Lin [a], Po-Ting Yeh [a], Cheng-Shiang Shiao [b], Fung-Rong Hu [a,*]

[a] *Department of Ophthalmology, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan*
[b] *Department of Pathology, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan*

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Two children with shield ulcer in vernal keratoconjunctivitis unresponsive to steroid therapy received plaque removal by superficial keratectomy, followed by amniotic membrane transplantation (AMT). Hematoxylin and eosin staining of the excised corneal specimen revealed a thick layer of eosinophilic material attached to the Bowman's layer. These deposits were positive for eosinophil granule major basic protein, as confirmed by an immunohistochemical study. The shield ulcer healed after the amniotic membrane was removed. No recurrent corneal plaque developed, although corneal opacity complicated in both cases. Lamellar keratectomy with AMT offers an effective management by removing the cytotoxic plaques and protecting the denuded stroma from deposition of inflammatory debris.

Introduction

Vernal keratoconjunctivitis (VKC) is an ocular allergy disease that predominantly affects young males in their first decade of life and subsides after puberty. This bilateral, seasonal, and recurrent disease usually presents with intense itching, photophobia, and copious mucoid discharge. Clinical characteristics of VKC include giant papillae on the upper tarsal conjunctiva or at the limbus,

* Corresponding author. Department of Ophthalmology, National Taiwan University Hospital, College of Medicine, National Taiwan University, 7 Chung-Shan South Road, Taipei 10002, Taiwan.
E-mail address: fungronghu@ntu.edu.tw (F.-R. Hu).

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superficial corneal erosion, shield ulcer of cornea, and corneal scar that may result in severe visual impairment.[1]

VKC was classified into three categories: palpebral, limbal, and mixed form.[2] Shield ulcer with corneal plaque is uncommon and is found only in 3.25% patients. However, it is a serious vision-threatening complication that puts patients at risk of occlusion amblyopia.[3] In these cases, secondary infection or perforation could also occur.[2,4–6] Surgical debridement is necessary in cases with corneal plaque unresponsive to conservative medical treatment.[2]

Amniotic membrane transplantation (AMT) has been used widely to treat ocular surface disease in the past decade.[7] Amniotic membrane has several functions including facilitation of epithelialization,[8] inhibition of fibrosis,[9] and anti-inflammatory[10] and antimicrobial[11] effects. AMT has successfully been applied in neurotrophic ulcers, persistent epithelial defects, microbial keratitis, band keratopathy, bullous keratopathy, following photorefractive keratectomy, chemical injury, pterygium, and symblepharon.[12]

In this report, we will demonstrate the effects of surgical management with AMT, describe the histopathologic findings of the ulcers, and discuss their possible role in VKC.

Case reports

Case 1

A 13-year-old boy had a history of atopic diseases including asthma, dermatitis, and rhinitis. Because of severe symptoms of eye itching, tearing, and diffuse papillary hypertrophy on the upper palpebral conjunctiva, VKC was diagnosed at the age of 7 years. Topical betamethasone and cromolyn sodium in both eyes and oral cetirizine were prescribed. Steroid-induced glaucoma was observed, and topical betaxolol was used to control the intraocular pressure.

Repeated episodes of grade 2 shield ulcer developed in the left eye since March 2005. Intensive topical dexamethasone was given. Surgical debridement, superficial keratectomy, and excimer laser phototherapeutic keratectomy with bandage contact lens were performed four times during June 2005 to January 2006 with partial effect. However, a corneal plaque developed at the upper part in March 2006 (Fig. 1). The best-corrected visual acuity (BCVA) decreased to 0.05 in the left eye. Lamellar keratectomy with AMT was then performed on March 15, 2006. Complete re-epithelialization was found within 1 week after surgery.

Another episode of shield ulcer occurred in the right eye of the patient in December 2006. The BCVA was 0.05. He had another surgery of lamellar keratectomy with AMT in January 2007. Due to severe itching and tearing, the amniotic membrane dislodged in postoperative 3 weeks prior to complete re-epithelialization. The corneal plaque at the nasal part was observed 3 months after the persistent epithelial defect (Fig. 1). Repeated surgeries of lamellar keratectomy with AMT were needed during February—April 2007. Complete re-epithelialization was achieved 1 month after the last surgery. The postoperative BCVA was 0.1 in the right eye and 0.2 in the left eye (Fig. 2).

Case 2

An 8-year-old boy presented with symptoms of eye itching and photophobia for 1 year. Oral antihistamine and topical drugs were given, but in vain. Bilateral shield ulcer had persisted for 4 months when the boy first came to our hospital on March 20, 2007. The BCVA was 0.7 in the right eye and 0.2 in the left eye. Examination revealed corneal plaques located at the lower part of the right eye and the upper part of the left eye (Fig. 3). The patient was treated with topical dexamethasone and oral levocetirizine. There was no response to the medical treatment. Hence, lamellar keratectomy in both eyes and AMT in the left eye were performed on March 28, 2007. Shield ulcer healed after the amniotic membrane was removed 6 weeks postoperatively. The BCVA improved to 1.0 in the right eye and 0.4 in the left eye (Fig. 3) 3 months after surgery.

Surgery

Surgeries were performed under general anesthesia. The corneal plaque was excised with a No. 64 crescent surgical blade. Lamellar keratectomy was performed to the depth to which the plaque invaded. The plaque and the surrounded superficial corneal stroma were removed in both cases. No remarkable surgical plane between the plaque and the Bowman's layer was identified. The entire cornea, including the area of denuded stromal bed, was then covered with the amniotic membrane. The amniotic membrane with stroma-side up and basement membrane-side down was secured to the episclera around the limbus

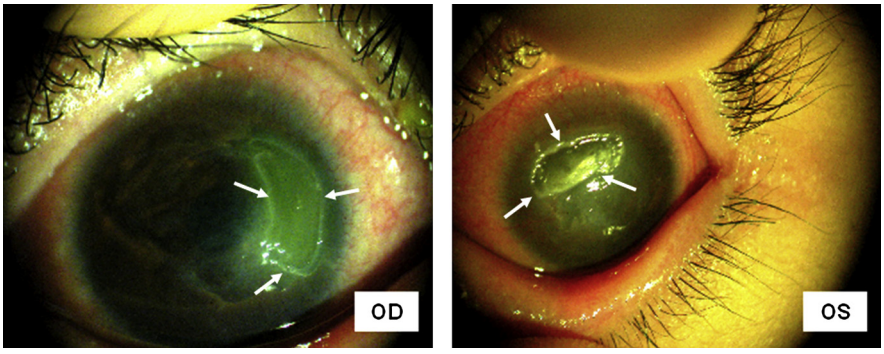

Figure 1 Case 1. Photographs prior to operation. Corneal epithelial defect with white corneal plaque (arrows) in both eyes.

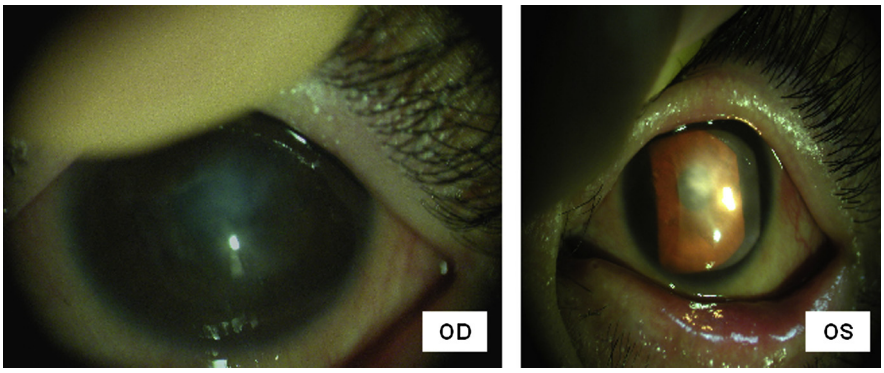

Figure 2 Case 1. Photographs after operation. Corneal opacity located at the central part of the cornea in both eyes.

by monofilament 10-0 nylon sutures. Interrupted circumferential sutures were used, and the knots were buried followed by application of a therapeutic soft contact lens. All the preoperative medications were continued in the postoperative period.

Histopathologic and immunohistochemical study

All the specimens taken during lamellar keratectomy were fixed in formalin, embedded in paraffin, and sent for histopathologic study. An immunohistochemical study was carried out to confirm the presence and location of eosinophil-derived major basic protein (MBP) on hematoxylin—eosin sections. We performed procedures similar to those mentioned earlier. The lymph node, which was filled with eosinophils, derived from a patient with Kimura disease served as a positive control. Von Kossa stain was also performed to confirm whether the calcification existed in the whitish plaque.

Microscopic examination of hematoxylin—eosin-stained sections revealed a thick layer of eosinophilic material attached to most surface of the Bowman's layer in both cases (Fig. 4, top). The laminated material tightly adhered to the underlying Bowman's layer. The epithelial cells were absent in the area where the Bowman's layer was coated with the eosinophilic material. The residual epithelium showed mild hyperkeratosis and parakeratosis. There was no inflammatory infiltration inside or below the eosinophilic plaque. Positive granular staining for eosinophil MBP was presented by an immunohistochemical study in the sections.

Discussion

VKC is an immune-mediated disorder of conjunctiva. The inflammatory disease has a multifactorial pathogenesis involving both Type I and Type IV hypersensitivity. Since the mechanism is unclear, most studies indicate that eosinophils, mast cells, lymphocytes, and a complex network of interleukins and cell mediators play major roles in the clinical presentation.[13] In the study of Bonini et al,[14] eosinophils were constantly detected in the epithelium of conjunctival scraping (85%). In contrast, basophils, neutrophils, and lymphocytes were observed rarely. They concluded that activated eosinophils with their mediators and adhesion molecules are the key factors in ocular surface inflammation and tissue damage.

Shield ulcer with corneal plaque is rare but can result in a severe visual development problem in children. Two hypotheses regarding shield ulcer were proposed in the literature. First is the mechanical hypothesis, according to which shield ulcer is caused by the friction from giant papillae on the upper tarsal conjunctiva and subsequent recurrent corneal abrasion.[1] This is why shield ulcer is more frequently located in the superior cornea (63.5% vs. 10.6% inferior) and is of palpebral type (85.4% vs. 2.4% limbal).[2] Second is the toxin hypothesis, which emphasizes the toxic effect of inflammatory mediators on the tear film.[15] These cytotoxic compounds may inhibit wound healing of shield ulcer and cause persistent corneal epithelial defects.

Cameron[2] proposed a classification system for shield ulcer based on the initial presentation. Patients having ulcers with a transparent base (grade 1) had rapid re-

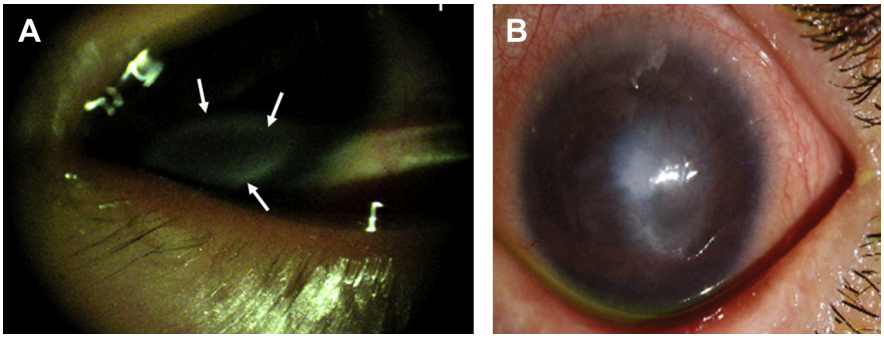

Figure 3 Case 2. (A) Photograph prior to operation. Large corneal plaque (arrows) in the left eye. (B) Photograph after operation. Corneal scar located in the central area.

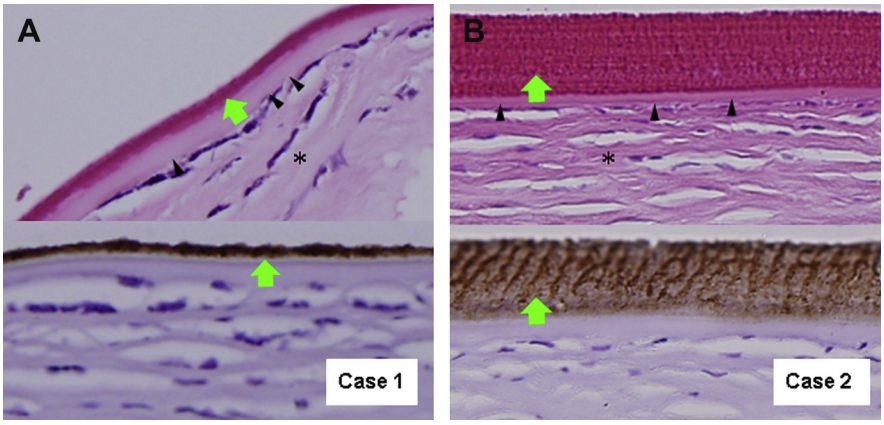

Figure 4 (A) Case 1, hematoxylin and eosin stain (100×). (B) Case 2, hematoxylin and eosin stain (400×). (A and B, top) Dense laminated layer of eosinophilic material (green arrows) adhered to Bowman's layer (black arrowheads) in both cases. Asterisks designate stroma. There is no residual epithelium under the thick eosinophilic layer. (A and B, bottom) Positive staining for eosinophil major basic protein (brown stain) in the plaque adhered onto Bowman's layer.

epithelialization under medical treatment alone. They had mild corneal scarring and favorable visual outcome. When opaque white or yellow deposits developed in part of the clear base (grade 2), poor medical response with delayed re-epithelialization usually presented at this stage. Dramatic re-epithelialization was followed by a simple surgical procedure. Two patients in our study had elevated plaques (grade 3) above the level of the surrounding epithelium. Worst visual prognosis in this group was caused by significant retardation of epithelial healing, corneal scarring, and high risk of concomitant bacterial keratitis. In Cameron's report, two out of 15 (13%) plaques recurred after surgical debridement.

Corneal plaque (grade 3) usually presented after a period of nonhealing epithelial defect. Immunochemical evidence of eosinophil granule MBP was found in the deposit.[16] The cytotoxic effect of MBP has been disclosed in human tissues.[17] In animal studies, corneal epithelial wound healing was inhibited by MBP.[18] The rate of epithelial cell migration during re-epithelialization was affected by MBP in proportion to the concentration. A higher concentration of MBP could nearly abort cell migration and protein synthesis.[16,18] Piled-up leading edge of epithelium was also observed, especially in high-concentration group, as a sign of poor wound healing. The abnormal findings strengthened the cytotoxic theory of MBP deposit complicated with prolonged shield ulcer.

Eosinophil granule MBP was identified in the ocular surface, such as tear film and conjunctiva, of patients with VKC.[15,19] Electron microscopy showed superficial erosion of the Bowman's layer, with collagen fibril extending into the corneal plaque.[20] In our cases, histopathologic studies confirmed that the Bowman's layer was firmly attached to the lamellar MBP in both patients with grade 3 shield ulcer, which might be devoid of epithelium coverage.[21]

Based on the clinical presentation, histopathologic findings of our cases, and previous studies,[11,14] we suggest that abundant MBP derived from tear and conjunctiva, especially the giant papillae, initiates the pathogenesis of shield ulcer. The interpapillary space of palpebral VKC can serve as a reservoir of the cytotoxic material. As a high concentration of the MBP accumulates on the ocular surface, the punctate erosions advance to a more severe injury—coarse epithelial keratopathy. Since the deposit hinders the migration of epithelial cells on the Bowman's layer and exerts cytotoxic effect on re-epithelialization, a persistent corneal wound develops. Once the epithelial defect persists for several weeks, the cytotoxic substances may precipitate on the denuded Bowman's layer and form a thick plaque. The vicious circle contributes to the poor wound healing in grade 3 shield ulcer with corneal plaque.

Management of corneal plaque in VKC should include both removal and prevention of deposition from the cytotoxic protein. Although a mechanical factor might contribute to the shield ulcer, papillectomy, which reduces the friction and probably decreases a small amount of MBP load, usually has limited effects on wound healing. Papillae regrowth was observed in most patients.[22] In our cases, therapeutic soft contact lens had been prescribed to protect corneal wound from friction, but in vain. Reduction of mechanical trauma without inhibiting cytotoxic effects was not enough.

Early surgical scraping or debridement of inflammatory deposit was suggested by Cameron.[2] In grade 2 ulcer, re-epithelialization usually occurs within 1 week. However, in grade 3 VKC plaques, firm adherence from corneal plaque to the underlying Bowman's layer was observed. Partial resection of the Bowman's membrane could not be avoided as no surgical plane was detected between the two layers on electron microscopy.[20] Excimer laser phototherapeutic keratectomy may help smoothen ocular surface and remove residual inflammatory deposits.[23]

The amniotic membrane patch served as a basement membrane that facilitates migration of epithelial cells, promotes adhesion of basal cells, and prevents epithelial apoptosis.[8] AMT in combination with debridement had been reported to be effective in treating shield ulcer.[24] However, in our case, early melting of the amniotic membrane was significant in severe ulcer due to profound inflammation. The exposure wound might lead to recurrence of cytotoxic deposits in ulcer base postoperatively prior to complete re-epithelialization. Instead of grafting, we covered the denuded stroma after lamellar keratectomy using the amniotic membrane as a therapeutic contact lens.

Satisfactory wound healing and visual outcome were obtained, especially in Case 2. No recurrence of corneal plaque was noted in both cases in the following 2 years. Successful results were also observed in a case study with a relatively shorter duration of epithelial defects.[25] We believed that the advantages include sheltering the ulcer base from deposition of cytotoxic protein and providing various growth factors.

MBP, one of the several cationic proteins secreted from activated eosinophils, plays an important role in the mechanism of shield ulcer with corneal plaque. Whether there is any other cytotoxic material within the plaque has not been demonstrated. Besides, strong adhesion between a corneal plaque and the underlying Bowman's layer observed in clinicopathological findings suggests close interaction between MBP and collagen fibers.[16] Further studies may focus on the target therapy of eosinophil cytotoxic proteins and the management of their tight connection with corneal collagen fibers.

In conclusion, shield ulcer with corneal plaque is derived from the vicious circle formed by cytotoxic protein and poor re-epithelialization. Successful management of the complicated VKC further confirmed the nature of the disease. Lamellar keratectomy with AMT offers an effective management by removing the cytotoxic plaques and protecting the denuded stroma from deposition of inflammatory debris.[2,24]

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