Chlorhexidine in the treatment of microsporidial stromal keratitis and the effect of host immunity: A case series and literature review

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Abstract: Microsporidial stromal keratitis is an increasingly well-known vision-threatening disease. A large proportion of cases are initially misdiagnosed as herpes simplex keratitis and treated with topical steroids. In most of such cases, medical treatment failed, and corneal transplantation was required. This study reported the results of 0.02% topical chlorhexidine used to treat three cases of microsporidial stromal keratitis and reviewed the literature on the outcomes of microsporidial stromal keratitis treatment. In the first case, histopathology of a specimen from penetrating keratoplasty (PK) revealed severe chronic inflammation involving the entire stromal layer but no microorganism activity after the application of topical chlorhexidine for 10 months. The second case exhibited complete resolution of keratitis after topical chlorhexidine. The patient in the third case did not respond to medical treatment, and therapeutic PK was performed. Histopathological examination revealed numerous microsporidial spores that had colonized in the mid and deep stroma, where few inflammatory cells were observed. These findings explain the variable microsporidial susceptibility to chlorhexidine, suggesting the crucial role of host immunity. In cases of host immunity, topical chlorhexidine may represent a promising option for the treatment of microsporidial stromal keratitis.

Keywords: Antimicrobial resistance; Chlorhexidine; Cornea; Microsporidia; Stromal keratitis

1. INTRODUCTION

Microsporidia corneal infection can manifest as either superficial keratoconjunctivitis or stromal keratitis.[1] Although superficial keratoconjunctivitis is self-limited,[2] stromal keratitis typically requires therapeutic penetrating keratoplasty (TPK) for infection control.[3–5] Treatment with topical antiparasitic or antifungal agents with or without oral albendazole or itraconazole is ineffective in most.[3,6–11] These patients are often initial misdiagnosed as having herpes simplex virus (HSV) stromal keratitis and treated with topical corticosteroids,[3] which can delay the proper diagnosis and negatively affect outcomes. In this paper, we describe the treatment responses to topical 0.02% chlorhexidine in three cases of microsporidial stromal keratitis. In patient 1, the infection was resolved but optical penetrating keratoplasty (OPK) was required for visual recovery. Patient 2 responded well to treatment, achieving complete visual recovery. Patient 3 underwent TPK because of uncontrolled keratitis. Histopathology demonstrated that microsporidial spores were few or absent in areas with numerous inflammatory cells and abundant in areas lacking such cells.

2. METHODS

This study protocol was approved by the Institutional Review Board and ethics committee of the Taipei Veterans General Hospital and followed the Declaration of Helsinki. Informed consent was obtained from all enrolled patients. This retrospective review included three patients diagnosed as having microsporidial stromal keratitis between October 2013 and March 2018. Microsporidia were confirmed using polymerase chain reaction (PCR) of corneal scrapings or biopsy specimens, and further sequencing was used to identify *Vittaforma corneae*.

3. RESULTS

3.1. Case 1

A 58-year-old man presented with complaints of redness and blurring of vision in the right eye and was diagnosed as having HSV keratitis. He had experienced intermittent episodes over the previous year, for which he had been prescribed oral and topical antivirals and topical corticosteroids. Slit-lamp examination of the right cornea revealed whole-layer diffuse stromal infiltrates and partial ring infiltrates. Best-corrected visual acuity (BCVA) in the right eye was 3/60. The patient was prescribed topical acyclovir ointment and topical 1% prednisolone five times per day. Two weeks later, more pronounced corneal infiltrates were observed (Fig. 1A). Analysis of corneal scrapings suggested the presence of an intracellular microorganism. Hence, corneal biopsy was performed, which revealed numerous microorganisms, which were

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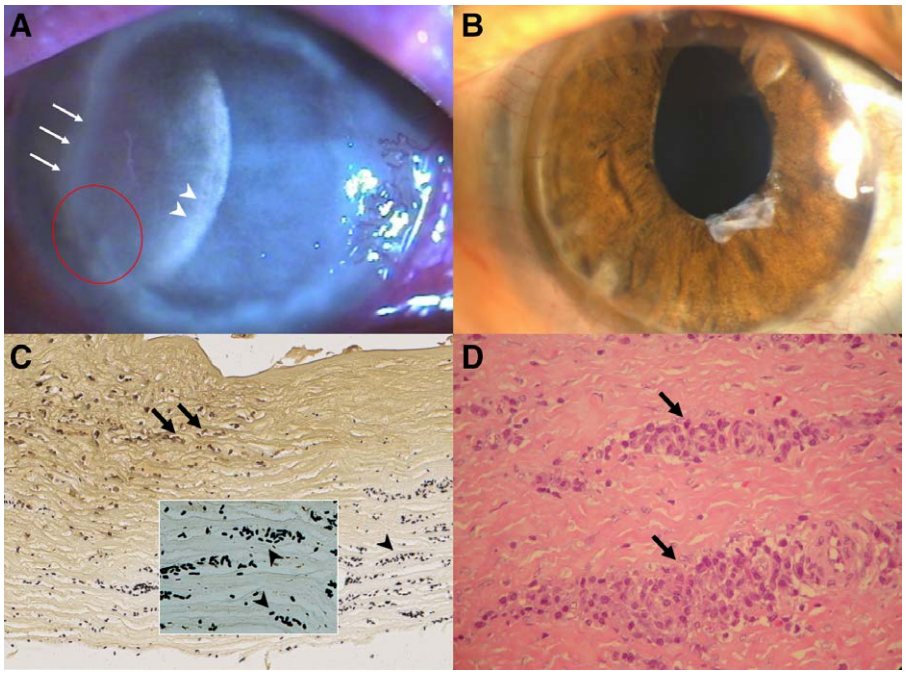

Fig. 1 Case 1. (A) Slit-lamp photograph of right cornea illustrating ring infiltrates (arrows) and diffuse coarse stromal infiltrates (arrowheads). The red circle indicates the biopsy area, including the ring infiltrates. (B) Disease resolution after PK and cataract surgery. (C) Biopsy specimen. On the left side of the image, the ring infiltrate area exhibits numerous inflammatory cells (arrows) but only a few spores in the anterior lamella. The right side of the image indicates few inflammatory cells but numerous microsporidial spores in the black oval shaped structures (arrowheads) in the posterior lamella (Warthin–Starry stain, 400× original magnification; higher magnification of microsporidia spores shown in insert). (D) Tissue sections obtained through PK indicate pronounced chronic inflammatory cells (arrows) distributed similarly to the microsporidial spores observed in biopsy sections. No microsporidial spores were observed in this specimen (H&E stain, 400× original magnification).

later identified as *V. corneae* based on PCR and sequencing. The microsporidia spores largely accumulated in interlamellar spaces, with few inflammatory cells (Fig. 1C).

TPK was performed, but it could not remove all of the spores because the infiltrates had already reached the perilimbal region. Therefore, 0.02% chlorhexidine eye drops (hourly) were prescribed. The inflammation ameliorated 10 months after treatment initiation, but the cornea became completely opaque. The patient then underwent OPK, and histopathological examination of the corneal button did not indicate the presence of microorganisms. PCR to detect microsporidia yielded negative results. The interlamellar spaces, which were filled with microsporidia spores, were occupied by chronic inflammatory cells, predominantly plasma cells, histiocytes, and some lymphocytes (Fig. 1D). The patient recovered well after OPK (Fig. 1B), with no sign of recurrence over a 5-year follow-up period. His postoperative BCVA was 6/6.

3.2. Case 2

A 34-year-old man presented with 1-month history of pain and redness in the right eye. He had been treated with topical dexamethasone for a short period. His BCVA was 6/6 in both eyes. The right cornea exhibited a well-demarcated area with mid-to-deep coarse stromal infiltrates that extended from the inferior limbus to the inferior pupillary border (Fig. 2A). We performed corneal biopsy, and histopathology revealed inflammatory cells but no evidence of microorganisms. However, PCR and sequencing confirmed the presence of *V. corneae*. On the basis of our experience with patient 1, this patient was administered topical 0.02% chlorhexidine eye drops (hourly) for nearly 2 months, which was gradually tapered off for 9 months. The infiltrates disappeared, and corneal scarring remained (Fig. 2B). Over a 2-year follow-up period, no recurrences were reported, and the patient's BCVA was maintained at 6/6.

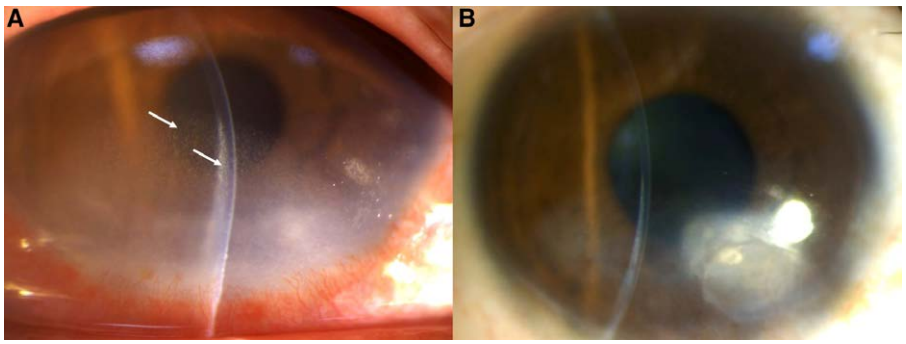

Fig. 2 Slit-lamp images in case 2 before and after treatment with 0.02% chlorhexidine. (A) Before treatment, the inferior cornea contained a well-demarcated area with mid-to-deep coarse stromal infiltrates (arrows). (B) One year after topical chlorhexidine was discontinued, the infiltrates were resolved, and scarring remained.

3.3. Case 3

A 65-year-old woman was referred to us with a 10-month history of persistent keratitis in her right eye. When she first presented to our clinic, she had been applying topical acyclovir ointment and 1% prednisolone eye drops hourly. The vision in her right eye was finger-counting at 20 cm. Coarse infiltrates were observed in the mid-to-deep right corneal stroma (Fig. 3A), and an analysis of smears of corneal scrapings revealed microsporidial spores, which were later identified as *V. corneae* through PCR and sequencing. We encouraged the patient to discontinue the 1% prednisolone eye drops and prescribed hourly topical 0.02% chlorhexidine. The ocular surface inflammation increased, and corneal epithelial defects developed, which improved after the use of 0.02% fluorometholone and therapeutic contact lenses. However, crystalline keratopathy with *Streptococcus mitis* and *oralis* infection developed. Although the infection was controlled with topical moxifloxacin, the patient's ulcers worsened after discontinuing use of the contact lenses. After 5 months of chlorhexidine treatment, we performed TPK. Histopathological examination of the host button revealed areas of ulcerative keratitis in the anterior stroma characterized by histiocytes and neutrophil infiltration but only a few microsporidial spores (Fig. 3C). However, numerous spores had colonized in the mid and deep stroma, where few inflammatory cells were present (Fig. 3D).

Because microsporidial spores were present, after PK, we switched the treatment to 0.02% polyhexamethylene biguanide (PHMB). At the 3-month follow-up, signs of recurrence and coarse mid-stromal infiltrates were noted, and the patient reported to have discontinued PHMB. The infiltrates were resolved after reapplication of PHMB eye drops, and this treatment was continued until 4 months after PK, at which time the patient had recovered well and underwent cataract surgery 6 months after PK (Fig. 3B). After surgery, her BCVA was 6/7.5. No recurrences were reported during the follow-up of more than 2 years.

4. DISCUSSION

Microsporidial stromal keratitis is often initially diagnosed as HSV keratitis because it usually presents with amorphous or multifocal, coarse, corneal stromal infiltrates.[10] The diagnosis requires the identification of microsporidial spores or DNA in corneal samples. Thus, analyzing empirical treatment failure can aid in the differential diagnosis of microsporidial stromal keratitis.

A review of the literature (Table 1) indicated that no treatment for microsporidial stromal keratitis has demonstrated consistent therapeutic efficacy.[3,5,8,10,12–15] Huang et al reported 14 cases of microsporidial stromal keratitis treated with either topical or oral fluoroquinolones and antifungal agents. None of the patients responded to treatment and eventually required surgical intervention.[13] Although the responses to treatment varied in this case study, we successfully controlled the infection with topical chlorhexidine in patients 1 and 2. The pathological results for patients 1 and 3 indicated that after the intensive topical chlorhexidine treatment, microsporidial spores were absent in areas with inflammatory cells but abundant in areas without inflammatory cells. This finding highlights the importance of host immunity in the effectiveness of treatment.

The reasons for the considerable variation in host immune responses to microsporidial stromal infection warrant consideration. According to previous reports, microsporidial stromal keratitis is often initially misdiagnosed as HSV keratitis, for which topical steroids are prescribed,[3,5,8,13,15] which increases the risk of treatment failure. Garg reported that 82% of patients in his series with available medical history were misdiagnosed as having HSV keratitis and treated with topical corticosteroids.[3] Only 2 of the 19 patients responded to the treatment. In Huang et al, 14 patients did not respond to treatment, and 42.9% received topical corticosteroids before admission.[13] For all the three cases of successful treatment, the initial diagnosis

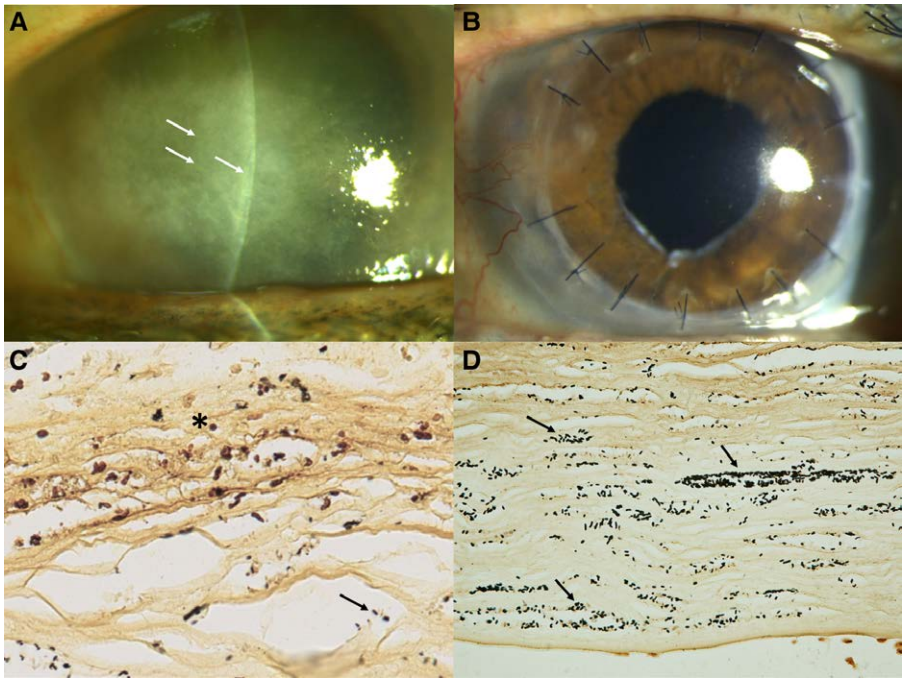

Fig. 3 Slit-lamp photographs and histopathology images of the corneal button from PK in Case 3. (A) Upon presentation, the patient's right cornea exhibited coarse infiltrates (arrows) in the mid-to-deep stroma. (B) Nineteen months after PK and 8 months after cataract surgery, the graft was clear except for scarring (arrows) due to recurrence. (C) Anterior stroma exhibit inflammatory cells (asterisk) and a few microsporidial spores (arrow), which appear as black oval shaped structures (Warthin–Starry stain, 400× original magnification). (D) In the mid and posterior stroma, numerous spores (arrow) and few inflammatory cells were noted (Warthin–Starry stain, 400× original magnification).

Table 1.

Outcomes of treatment in studies on microsporidial stromal keratitis

Author	Date of Publication	Study Design/ Sample Size	Previous Diagnosis/ Treatment	Medical Treatment	Total Treatment Duration	Outcome	Final Visual Acuity
Font et al[15]	2000	Case Report; 1 case	HSK/topical fluorometholone	Topical fumagillin and oral albendazole	14 wks	TLK then TPK with complete resolution	20/40
Vemuganti et al[14]	2005	Case Series; 5 cases	Infectious keratitis/topical steroids, antiviral, and antifungal medications	Oral itraconazole (2 cases); topical ciprofloxacin (1 case)	–	1 cornea scar; 4 TPK with complete resolution	–
Sangit et al[10]	2011	Case Report; 1 case	Bacterial keratitis/moxifloxacin	Topical chlorhexidine and oral albendazole and later topical loteprednol	12 wks	Complete resolution	20/30
Grag[3]	2013	Case Series; 29 cases	82% HSK /topical steroid and acyclovir	Topical PHMB and chlorhexidine ± oral albendazole or itraconazole	> 2 wks	2 medical treatment resolution, 1 LK with recurrence, 20 TPK with complete resolution	–
Gautam et al[11]	2013	Case Report; 1 Case	Infectious keratitis post cross-linking surgery/moxifloxacin, tobramycin, natamycin	Topical moxifloxacin and oral albendazole	6 wks	Pending LK	20/60
Coca et al[8]	2016	Case Report; 1 Case	CL associated keratitis/ antibiotics and dexamethasone ointment	Topical voriconazole and oral itraconazole	8 wks	Complete resolution	–
Sabhapandit et al[5]	2016	Case Series; 34 Cases	>25% HSK, 18% fungal keratitis/ antiviral therapy + topical corticosteroids (37.5%)	Topical PHMB and chlorhexidine ± oral albendazole	2–120 d	5 cases responded to medical treatment; 3 DALK, 22 PK; 1 evisceration	–
Pariyakanok et al[12]	2018	Case Report; 1 Case	Unknown keratitis; nil	Topical fumagillin and oral albendazole	6 mo	No recurrence on graft; Post-PK endophthalmitis	HM
Huang et al[13]	2020	Case Series; 14 Cases	57% Fungal keratitis, 14% HSK, 14% MSK, 14% bacterial keratitis/topical corticosteroids (42.9%)	Topical or oral fluoroquinolones or antifungals	>3 wks	All cases failed medical treatment and required surgical treatment (7 TLK; 6 PK; 1 TLK+PK)	20/800

CL = contact lens; DALK = deep anterior lamellar keratoplasty; HM = hand motion; HSK = herpes simplex keratitis; LK = lamellar keratoplasty; MSK = microsporidial stromal keratitis; PK = penetrating keratoplasty; TLK = therapeutic lamellar keratoplasty; TPK = therapeutic penetrating keratoplasty.

was not HSV keratitis.[8,10,11] In this study, the patient who did not respond to topical chlorhexidine (patient 3) had been applying 1% prednisolone eye drops hourly when we first examined her. Therefore, the use of potent corticosteroids before diagnosis was suspected to cause the microsporidial stromal keratitis treatment to fail.

We selected topical chlorhexidine as the treatment agent because it had produced positive results in previous studies.[3,10] It can disrupt the microbial cell membrane through interaction between the electropositive biguanide groups and plasma membrane to penetrate the sealed ostioles of parasitic cysts.[16] Topical chlorhexidine combined with oral albendazole was used in the first case report of successful medical treatment but failed in the other 24 cases. Limitation of the infection to the mid stroma was identified as the reason for success.[10] However, in the second case, the infection, mainly in the mid-to-deep stroma, was resolved after topical chlorhexidine treatment, which supports the claim that topical chlorhexidine is effective in treating deep stroma infection.

This case series also underscores the importance of prompt diagnosis and timely treatment. In advanced cases, although medical treatment can potentially eradicate microsporidial spores, OPK might still be recommended to restore vision damaged by scars secondary to inflammation. In addition, medical treatment should be continued after TPK to prevent recurrence. Our study highlights the importance of host immunity and the potential benefits of topical chlorhexidine in the treatment of microsporidial stromal keratitis.

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