BMJ Open Ophthalmology

Microsporidial stromal keratitis: treatment outcomes, clinical manifestations, confocal microscopy and histopathology findings: a retrospective observational study

Thurdkiat Saksurakan,[1] Vilavun Puangsricharern,[1,2] Buravej Assavapongpaiboon,[1] Thanachaporn Kittipibul,[1,2] Urassaya Pattanawong,[3] Vannarut Satitpitakul ,[1,2] Usanee Reinprayoon,[1,2] Ngamjit Kasetsuwan ,[1,2] Somchai Jongwutiwes,[3] Wasee Tulvatana [1]

To cite: Saksurakan T, Puangsricharern V, Assavapongpaiboon B, *et al*. Microsporidial stromal keratitis: treatment outcomes, clinical manifestations, confocal microscopy and histopathology findings: a retrospective observational study. *BMJ Open Ophthalmology* 2024;**9**:e001581. doi:10.1136/bmjophth-2023-001581

This research was partly presented on 24 November 2022 at the 8th Asia Cornea Society Biennial Scientific Meeting in Bangkok, Thailand.

Received 15 November 2023
Accepted 20 August 2024

For numbered affiliations see end of article.

Correspondence to
Dr Wasee Tulvatana;
waseetulvatana@chula.md

ABSTRACT

Objective Microsporidial stromal keratitis (MSK) is an uncommon disease. Only several case series have been reported. We aimed to describe the clinical manifestations, histopathology and treatment outcomes of MSK.

Methods and analysis Retrospective data of MSK diagnosed between January 2009 and December 2020 at the King Chulalongkorn Memorial Hospital, Bangkok, Thailand were retrieved. The diagnosis was made based on corneal scraping, corneal biopsy and corneal button histopathology findings. Detailed clinical characteristics, histopathological findings and treatment outcomes were reviewed and analysed.

Results 21 patients with MSK with a mean age of 63.8 years (SD 12.2) had an indolent disease onset with a median of 9 months (IQR 2.2–12.0). Five patients (23.8%) experienced ocular traumas. Herpes stromal keratitis was the most common preliminary diagnosis (33.3%), followed by non-specific ulcers and fungal keratitis. The most common corneal finding was multifocal grey-white lesions with anterior to mid-stromal infiltration and fluffy borders (66.7%). Pathogens were identified by modified trichrome staining of corneal scrapings in 11 of 14 cases (78.6%). Histopathological examination showed positive Ziehl-Neelsen staining in 17 of 19 cases (89.5%). All patients received surgical treatment, with 18 receiving therapeutic penetrating keratoplasty (TPK), 2 undergoing deep anterior lamellar keratoplasty and 1 undergoing femtosecond laser-assisted anterior lamellar keratoplasty. The overall cure rate was 76.2% after the first surgery and 95.2% after the second surgery.

Conclusion MSK can be easily underdiagnosed. Clues to diagnosis included a history of chronic refractory stromal infiltration and typical corneal findings of deep stromal infiltration, without epithelial defects. TPK is the preferred treatment for MSK.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Microsporidial stromal keratitis (MSK) is a rare disease and can be easily unnoticed because of indolent disease onset, and is often misdiagnosed because the corneal infiltration pattern resembles herpes simplex stromal keratitis. Various modalities of treatment including medical and surgical treatments have been reported.

WHAT THIS STUDY ADDS

⇒ Therapeutic penetrating keratoplasty was the preferred treatment, rather than medications or anterior lamellar keratoplasty. Rapid progression of the disease may occur after surgical manipulation of a corneal lesion, such as scraping and biopsy, and clinicians should be prepared for intervention.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Corneal biopsy should be performed with great postoperative care in MSK. Therapeutic penetrating keratoplasty should be the mainstay of treatment for this disease.

INTRODUCTION

Microsporidia are obligate intracellular eukaryotic parasites that have recently been classified as fungi. They have been identified as causative microorganisms for ocular, sinus, pulmonary, intestinal, renal and muscular diseases.[1] Previously, this pathogen was commonly reported in immunocompromised hosts, but in recent years, the reported incidence in immunocompromised patients has declined.[2]

The first case of microsporidial ocular infection was reported in 1973. The patient was injured by a goat in the lid area and presented with stromal keratitis 6 years later.[3] From then on, microsporidia have been considered an emerging ocular infection in humans, as reports have been increasing.[4] Ocular manifestation of microsporidia has

been reported to be in the forms of keratoconjunctivitis, stromal keratitis, endophthalmitis and scleritis.[5 6]

Microsporidial keratitis has been divided into two distinctly different clinical patterns: microsporidial keratoconjunctivitis (MKC) and microsporidial stromal keratitis (MSK). The awareness of MKC is increasing since more case series have been reported from south and southeast Asia in the past two decades.[7–10] The clinical manifestation includes acute red eye, papillary conjunctivitis, greyish white raised superficial punctate keratitis of various shapes all over the cornea and preauricular lymphadenopathy. It is a self-limiting disease with a good visual prognosis, and treatment includes topical fluoroquinolones, polyhexamethylene biguanide (PHMB), oral albendazole and corneal debridement.

In contrast to MKC, MSK is more severe and resistant to medical treatment. This entity often results in poor visual outcomes[11] and remains a challenge to diagnose because of its rarity and lack of specific clinical presentation. MSK is usually misdiagnosed as a herpetic, bacterial or fungal keratitis.[12] Furthermore, spores of microsporidia are poorly stained with Gram stain, which means that it can be easily unnoticed, and this organism does not grow in any commonly used culture media.[6] Despite a narrative review published in 2020,[13] comprehensive knowledge of MSK is limited, and most previous studies were case reports. There were only two large case series from India and Taiwan.[5 14] No studies have been conducted in Thailand.

Our setting is a tertiary care ophthalmic centre, where we encounter a number of MSK cases. Therefore, we conducted a retrospective case series of 21 cases of MSK, including an analysis of clinical history, chronicity, ophthalmic examination, rapid progression after corneal biopsy, investigation, pathological findings, inflammatory response, medical treatment, surgical treatment and treatment outcomes.

MATERIALS AND METHODS

This study conformed to the tenets of the Declaration of Helsinki. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Our study was a retrospective data collection of patients diagnosed with MSK by corneal scraping, corneal biopsy or corneal button pathological sectioning at King Chulalongkorn Memorial Hospital between January 2009 and December 2020, retrieved from previously recorded data of the hospital information system database.

Generally, all patients diagnosed with chronic keratitis at our institute were evaluated on a case-by-case basis for further investigation. In vivo confocal microscopy was performed for each case. Corneal scrapings were routinely performed in all cases and sent for Gram stain and potassium hydroxide (KOH) preparation, as well as aerobic, anaerobic and fungal cultures. Additional modified trichrome staining was done in cases with clinically suspected microsporidial infection. Corneal biopsies were performed in cases with deep stromal lesions with no epithelial defect and cases in which causative organisms cannot be identified. Tissues were sent for H&E and Ziehl-Neelsen staining. In some severe cases, a primary therapeutic penetrating keratoplasty (TPK) was needed.

The clinical history, clinical findings and histopathological findings were recorded. Treatment outcomes were defined as follows: 'failed medical treatment' designating the cases that showed the progress of the lesions after medical treatment; 'cured' designating the cases that the infection was finally eliminated; and 'uncontrolled infections' designating the cases which the infection was not able to be eliminated and then needed further surgical interventions such as evisceration, second TPK or vitrectomy. All data were entered into the REDCap (Vanderbilt University, Nashville, Tennessee, USA). Data were double-checked to ensure accuracy and to minimise the potential information bias (TS and WT). Descriptive statistical analyses were performed using REDCap.

RESULTS

A total of 21 cases with an average age of 63.8 years (SD 12.2) and predominantly female patients (81%) were collected. The presenting visual acuity ranged from 20/40 to light perception. All demographic data and baseline characteristics are shown in table 1. Two immunocompromised hosts were observed in this case series: one case was a female patient with HIV infection, and another was a female patient with systemic lupus erythematosus, treated with oral methotrexate and prednisolone.

Corneal findings

Corneal findings were carefully reviewed and categorised, as shown in figure 1. The most frequently observed corneal finding was the presence of central faint grey-white stromal lesions with fluffy borders, often accompanied by multiple paracentral stromal infiltrations and intact corneal epithelium (66.7%), as shown in figure 1A. In the remaining cases, 16.7% exhibited multifocal stromal lesions that shared the same characteristics as previously described (figure 1B). Another 16.7% presented with a significant, dense, homogeneous central infiltration involving all corneal layers. This infiltration was accompanied by a thin and necrotising stroma with an overlying epithelial defect, as depicted in figure 1C.

Five patients underwent corneal biopsy. Interestingly, we observed rapid clinical progression of the disease after surgical manipulation of the cornea in two cases.

Confocal microscopy

In vivo confocal microscopy was performed for each case using two machines: Confoscan 4 (Nidek Technologies, Gamagori, Japan) and HRT3 (Heidelberg, Germany). Confoscan showed good image resolution and details of the organisms as small (2–4 µm) hyper-reflective dots lining along the corneal lamellae (figure 2A). Confocal microscopy data were retrieved from 11 cases and demonstrated microsporidia in the stroma in every case.

Table 1 Demographic data and baseline characteristics* (total n=21 patients)

Mean age in years (SD)	63.8 (12.2)
Female	17, 81%
Median duration of onset in months (IQR)	9 (2.2–12.0)
Unilateral disease	21, 100%
Immunocompromised hosts	2, 9.5%
History of topical steroid eye-drops (n=15)	5, 33.3%
History of ocular injuries	5, 23.8%
Presenting visual acuity (n=20)	
Worse than 20/200	12, 60%
20/200 to 20/70	4, 20%
Better than 20/70	4, 20%
Presence of conjunctival injection (n=17)	15, 88.2%
Intraocular pressure higher than 21 mm Hg (n=14)	3, 21.4%
Presence of epithelial defect (n=15)	2, 13.3%
Presence of hypopyon (n=18)	3, 16.7%
Clinical diagnoses before referral to our institute	
Herpes stromal keratitis	7, 33.3%
Non-specific ulcer	2, 19.0%
Fungal keratitis	4, 9.5%
Cytomegalovirus keratouveitis	1, 4.8%
Others	4, 19.0%
No data	3, 14.3%

*Data presented as the number of patients, percentage.

To make a definite diagnosis of MSK, a positive stain for microsporidia from corneal scraping (figure 2B), corneal biopsy or corneal button pathology (figure 2C,D) was required. Microsporidia were detected in 11 of 14 patients by corneal scraping (78.6%), in 2 of 5 patients by corneal biopsy (40%) and in 17 of 19 patients by histopathology (89.5%). PCR was not routinely performed due to laboratory limitations.

Histopathological findings

The most vivid histopathological staining to detect microsporidia in corneal buttons was in the Ziehl-Neelsen-stained sections (figure 2D). Of the 19 corneal buttons collected, 10 demonstrated full-thickness infection and four showed positive organisms at the surgical cut end.

Medical treatment

Every case diagnosed with MSK was scheduled for emergency TPK. However, due to a shortage of donors in Thailand, there were some patients who required medical treatment for several days while waiting for surgical treatment. The mean duration of medical treatment before surgical treatment was 17 days (SD 12.5), ranging from 1 to 43 days, depending on the donor availability. Various topical and systemic regimens were prescribed after definite diagnosis, including topical 0.02% fumagillin, 0.5% moxifloxacin and 0.02% chlorhexidine, combined with oral albendazole (200 mg/day), itraconazole (200–400 mg/day) or voriconazole (800 mg/day). None of the patients showed resolution or improvement of symptoms after medical treatment.

Surgical treatment

All cases underwent surgical intervention due to medical treatment failure. 18 patients were treated with TPK, 2 with deep anterior lamellar keratoplasty (DALK) and 1 with femtosecond laser-assisted anterior lamellar keratoplasty (FS-ALK).

Manual DALKs were performed in two cases with anterior to mid-stromal infiltration. After successful elimination of microsporidial infection, one case in this group developed streptococcal corneal ulcer in the fourth month, necessitating TPK, and the other case had a brief follow-up period. Due to the availability of the new technology at that moment, one case of FS-ALK was performed at a depth of 429 µm, guided by the infiltration

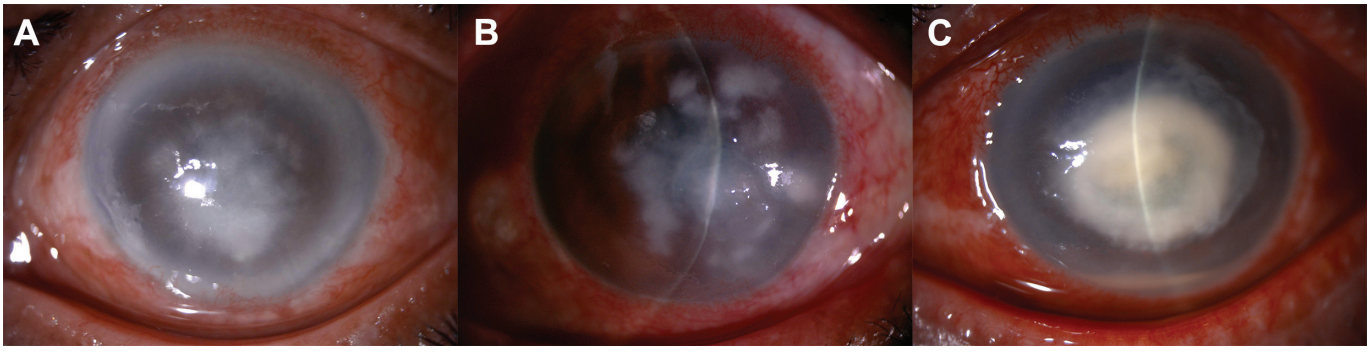

Figure 1 Three types of corneal infiltration patterns in microsporidial stromal keratitis. (A) Central faint grey-white stromal lesions with fluffy borders, often accompanied by multiple paracentral stromal infiltrations and intact corneal epithelium. (B) Multifocal faint grey-white stromal lesions with fluffy border and intact epithelium. (C) A significant, dense, homogeneous central infiltration involving all corneal layers, accompanied by a thin and necrotising stroma with overlying epithelial defect. Hypopyon and anterior chamber inflammation were also observed.

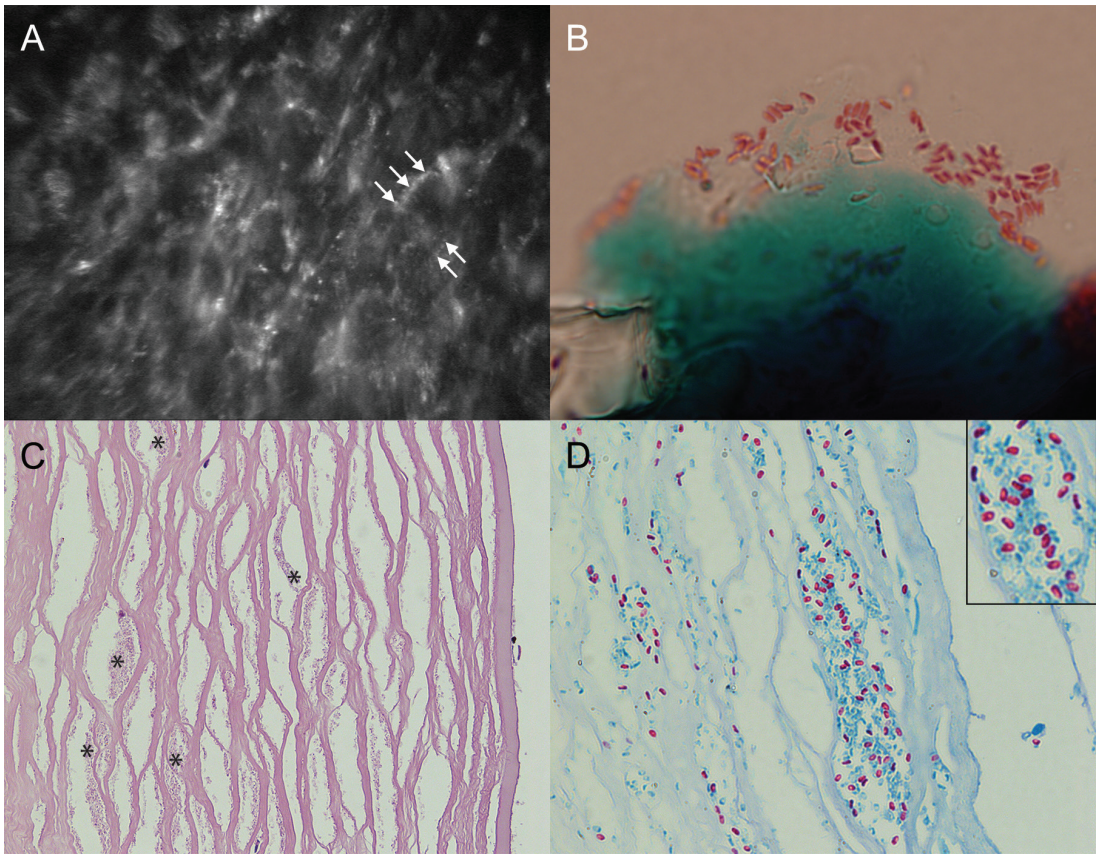

Figure 2 (A) Confocal microscopy demonstrates hyper-reflectivity dots (white arrows) lining along corneal lamellae (original magnification ×40). (B) Corneal scraping specimen shows red oval-shaped spores (modified trichrome stain, original magnification ×1000). (C) Histopathology shows small eosinophilic organisms aggregating inside the stromal clefts (asterisks) involving full thickness of corneal button (H&E, original magnification ×400). (D) Histopathology shows bright red oval organisms, densely packed in the stromal clefts with few inflammatory cells (Ziehl-Neelsen, original magnification ×600); inset shows higher magnification of the organisms with violet polar bodies (digitally magnified).

depth of 399 µm from anterior segment optical coherence tomography.[11] This technology offers advantages in terms of greater precision in cutting the host cornea, as well as in preparing the precise donor size and thickness. Nonetheless, the recurrence of MSK in the FS-ALK case, which may result from presiding organism spores at the interface, eventually required TPK to cure the infection. Later, the patient underwent multiple surgeries and ended with a poor visual outcome. The clinical course of this recurrent case as well as a brief period of documented successful visual results in manual DALK patients has led us to choose TPK as the treatment of choice in the subsequent cases.

Postoperative medical regimen was topical fumagillin and moxifloxacin, combined with oral albendazole and itraconazole. The mean duration of treatment was 4.6 months (SD 1.9), ranging from 2 months to 6 months based on the clinicians' decisions regarding the patients' conditions.

Treatment outcomes

The overall cure rate and uncontrolled infections are shown in figure 3. In the TPK group: one patient developed recurrence on graft with subsequent corneal perforation, leading to evisceration; one patient developed a recurrent infection and was successfully treated with a second TPK; two patients developed acute postoperative endophthalmitis and ended up with poor visual acuity following pars plana vitrectomy and multiple intravitreal antibiotic injections. Microsporidia were identified by PCR from vitreous fluid in one of them. Vitreous culture and other investigations in the other case could not identify causative pathogens. Fortunately, the infection in both eyes was successfully eliminated. Meanwhile, in the ALK group, the patient who received FS-ALK developed recurrence of MSK at 25 months after surgery. A second TPK was performed, resulting in complete cure. The overall cure rate was 76.2% after the first surgery and 95.2% after the second surgery. The mean duration of follow-up after surgery was 46 months (SD 49.7; median 21.6, IQR 8.6–56.7). The final visual outcome ranged from 20/40 to light perception. Graft failure was observed in eight eyes.

Rapid clinical progression of the disease after corneal biopsy in two cases

The first case was a patient with a history of eye injury from a papaya leaf that developed symptoms of a foreign

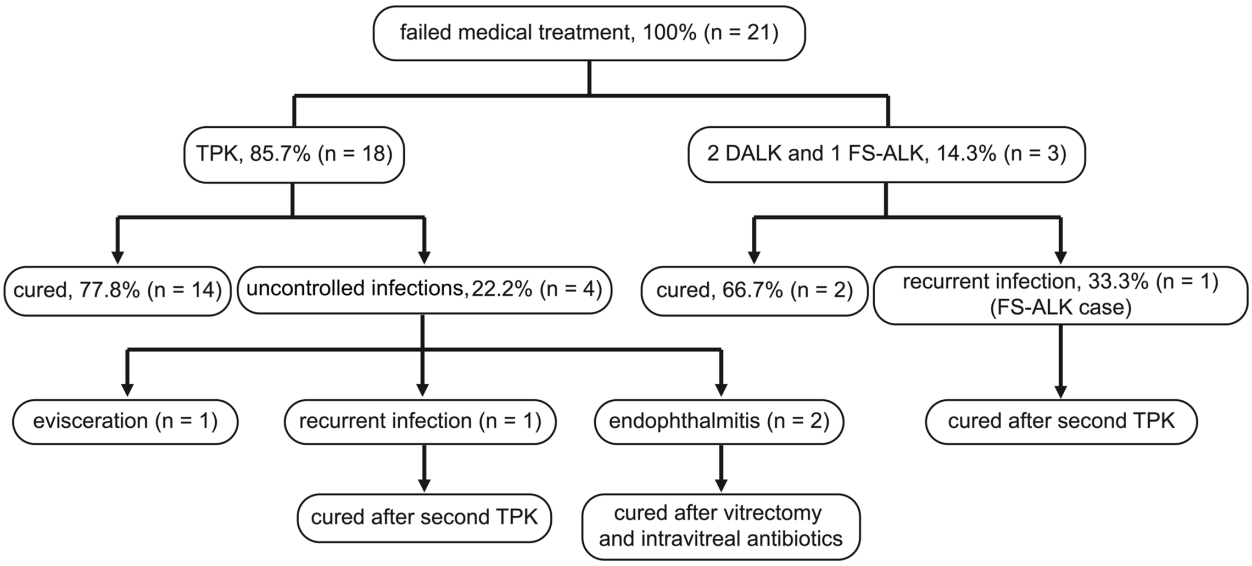

Figure 3 Treatment outcomes. DALK, deep anterior lamellar keratoplasty; FS-ALK, femtosecond laser-assisted anterior lamellar keratoplasty; TPK, therapeutic penetrating keratoplasty.

body sensation in the left eye for 12 months. He was previously diagnosed with bacterial and herpes simplex virus stromal keratitis and was treated accordingly. On his first outpatient visit, multifocal soft grey-white deep stromal infiltrations with no epithelial defect and minimal conjunctival injection were seen (figure 4A). A corneal scraping was conducted, and Gram staining and KOH preparation failed to reveal the presence of any microorganisms. The aerobic bacteria, anaerobic bacteria and fungal cultures were negative. Two days later, he returned for admission and further assessment, during which an increase in conjunctival injection, enlargement of the corneal infiltration and the presence of a hypopyon were observed (figure 4B). On the same day, a corneal biopsy was performed, revealing microsporidia spores through Ziehl-Neelsen staining in the tissue. Treatment with topical moxifloxacin, topical fumagillin and oral albendazole was started. However, the lesion exhibited rapid progression, necessitating TPK within 14 days of admission. The clinical event correlated with the histopathological findings, as shown in figure 4C,D. During his last 4-year follow-up, the patient experienced graft failure, and he is currently awaiting a repeat corneal transplantation.

In the second case, a patient with a history of a corneal scar that had persisted for 5 years was referred to our institute. On admission, the patient's cornea exhibited a central, small, faint, grey-white stromal infiltration characterised by a fluffy border and minimal inflammation. Due to suspicion of MSK, corneal scraping and biopsy were conducted on the first day of admission, revealing positive results for microsporidia spores using modified trichrome staining. The aerobic bacteria, anaerobic bacteria and fungal cultures were all negative. Despite treatment with topical fumagillin and oral albendazole, the lesion enlarged and became much denser on the third day after biopsy. TPK was performed on the 27th day after the biopsy, and there has been no recurrence in the 5 months following the surgery.

DISCUSSION

MSK is a rare disease, and only a limited number of comprehensive case series have been documented in the medical literature. Our study involved retrospective data collection over 12 years in a tertiary care setting in Thailand. Earlier reports have shown that a high percentage of previous ocular trauma is a predisposing factor, ranging from 47.1% to 92.9%.[5 14] In this case series, only five patients (23.8%) had a history of ocular injury, including insects, plants, dust and soil. We believe that because most patients in our study had a long-onset duration and had visited many hospitals, the incidence of injury may have been underestimated.

Our data highlight the chronicity of MSK, with patients experiencing a median symptom duration of 9 months (IQR 2.2–12.0). Previous literature has reported symptom durations ranging from 4 months to 2 years.[5 15 16] However, the true nature of the chronic and subtle course of MSK remains incompletely understood. The immune response to microsporidia infection in other tissues is well documented in the literature, involving both the innate and adaptive immune systems, mediated by T cells, macrophages, dendritic cells and secreted cytokines via surface molecules.[17] In MSK, according to Huang *et al* based on electron microscopic findings, the primary pathway of infection spread in MSK involves the proliferation of microsporidia spores inside infected keratocytes.[14] Subsequently, the infected keratocytes rupture, leading to the release of the spores into the corneal stroma.

Regarding the chronic course observed in MSK, we postulated that there are several possible explanations. First, the cornea is an immune-privileged site due to the avascularity of the central part. This immune tolerance may allow microsporidia to persist in the cornea without

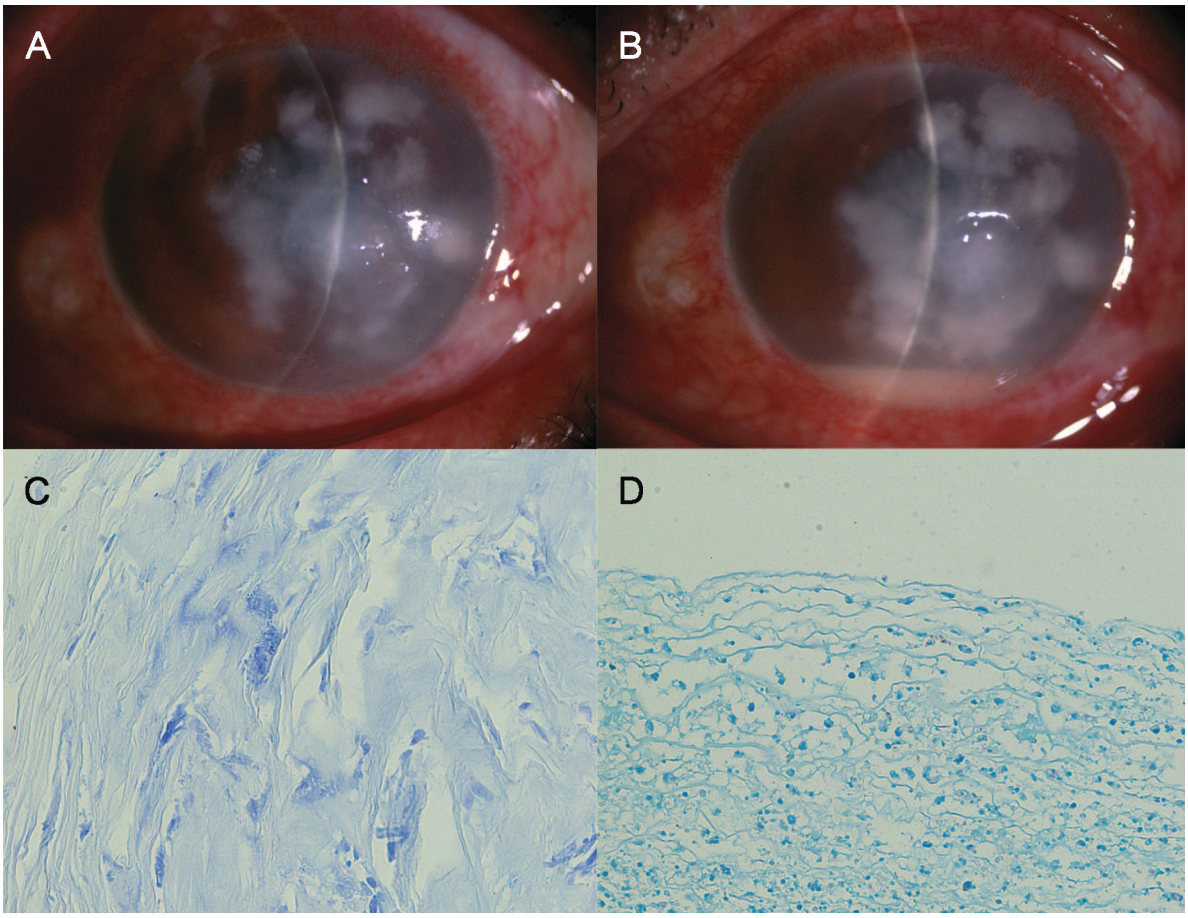

Figure 4 Rapid progression of the disease after biopsy. (A) Multiple grey-white, full-thickness infiltration with fluffy borders. (B) After corneal biopsy, the stromal infiltration rapidly progressed and hypopyon was observed in 2 days, requiring urgent therapeutic penetrating keratoplasty (TPK). (C) Corneal biopsy pathology at presentation showed no inflammatory cells with few unstained intrastromal microsporidial spores (Ziehl-Neelsen, original magnification ×400). (D) Corneal button pathology of the same patient after urgent TPK showed loss of epithelium and Bowman's layer, and numerous acute inflammatory cells with necrosis in the stroma (Ziehl-Neelsen, original magnification ×400).

eliciting a robust immune reaction. Also, microsporidia are known for their ability to persist within host cells.[14] The chronicity of MSK may be attributed to the ability of these parasites to evade the host immune response by residing within keratocytes for extended periods. Furthermore, the chronic course of MSK could also be influenced by delayed diagnosis and treatment challenges. Microsporidia infections are often overlooked or misdiagnosed, leading to delays in appropriate intervention.[5 14] Finally, it is noteworthy that 33% of the patients in our series had previously been diagnosed with herpes stromal keratitis (HSK) and was managed with topical steroids. This treatment approach, aimed at suppressing the inflammatory response, could potentially have contributed to the chronicity observed in these cases. Understanding these factors is crucial for developing effective diagnostic and treatment strategies for this challenging ocular infection.

Most cases in our centre were misdiagnosed as HSK, followed by non-specific ulcers and fungal keratitis. A previous case series has reported similar findings.[5 14] HSK can mimic MSK because both share common characteristics such as indolent history, white deep central stromal infiltrations and partial response to topical antibiotics and steroids.[5 14 18] In our series, coinfection of HSK was not found during the clinical courses and in the pathological findings. Recently, Mohanty *et al* reported 20 cases of microsporidia-induced stromal keratitis following MKC and proposed that this condition should be recognised as a new cause of presumed immune stromal keratitis.[19] Nonetheless, none of our cases reported previous symptoms and signs of MKC.

An interesting finding from our study is the rapid disease progression observed in two patients with MSK following corneal biopsies, a phenomenon not previously reported in the literature. The surgical manipulation of the infected corneal stroma, as in the case of corneal biopsy or even corneal scraping, may potentially expose microsporidia spores to the host environment, resulting in the activation of the immune system and an elevated inflammatory response, as observed in these two cases. This observation underscores the need for specific considerations when planning a surgical diagnostic procedure on a cornea suspected of having MSK. Clinicians should be prepared for the potential disease

progression, suggestively by daily follow-up examination, and contemplate subsequent therapeutic interventions, such as TPK if required.

Multiple stains have been proposed as effective methods for detecting microsporidial spores under light microscopy.[20] Our study showed high sensitivity of modified trichrome stains from corneal scrapings, with a positivity rate of 78.6%. We also found a high positivity rate of 89.5% using Ziehl-Neelsen-stained histopathological sections, which is comparable to a previous case series.[5 15] Huang *et al* reported that a combination of Gram and modified Ziehl-Neelsen staining had a high positivity rate of 92.9%.[14]

Although MSK is a stromal disease, corneal scraping with modified trichrome staining in our study resulted in a high positivity rate for the diagnosis. The method for detecting microsporidia in corneal scrapings is generally accessible and can be performed without the need for a specialised laboratory. However, an experienced interpreter is required to detect microsporidial spores due to their small size and limited staining, which can make them difficult to detect. In some cases, the spores may also be obscured by tissue debris. Despite these challenges, our study highlights the importance of having skilled interpreters to accurately identify microsporidia in corneal scrapings, which can lead to timely and appropriate treatment for patients with MSK.

None of the patients in our study were successfully treated with medication. Many medical regimens have been reported as successful treatments for MSK, including combination therapy of 0.02% PHMB with chlorhexidine 0.02% and oral albendazole,[5] topical 1% voriconazole with oral itraconazole 200 mg,[5 21] and 0.02% topical chlorhexidine gluconate with 400 mg oral albendazole.[22] Our results are consistent with those of previous studies[5 14 15] in that TPK is the definitive treatment for MSK with a low recurrence rate.

There are few case reports of microsporidia endophthalmitis, one of which was a post-TPK for corneal scar and the other was penetrating trauma from a screwdriver.[23 24] In our study, two cases developed acute endophthalmitis after TPK. Microsporidia were detected by PCR from vitreous fluid. Both patients underwent pars plana vitrectomy and intravitreal antibiotic injections, and finally had poor outcomes at the final visits. The histopathological examination of both corneal buttons did not show Descemet's membrane penetration by the organisms. Therefore, we postulate that during the TPK, the microsporidia spores might spill into the anterior chamber, and then penetrate to the posterior segment. To prevent the occurrence of endophthalmitis after TPK, we recommend surgeons to exercise caution and careful manipulation of corneal tissue during the procedure. However, in cases where endophthalmitis does occur, it can be difficult to determine the exact onset of the infection, and whether it occurred before or after the TPK. Further studies are needed to identify the risk factors for endophthalmitis following TPK, and to develop effective preventive measures to reduce the incidence of this serious complication.

This study has some limitations, such as the lack of using advanced diagnostic techniques like transmission electron microscopy or molecular diagnosis in every case due to laboratory limitations, which prevented identification of the specific microsporidia species responsible for the infection. The retrospective nature of data collection also resulted in missing data, and the small sample size, due to the rarity of the disease, limited the ability to perform further analysis such as regression analysis for risk factors. However, despite these limitations, we believe that our findings can be useful for corneal physicians in the management of patients with MSK. Future studies using advanced diagnostic methods and larger sample sizes are needed to confirm our findings and to further explore the risk factors for this condition.

In summary, MSK is a rare corneal infection and we found that most patients had an indolent onset. The suggestive corneal infiltration pattern was multifocal grey-white lesions with anterior to mid-stromal infiltration and fluffy borders. MSK lesions might progress rapidly after corneal biopsy or corneal scraping. The modified trichrome stain was effective to detect microsporidia spores under light microscopy in the cornea scraping specimens. Ziehl-Neelsen-stained histopathology was a practical method for detecting microsporidia from corneal buttons. None of the patients were successfully treated with medication, and TPK was the preferred treatment for MSK.

Author affiliations
[1]Ophthalmology, Chulalongkorn University Faculty of Medicine, Bangkok, Thailand
[2]Centre of Excellence for Cornea and Stem Cell Transplantation, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand
[3]Parasitology, Chulalongkorn University Faculty of Medicine, Bangkok, Thailand

Acknowledgements We thank Ms Sranya Phaisawang and the English editing service, Research Affairs, Faculty of Medicine, Chulalongkorn University, for their assistance with the manuscript preparation.

Contributors Planning, conduct, reporting, conception and design: all authors. Acquisition of data and analysis: TS, WT. Interpretation of data: TS, VP, BA, TK, UP, SJ, WT. Full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish: WT.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (Certificate of Approval number 149/2021).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Deidentified individual participant-level data can be provided by the corresponding author upon reasonable request.

ORCID iDs
Vannarut Satitpitakul http://orcid.org/0000-0002-1020-6833
Ngamjit Kasetsuwan http://orcid.org/0000-0003-1926-4666
Wasee Tulvatana http://orcid.org/0000-0002-5421-5619

REFERENCES

1 Joseph J, Vemuganti GK, Sharma S. Microsporidia: emerging ocular pathogens. *Indian J Med Microbiol* 2005;23:80–91.
2 Didier ES, Weiss LM. Microsporidiosis: not just in AIDS patients. *Curr Opin Infect Dis* 2011;24:490–5.
3 Ashton N, Wirasinha PA. Encephalitozoonosis (nosematosis) of the cornea. *Br J Ophthalmol* 1973;57:669–74.
4 Sharma S, Das S, Joseph J, *et al*. Microsporidial keratitis: need for increased awareness. *Surv Ophthalmol* 2011;56:1–22.
5 Sabhapandit S, Murthy SI, Garg P, *et al*. Microsporidial Stromal Keratitis: Clinical Features, Unique Diagnostic Criteria, and Treatment Outcomes in a Large Case Series. *Cornea* 2016;35:1569–74.
6 Mietz H, Franzen C, Hoppe T, *et al*. Microsporidia-induced sclerouveitis with retinal detachment. *Arch Ophthalmol* 2002;120:864–5.
7 Chan CML, Theng JTS, Li L, *et al*. Microsporidial keratoconjunctivitis in healthy individuals: a case series. *Ophthalmology* 2003;110:1420–5.
8 Joseph J, Sridhar MS, Murthy S, *et al*. Clinical and microbiological profile of microsporidial keratoconjunctivitis in southern India. *Ophthalmology* 2006;113:531–7.
9 Thanathanee O, Athikulwongse R, Anutarapongpan O, *et al*. Clinical Features, Risk Factors, and Treatments of Microsporidial Epithelial Keratitis. *Semin Ophthalmol* 2016;31:266–70.
10 Agashe R, Radhakrishnan N, Pradhan S, *et al*. Clinical and demographic study of microsporidial keratoconjunctivitis in South India: a 3-year study (2013-2015). *Br J Ophthalmol* 2017;101:1436–9.
11 Pariyakanok L, Satitpitakul V, Putaporntip C, *et al*. Femtosecond laser-assisted anterior lamellar keratoplasty in stromal keratitis caused by an Endoreticulatus-like microsporidia. *Cornea* 2015;34:588–91.
12 Pradhan S, Mascarenhas J, Srinivasan M. Microsporidial stromal keratitis masquerading as acute graft rejection. *Cornea* 2015;34:353–4.
13 Moshirfar M, Somani SN, Shmunes KM, *et al*. A Narrative Review of Microsporidial Infections of the Cornea. *Ophthalmol Ther* 2020;9:265–78.
14 Huang H-Y, Wu C-L, Lin S-H, *et al*. Microsporidial stromal keratitis: characterisation of clinical features, ultrastructural study by electron microscopy and efficacy of different surgical modalities. *Br J Ophthalmol* 2020;104:1613–20.
15 Vemuganti GK, Garg P, Sharma S, *et al*. Is microsporidial keratitis an emerging cause of stromal keratitis? A case series study. *BMC Ophthalmol* 2005;5:19.
16 Pinnolis M, Egbert PR, Font RL, *et al*. Nosematosis of the cornea. Case report, including electron microscopic studies. *Arch Ophthalmol* 1981;99:1044–7.
17 Han Y, Gao H, Xu J, *et al*. Innate and Adaptive Immune Responses Against Microsporidia Infection in Mammals. *Front Microbiol* 2020;11:1468.
18 Matoba A, Goosey J, Chévez-Barrios P. Microsporidial Stromal Keratitis: Epidemiological Features, Slit-Lamp Biomicroscopic Characteristics, and Therapy. *Cornea* 2021;40:1532–40.
19 Mohanty A, Behera HS, Barik MR, *et al*. Microsporidia-induced stromal keratitis: a new cause of presumed immune stromal (interstitial) keratitis. *Br J Ophthalmol* 2023;107:607–13.
20 Weber R, Bryan RT, Owen RL, *et al*. Improved Light-Microscopical Detection of Microsporidia Spores in Stool and Duodenal Aspirates. *N Engl J Med* 1992;326:161–6.
21 Coca M, Kim J, Shenoy S, *et al*. Microsporidial Stromal Keratitis: Successful Treatment with Topical Voriconazole and Oral Itraconazole. *Cureus* 2016;8:e934.
22 Sangit VA, Murthy SI, Garg P. Microsporidial stromal keratitis successfully treated with medical therapy: a case report. *Cornea* 2011;30:1264–6.
23 Sood AB, Debiec MR, Yeh S, *et al*. Microsporidial stromal keratitis and endophthalmitis in an immunocompetent patient. *J Ophthalmic Inflamm Infect* 2016;6:30.
24 Rodrigues G, Rao P, Vepakomma T, *et al*. Case Report: Microsporidial Endophthalmitis after Penetrating Eye Trauma. *Optom Vis Sci* 2022;99:830–2.