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CASE REPORT

Clinical Analyses of 4 Cases of Microsporidial Keratoconjunctivitis

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Abstract

Objective To report four cases of microsporidial keratoconjunctivitis (MKC) from The Affiliated Eye Hospital of Nanjing Medical University (from May 2023 to October 2024) and to aid ophthalmologists in diagnosing and treating MKC, as MKC has been increasingly reported in Asian healthy individuals but not much in Mainland China.

Methods Four patients with MKC were studied. Demographic information, symptoms, and clinical data were collected. Diagnosis involved ophthalmic examinations, corneal scraping microscopy (including Giemsa staining, modified Ziehl-Neelsen staining, Calcofour white staining), bacterial and fungal cultures, and metagenomic next-generation sequencing (mNGS). Treatment included various topical medications like polyhexamethylene biguanide (PHMB), fluconazole, tacrolimus, sodium hyaluronate, and systemic medication such as albendazole.

Results Three cases were caused by Encephalitozoon hellem proved by mNGS. Patients had symptoms like eye redness, swelling, pain, foreign body sensation, and vision loss. Risk factors included improper contact lens - wearing habits, contact with birds, or exposure to potentially contaminated environments. All patients showed improvements after treatment, with 3 cases cured and 1 case improved.

Conclusion MKC is a unilateral, acute, non-purulent ocular surface infectious disease. Clinicians should be more aware of it. Diagnosis depends on recognizing clinical signs, exploring risk factors, and laboratory tests. There is no consensus on treatment, but combined topical and systemic anti-protozoal drugs showed good results. Further large-scale validation is needed. Relevant departments should strengthen water source management, and patients should pay attention to personal hygiene.

Keywords Microsporidia · Microsporidial keratoconjunctivitis · Corneal scraping · Metagenomic next-generation sequencing

Introduction

Microsporidia are unicellular eukaryotes that are intracellular parasites and spore formers [1]. They infect diverse hosts, including humans. Microsporidiosis has been associated with immunocompromised patients [2, 3], although cases in healthy individuals have been increasingly reported in recent years [4, 5]. These organisms can affect various human organs, notably the digestive system, eyes, respiratory system, kidneys, and the central nervous system [3, 6]. Ocular infections typically present as microsporidial keratoconjunctivitis (MKC) or microsporidial keratitis (MSK) [7], of which MKC is the most common. While MKC has been frequently reported in several Asian countries, such as India, Thailand, and Singapore [8–10], few studies have been conducted in mainland China. This may stem from the difficulties in its clinical, imaging, and laboratory diagnoses. This study reported four cases of MKC from the Eye Hospital (from May 2023 to October 2024) to aid ophthalmologists in diagnosing and treating MKC. The study was approved by the Ethics Committee of the Eye Hospital. Informed written consent was obtained from each participant before inclusion in the study.

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Table 1 Demography and symptoms of all patients

Case No.	Age (years)	Gender	Eye Affected	Risk Factors	Symptoms
1	10	Female	Right eye	Contact lenses, sewage	Eye redness, swelling, pain, foreign body sensation, vision loss
2	13	Female	Right eye	Contact lenses, parrot-keeping	Eye redness, swelling, tearing, foreign body sensation, discharge
3	29	Female	Right eye	Contact lenses	Eye redness, foreign body sensation, vision loss
4	28	Male	Both eyes	History of living in pastoral areas	Eye redness, tearing, foreign body sensation, vision loss

Table 2 The demography, clinical data and mNGS of 4 cases

Case No.	Signs	Corneal scrapings	mNGS	Duration of treatment	Treatment	Prognosis
1	Diffuse, multifocal, rough, punctate, protruding corneal epithelial lesions; conjunctival hyperemia; Nipple reactions	Giemsa staining (+); Modified Ziehl-Neelsen staining (+)	(+) *E. hellem*	85 days	0.02% PHMB + 0.5% fluconazole + 0.1% sodium hyaluronate + oral albendazole	cured
2	Multifocal, rough, punctate, protruding corneal epithelial lesions; conjunctival hyperemia; Follicular reaction	-	(+) *E. hellem*	90 days	0.02% PHMB + 0.5% fluconazole + 0.1% sodium hyaluronate + oral albendazole	cured
3	Diffuse, multifocal, rough, punctate, protruding corneal epithelial lesions; Conjunctival hyperemia	Giemsa staining (+); Calcofour white staining (+)	(-)	60 days	1% tacrolimus, 0.02% PHMB + 0.1% sodium hyaluronate	cured
4	Diffuse, multifocal, rough, punctate, protruding corneal epithelial lesions; Conjunctival hyperemia	Giemsa staining (-); Calcofour white staining (+)	(+) *E. hellem*	125 days	0.5% fluconazole + 1% tacrolimus + 0.02% PHMB, oral albendazole	improved

mNGS: Metagenomic next-generation sequencing. PHMB: polyhexamethylene biguanide

Case Report

The demographic information, symptoms, and clinical data are available in Tables 1 and 2.

Case 1

A 10-year-old female presented with redness and itchiness in her right eye for 3 weeks. She had worn orthokeratological lenses in both eyes for 14 months with improper wearing habits. The protein-removing care solution was discontinued for 7 weeks. She was initially diagnosed with allergic and viral keratoconjunctivitis in the right eye without improvement after treatment.

Ophthalmic examination showed a corrected visual acuity of 0.8 and an intraocular pressure of 13 mmHg (1 mmHg = 0.133 kPa) in the right eye. The palpebral conjunctiva was hyperemic with small papillary hyperplasia in the upper eyelid and mixed hyperemia in the bulbar conjunctiva. Diffuse, multifocal, rough, punctate, protruding epithelial lesions were seen in the central cornea and limbus (Fig. 1A), and corneal fluorescein staining (CFS) was positive (+) (Fig. 1B). The patient's left eye was normal.

Upon referral to the Beijing Institute of Ophthalmology for etiological examination, two drops of topical 0.5% Proxymetacaine was given. A corneal curette was then used to scrape the fluorescein-stained corneal epithelial lesions, which was smeared on a slide. Once the epithelial tissue was driy, apply a single drop of undiluted methanol to fix the tissue.Giemsa and modified Ziehl-Neelsen staining were performed in sequence. Oval spore-like structures were visible under Giemsa staining (Fig. 1C). Purplish-red spore-like structures appeared with modified Ziehl-Neelsen staining (Fig. 1D). Bacterial and fungal cultures of corneal swab were both negative. Metagenomic next-generation sequencing (mNGS) included following steps. The removed epithelium was collected into DNA/RNA-Shield and temporarily stored at -20 °C. DNA was extracted and purified from the sample using the QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany). Subsequently, the DNA library was constructed with the QIAseq™ Ultralow Input Library Kit (QIAGEN, Hilden, Germany) and sequenced on the NovaSeq6000 instrument (Illumina, San Diego, USA). During the quality-control process, the raw sequencing data were processed to remove human reads using the SNAP software. The remaining sequences were then aligned to the Microbial Genome Databases (https://ftp.ncbi.nlm.nih.gov/genomes/). mNGS identified the sequence of *Encephalitozoon hellem*, confirming MKC in the right eye.

Treatment included 0.02% polyhexamethylene biguanide (PHMB) eye drops q6d, 0.5% fluconazol eye drops q6d, and 0.1% sodium hyaluronate eye drops qid in the right eye, along with oral albendazole 400 mg bid. After 1 week of treatment, conjunctival hyperemia in the right eye decreased,

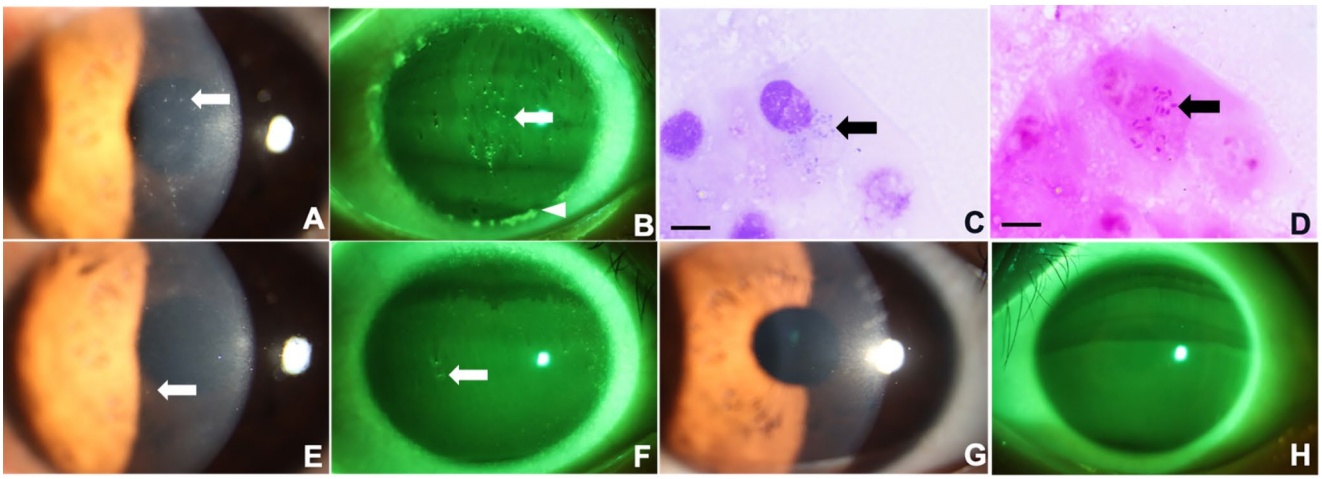

Fig. 1 Slit lamp photographs and corneal scrapings of case 1. **A**, **B**: Before diagnosis (arrow, corneal lesion; arrowhead, limbal lesion); **C**, **D**: Microscopic examination of corneal scrapings. **C**: spores in the epithelial cell (arrow) under Giemsa staining at ×1000 magnification (scale bar = 20 μm); **D**: spores in the epithelial cell (arrow) under modified Ziehl-Neelsen staining at ×1000 magnification (scale bar = 20 μm); **E**, **F**: 1 week after diagnosis (arrow, corneal lesion); **G**, **H**: 85 days after diagnosis

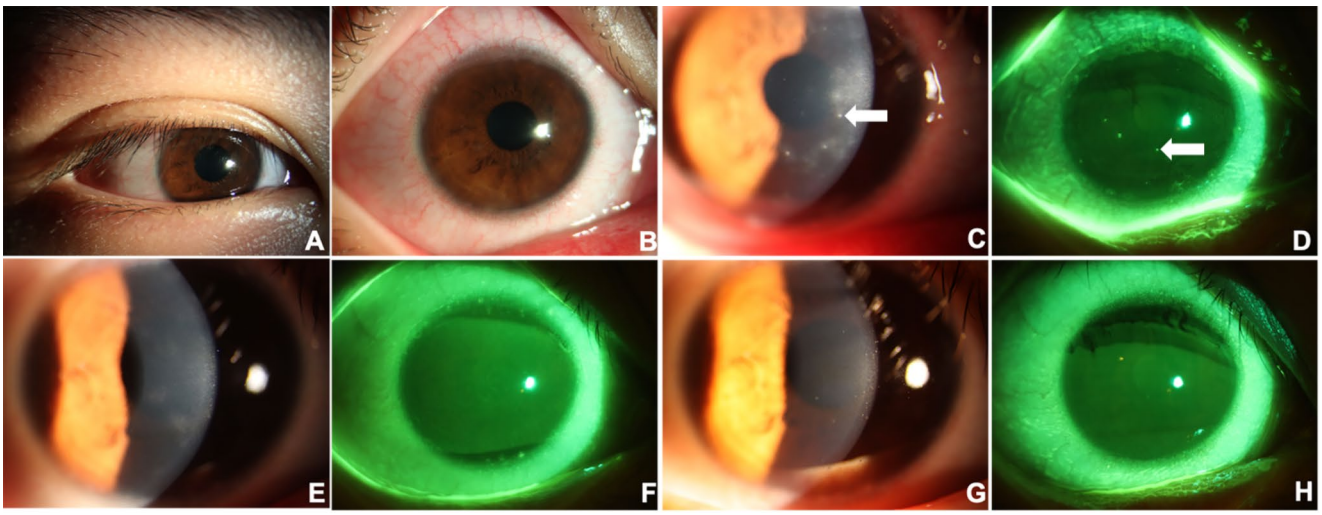

Fig. 2 Slit lamp photographs of case 2. **A**-**D**: before diagnosis (arrow, corneal lesion); **E**, **F**: 1 week after diagnosis; **G**, **H**: 3 months after diagnosis

and central corneal infiltration was relieved (Fig. 1E) with peripheral punctate CFS (Fig. 1F). After 85 days of treatment, the corrected visual acuity of the right eye was 1.0, the bulbar conjunctiva was not hyperemic, and the cornea was transparent (Fig. 1G) and CFS (-) (Fig. 1H). Medication was discontinued, and no recurrence was observed during the 12-month follow-up.

Case 2

A 13-year-old female with a 10-day history of redness and tearing in the right eye was diagnosed with viral keratitis that was refractory to antiviral therapy. She had been wearing orthokeratology lenses for over three years, had been raising parrots for a year, and recently experienced parrot diarrhea. She often neglected hand hygiene and eye protection after contact with birds. Ophthalmic examination revealed a corrected visual acuity of 0.8; eyelid edema (Fig. 2A); upper palpebral conjunctival follicles; bulbar conjunctival hyperemia (Fig. 2B); and diffuse, multifocal, rough, punctate, protruding epithelial lesions in the cornea and limbus (Fig. 2C). CFS was positive in the right eye (Fig. 2D) with no abnormalities in the left eye. Due to the child's young age, after communicating with parents, mom worried about eye discomfort from corneal scraping. Her parents chose mNGS (corneal swabbing) and bacterial and fungal cultures (corneal swabbing) first, corneal scraping for pathogens if mNGS was negative. Bacterial and fungal cultures of corneal swab were both negative. mNGS of the

corneal swab identified sequences of E. hellem in the right eye, suggesting MKC in the right eye.

Treatment with 0.02% PHMB qid, 0.5% fluconazole tid, then sodium hyaluronate 0.1% tid was initiated, and 400 mg of albendazole twice daily was added. One week after treatment, conjunctival hyperemia and corneal lesions were relieved (Fig. 2E and F). At the 3-month follow-up, the right eye's corrected visual acuity was 1.0. A transparent cornea without bulbar conjunctival hyperemia (Fig. 2G) and fine dot-like CFS in the inferior limbus was observed (Fig. 2H). The medication was then discontinued, and there was no recurrence over the subsequent 2.5 months of follow-up.

Case 3

A 29-year-old female presented with persistent redness and foreign body sensation in her right eye for 5 months and a history of soft contact lens use for over 2 years. She had been diagnosed with a corneal epithelial defect and keratitis and treated with fluorometholone, tobramycin, and artificial tears but experienced recurrence.

Ophthalmic Examination: the right eye had corrected visual acuity of 0.8, intraocular pressure of 13 mmHg, bulbar conjunctival hyperemia, and punctate keratopathy (Fig. 3A) with CFS (+) (Fig. 3B). The patient's left-eye test results were negative. Giemsa staining revealed oval spore-like structures (Fig. 3C), and Calcofour White staining identified sparse oval or pear-shaped spores (Fig. 3D). Bacterial and fungal cultures of corneal swab were both negative. Although mNGS results were negative for microsporidia, MKC was still diagnosed based on scraping results.

1% tacrolimus tid, 0.02% PHMB tid, and 0.1% sodium hyaluronate tid were administered to the right eye. After 10 days of treatment, conjunctival hyperemia and corneal punctate keratopathy were relieved (Fig. 3E), with the remaining peripheral scattered punctate CFS (Fig. 3F). At the 2-month follow-up, corrected visual acuity was 1.0 in the right eye, with corneal clarity and without bulbar conjunctival hyperemia (Fig. 3G), CFS (-) (Fig. 3H). The medication was discontinued, and no recurrence was observed over the subsequent 2-month follow-up.

Case 4

A 28-year-old male presented with bilateral recurrent redness, tearing, foreign body sensation, and decreased visual acuity for >4 months. He had traveled to pastoral area one week prior to symptom onset. The patient was diagnosed with bilateral viral keratitis, but the antiviral and pro-repair therapies failed, and the condition worsened.

Ophthalmic examination: uncorrected visual acuity of 0.5 right and 0.5 left, intraocular pressure of 12 mmHg right and 17 mmHg left, bilateral conjunctival hyperemia, diffuse punctate protruding keratopathy, and CFS (+) (Fig. 4A and D). Calcofluor white staining revealed oval, spore-like structures (Fig. 4E), whereas Giemsa staining was negative. Bacterial and fungal cultures of corneal swab were both negative. mNGS of bilateral corneal epithelial tissue identified sequences of *E. hellem*, thereby confirming the presence of bilateral MKC. Initially, 0.5% fluconazole tid, 0.3% gatifloxacin tid, 1% sodium bromophenolate 1% bid, and oral albendazole 400 mg bid were prescribed. After 10 days of treatment, the patient's visual acuity was further reduced, with the uncorrected visual acuity at 0.3 right and 0.4 left. Palpebral conjunctival and bulbar conjunctival hyperemia were aggravated. Increased white protruding

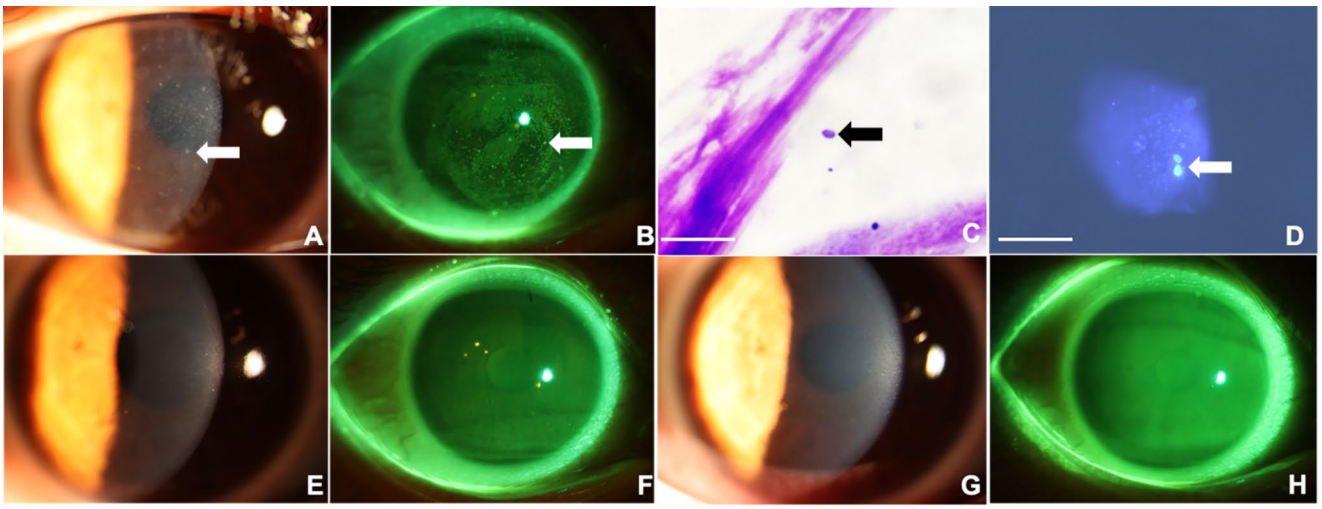

Fig. 3 Slit lamp photographs and corneal scrapings of case 3. **A**, **B**: Before diagnosis (arrow, corneal lesion); **C**, **D**: Microscopic examination of corneal scrapings. **C**: a spore (arrow) under Giemsa staining at ×1000 magnification, (scale bar =20 μm); **D**: a spore (arrow) under calcoflour white staining at ×1000 magnification (scale bar =20 μm); **E**, **F**: 10 days after diagnosis; **G**, **H**: 2 months after diagnosis

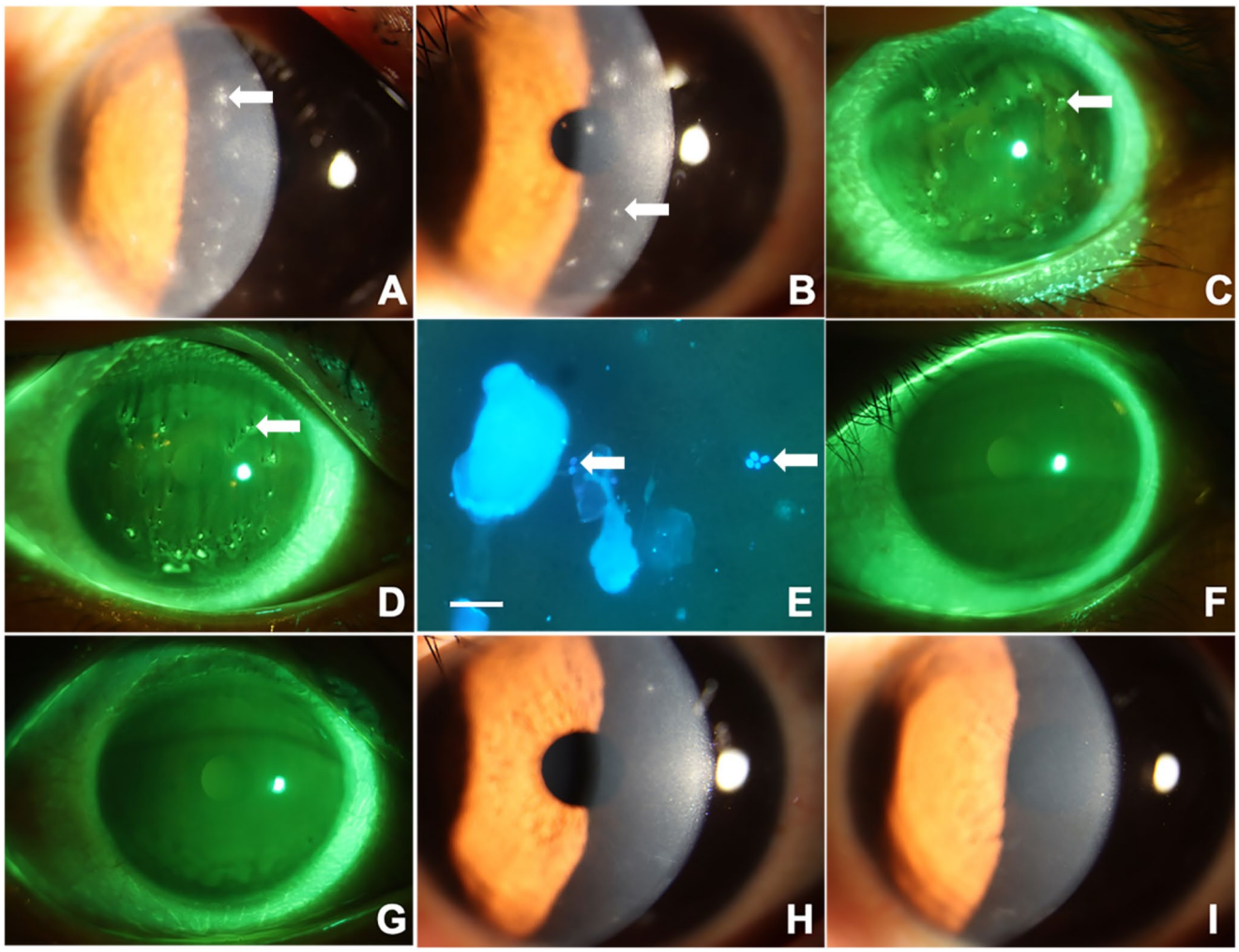

Fig. 4 Slit lamp photographs and corneal scrapings of case **4. A**- **D**: Before diagnosis (arrow, corneal lesion). **A**, **D**: Right; **B**, **C**: Left; **E**: spores (arrow) under Calcoflour white staining at ×1000 magnification (scale bar = 20 µm); **F**-**I**: 2 months after diagnosis. **F**, **H**: Left; **G**, **I**: Right

lesions were observed in the middle and periphery of the cornea, which was CFS (+). Subsequently, PHMB 0.02% qid and tacrolimus qid were added. After an additional 10 days, uncorrected visual acuity was improved to 1.0 right and 0.8 left. Palpebral conjunctival and bulbar conjunctival hyperemia were relieved. The number of white protruding lesions decreased. One month after the initiation of treatment, the patient, feeling a reduction in symptoms, discontinued the medication. Subsequently, both the symptoms and signs exacerbated and medication was then continued. After two months of treatment, the bilateral distance visual acuity was 1.0, the palpebral and bulbar conjunctiva were not hyperemic, and the cornea was transparent. Only one white protruding lesion remained in the superotemporal region of the left eye, which was CFS (+) (Fig. 4F and I). 1% Tacrolimus qd and 0.02% PHMB qd were continued for 1 month before discontinuation of the medication. No recurrence was observed after one month of follow-up.

Discussion

In 1973, Ashton et al. [11] reported the first microsporidia keratitis case in an 8-year-old boy following a goat injury. Over 1700 species across 220 genera of microsporidia are known, with seven genera related to ocular infections, notably the genera *Encephalitozoon* and *Vittaforma* [12]. *Encephalitozoon hellem* and *Vittaforma corneae* are the two main species of MKC [13]. Uematsu et al. [14] identified *V. corneae* as the causative agent of the 2022 MKC outbreak among five Japanese football players, and Schwartz et al. [15] found *E. hellem* to be the leading cause of ocular infections in seven AIDS patients. This case series confirmed *E. hellem* as the causative agent in 3 cases, consistent with a report by Han et al. [13]. MKC is also associated with *Anncaliia algerae*, *Encephalitozoon cuniculi*, *Encephalitozoon intestinalis*, *Trachipleistophora hominis*, *Nosema ocularum*, and other species. The variability in the treatment

and clinical outcomes of MKC caused by different microsporidian species requires further investigation.

Microsporidia are opportunistic waterborne pathogens that infect the human eye through contact with contaminated water or soil [16–18]. This case series suggested improper hygiene, such as not drying hands after bathing before handling orthokeratology lenses, may lead to MKC. This organism is also known to parasitize birds. Indeed, *E. hellem* was isolated from domestic bird feces by Slodkowicz-Kowalska et al. [19]. Ma et al. [20] described the case of an 18-year-old female who developed MKC in her left eye after exposure to bird cage fragments. In Case 2, a patient who kept parrots and wore orthokeratology lenses was suspected of contracting MKC due to the microsporidia potentially carried by the birds and improper lens-wearing habits. Further testing is needed to confirm the infection route. Case 3 was similar to the findings of Theng et al. [21], who found that contact lens use was a risk factor for MKC. Lenses can cause corneal epithelial damage, disrupt the barrier, and facilitate the contact of microsporidia with the cornea, thereby increasing the risk of infection. Leroy et al. [22] reported a case of MKC in a French patient who traveled to India during the monsoon. Loh et al. [9] found that 50% of the patients with MKC engage in outdoor activities that may expose them to contaminated environments. Case 4 involved a patient who developed MKC after visiting a pastoral area, suggesting that exposure to contaminated water or soil and inadequate sanitation were risk factors. To prevent MKC, it is crucial to standardize contact lens wear, avoid contact with contaminated water and animals (mainly birds), and maintain ocular hygiene.

MKC was mostly monocular with rough, multifocal, protruding round or oval corneal epithelial lesions, with or without anterior stromal infiltrates; lesions varied in degree of staining, often in a peripheral, paracentric, or diffuse distribution, often with mild nonpurulent papillae or follicular conjunctivitis [12, 23]. This case series included three unilateral cases and one bilateral case, and the cornea showed similar lesions, consistent with previous reports. Wang et al. [24] classified MKC into four stages based on the pattern of corneal epithelial lesions. Based on Wang's method, this case series identified one case of stage II, one of stage III, 2 cases of stage IV, and no case of stage I. This suggests that ophthalmologists should enhance their differential diagnostic ability and awareness of early diagnosis and treatment of MKC. Moreover, MKC presents with nonspecific symptoms and is frequently misdiagnosed as adenoviral keratoconjunctivitis or Thygeson superficial punctate keratitis [25–27]. Therefore, recognizing clinical signs, comprehensive exploration of potential risk factors, and laboratory tests constitute three crucial aspects for averting misdiagnosis of MKC in the initial stage.

Accurate etiological diagnosis of MKC was crucial for effective treatment. This involved transmission electron microscopy, corneal scraping microscopy, and molecular biology tests. Transmission electron microscopy is the best but is costly and technically demanding for clinical use. Corneal scraping microscopy is a supplementary method that cannot be used to identify species. Using multiple stains together can improve sensitivity, although caution should be exercised when differentiating microsporidia from yeast spores using microscopy. Joseph et al. [28] reported that modified Ziehl-Neelsen and fluorescent staining were effective for MKC diagnosis, with sensitivities of 96.7% and 93.3%, respectively, whereas Giemsa staining was only 64.5%. As previously reported, this case series used these three staining methods to observe typical microsporidia spores [29, 30]. It is worth noting that the amount of samples obtained from corneal scraping is usually small, which poses a challenge to obtaining positive results in corneal scraping examinations.In molecular biology tests, specific polymerase chain reaction (PCR) primers have been used to identify Microsporidia species; however, false negatives may occur in small epithelial lesions and it has not been implemented widely in most parts of mainland China. In contrast, mNGS is a novel and efficient method for rapidly and accurately identifying microbes. It can detect pathogens in a small sample, has broad coverage, and is increasingly used in clinics [31, 32]. In this study, mNGS detected *E. hellem* in cases 1, 2, and 4 but not in case 3, possibly because of extensive medication that increased sampling difficulties. For patients suspected of having MKC, corneal scraping microscopy should be performed at the onset of the disease, followed by mNGS or PCR to determine the Microsporidia species. Further investigation is needed to determine the optimal sampling sites and methods for enhancing microsporidia detection.

No consensus exists on treatment of MKC currently. The treatment methods reported in the literature, including topical medication, systemic medication, and corneal epithelial debridement, are all effective for the treatment of MKC [23]. Topical drugs include fluoroquinolone antimicrobials, fumagillin, antifungals, biguanides, immunosuppressants, and eye moisturizers [12, 23]. For systemic medication, albendazole, a broad-spectrum anti-protozoal drug, inhibits the formation of microtubules in microsporidia to impede cell division and has also been proven to be effective in the treatment of MKC [33]. Previously, single-drug or combination-drug regimens were mostly used to treat MKC. Most patients had a favorable prognosis, with no significant decline in visual acuity [34]. In this case series, patients 1 and 2 were cured using a combination of topical 0.5% fluconazole, 0.02% PHMB, 0.1% sodium hyaluronate and oral albendazole. In Case 3, the condition improved after using

0.02% PHMB and 1% tacrolimus eye drops. Das et al. [34] found that treatment of MKC with PHMB does not offer any significant advantage over placebo, suggesting the self-limiting nature of the disease. Whether MKC is a self-limiting disease is certainly doubtful. On one hand, In Case 4, the patient was treated with topical antibacterial and antifungal agents, plus oral albendazole after confirmed diagnosis, but symptoms worsened. After adding topical immunosuppressant eye drops and 0.02% PHMB, the condition improved greatly. On the other hand, four patients in this study took various medications including lubricant eye drops before they were definitely diagnosed with MKC. Yet, their conditions worsened instead of improving, which was inconsistent with Das et al. [34].

Joseph et al. [35] treated 19 cases of MKC effectively by using topical or oral medications combined with corneal debridement. Das et al. [36] demonstrated that debridement does not have any significant advantage in terms of resolution of the corneal lesions and final visual outcome in cases of microsporial keratoconjunctivitis. Although corneal epithelial debridement can reduce the parasitic load, it will increase the risk of pathogens invading the corneal stroma layer [7], and exacerbate the ocular irritation symptoms such as pain. In this case series, all four patients demonstrated improvements following topical and/or systemic medications. Therefore, there is still a controversy about the necessity of corneal debridement in clinical practice of MKC. The treatment of MKC may be influenced by various factors, including the infecting species and patient's immune status [22]. Moreover, geographical disparities may also play a role. Consequently, multi-center randomized controlled trials (RCTs), spanning different regions and even countries, hold greater potential in providing comprehensive and generalizable insights and offering a more accurate understanding of the disease's characteristics.

Conclusion

MKC is a unilateral, acute, and nonpurulent ocular surface infectious disease. Clinicians need to raise awareness about this disease. Accurate diagnosis relies on recognizing the patient's typical clinical signs and epidemiological history and confirming with corneal scraping microscopy and molecular biology testing, though clinical and laboratory diagnoses present some difficulties. Currently, no consensus has been reached regarding the treatment of MKC. In our practice, the combined use of topical and systemic anti-protozoal drugs for MKC treatment seems to have yielded relatively good results. Further validation on a larger scale is needed. In addition, relevant departments should strengthen water source management. Patients are urged to focus on personal hygiene, especially the proper use of contact lenses by adolescents, and avoid contact with sewage, soil, and pets.

Author Contributions Y.Z. participated in microbiological work up, carried out the analysis, and drafted the manuscript. S.X. was involved in collectining data and drafting the manuscript. Y.X. carried out the studies, provided the clinical information and edited the manuscript. All the authors read and approved the final manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

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