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ORIGINAL ARTICLE

# Demographic and clinical profile of microspodial keratitis in North India: an underreported entity

**Sumeeta Khurana[1] · Sonu Kumari Agrawal[1] · Kirti Megha[1] · Sujata Dwivedi[2] · Neha Jain[1] · Amit Gupta[3]**

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**Abstract** Ocular microsporidiosis was first described in immunocompromised subjects but recent reports have also shown cases in immunocompetent hosts. The database of existing clinical studies is very limited. The aim of present study was to analyse demographic profile, clinical features, microbiological profile, treatment and outcome of ocular microsporidiosis in northern India. The present study was carried out in the Department of Medical Parasitology, Advanced Eye Center, Postgraduate Institute of Medical Education and Research, and Bharat Vikas Parishad Charitable trust, Chandigarh. A total of 250 patients during year 2013–17 and suspected of microsporidial keratitis (either clinically or after exclusion of bacterial, viral or fungal agents). Corneal scraping were taken and subjected for various staining methods and PCR. 8 patients of microsporidial keratitis were confirmed, based on positivity by at least any two of the above mentioned techniques. None of the patients had history of contact lens wear or exposure to swimming pool. All these patients were systemically healthy and HIV serology was negative except one had history of diabetes mellitus. This study is a reminder to clinicians that when multifocal diffuse epithelial keratitis and culture-negative keratoconjunctivitis not responding to medical therapy, regardless of immune status are found in patients with symptoms suggesting an infectious keratitis, microsporidial keratoconjunctivitis should be included in the differential diagnosis.

✉ Sumeeta Khurana
sumeetakhurana@hotmail.com

[1] Department of Medical Parasitology, PGIMER, Chandigarh, India

[2] Bharat Vikas Charitable Trust, Chandigarh, India

[3] Advanced Eye Center, PGIMER, Chandigarh, India

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## Introduction

Microsporidia are unicellular, obligate spore-forming intracellular eukaryotes that are closely related to fungi. Spores are the resistant infective form of microsporidia (Sharma et al. 2011). This pathogen can cause variety of human infections including intestinal, ocular, sinus, pulmonary, muscular and renal, in both immunocompetent and immunocompromised patients (Malhotra et al. 2017). Systemic immunosuppression, contact lens wear, trauma, prior refractive surgery, exposure to contaminated water and soil/mud from activities such as soccer, golf, trail biking and army field exercises are risk factors of ocular microsporidiosis (Tham and Sanjay 2012). Ocular involvement has been reported in the form of keratitis, keratoconjunctivitis, deep stromal keratitis, and rarely scleritis or endophthalmitis (Mietz et al. 2002; Yoken et al. 2002). Although microsporidial ocular infection was first described in immunocompromised subjects but recent reports have also shown cases in immunocompetent hosts. The exact prevalence rate of ocular microsporidiosis is not known as only a handful of studies from South India are available (Joseph et al. 2006a). The clinical picture is often misdiagnosed as adenoviral keratoconjunctivitis and other infections that are more common (Rauz et al. 2004; Davis et al. 1990). Therefore, high index of suspicion is required because microbiological investigations are often be negative in these cases. Although correct identification of ocular microsporidiosis have been challenging but with the recent use of molecular techniques in routine diagnostics, correct

identification to species level has been possible. Nonetheless, identification of this organism presents challenging scenarios to the microbiologist.

In addition, the database of existing clinical studies is very limited therefore, treatment modalities varied among ophthalmologist and is guided by the clinical response of each patient. Several options have been suggested; for example, corneal debridement with topical medications or a combination of topical and oral drugs (Agashe et al. 2017). Any delay in definitive diagnosis and treatment of ocular microsporidiosis can result in complications like visual loss.

Thus, aim of present study was to analyse demographic profile, clinical features, microbiological profile, treatment and outcome of ocular microsporidiosis in northern India. We believe, publishing this study will provide more clarity among ophthalmologists and microbiologists regarding the clinical significance of the disease, including associated risk factors and appropriate treatment.

## Materials and methods

### Study area, population, and period

The present study was carried out in the Department of Medical Parasitology, Advanced Eye Center, Postgraduate Institute of Medical Education and Research, and Bharat Vikas Parishad Charitable trust, Chandigarh. A total of 250 patients during year 2013–17 and suspected of microsporidial keratitis either clinically (antecedent history of surgery, trauma, steroid use, and comorbid ocular/systemic illness) or after exclusion of bacterial, viral or fungal agents. The patients' demographic characteristics including age, gender, predisposing factor and occupational status as well as clinical symptoms were evaluated and a slit lamp bimicroscopic examination was performed. A complete blood count, blood sugar count and HIV test were done in all the cases. The informed consent from participants as well as parents in case of (age less than 16) was taken before sample collection.

### Sample collection and microbiological investigations

Under 0.5% proparacaine topical anaesthesia, corneal scrapings were taken from all patients having clinical features of keratoconjunctivitis using a sterile no. 15 surgical blade on a Bard-Parker handle. The scraping was smeared on clean glass slide and subjected for Gram stain, modified trichrome stain and calcofluor white stain. The sample was also inoculated onto 5% sheep blood agar, chocolate agar, Sabouraud's dextrose agar and non-nutrient agar with *Escherichia coli* overlay to rule out mixed infection. The scraped material collected was also subjected to microsporidium PCR performed as per previously published protocol (Joseph et al 2006b).

The study was approved by the Ethics Committee, Post graduate institute of medical education and research, Chandigarh, India (Memo No. 9216/PG/2Trg/12, Endst. No. 9216-PG/2Trg/12).

## Results

During the period between 2013 and 2017, 8 patients of microsporidial keratitis were confirmed, based on positivity by at least any two of the above mentioned techniques. Clinical and demographic details are summarized in Table 1. The age of the patients ranged from 12 to 70 years (mean 32 years). Among these 8 patients, 6 (75%) were males and 2 (25%) were females and the male-to-female ratio was 3:1. Five cases presented with unilateral infection; 3 complained of symptoms in the left eye and 2 complained of symptoms in the right eye. Three cases presented with bilateral infection. All cases described pain, redness, irritation, watering, foreign body sensation and worsening of symptoms with blurring or diminution of vision. The duration of presenting complaints ranged from 2 to 10 days. The predisposing factor related to trauma was identified in three patients which included injury due to non-vegetative matter in 1, vegetative matter in 2, whereas one patient was diabetic and one had a history of steroid use. In three patients predisposing factor could not be ascertained. None of the patients had history of contact lens wear or exposure to swimming pool. All these patients were systemically healthy and HIV serology was negative except one had history of diabetes mellitus.

Slit-lamp microscopy examination revealed diffuse, multiple, pin-point to pin head epithelial lesions stained with fluorescein with a characteristic 'stuck on' appearance in 7 patients and sub epithelial infiltrates in 1 patient. The underlying stroma was normal in all the cases. Conjunctival hyperaemia was seen in all the cases (Fig. 1).

The details of microbiological analysis are shown in Table 2. The bluish white 1–3 μm oval structures characteristic of microsporidia spores were detected by calcofluor stain in 8 cases; red/pink oval structure with diagonal stripe were detected by modified trichrome stain in 4 samples; and the spores stained Gram positive in 5 samples. All the cases were confirmed as microsporidia by PCR amplification. These cases were negative for bacterial and fungal culture. However, the scraping from one case showed the presence of *Acanthamoeba* on culture and PCR.

Most of the patients were on topical antibiotics before the definitive diagnosis of microsporidia keratitis. After diagnosis, the patients were started on voriconazole 1%,

**Table 1** Clinical and demographic details of 8 patients with microsporidial keratoconjunctivitis

| S. no. | Age and sex | Predisposing factor | Eye involvement | Corneal signs | Duration of illness | Treatment after diagnosis (surgical debridement was performed in all the cases) | Outcome |
|---|---|---|---|---|---|---|---|
| 1 | 16 M | Trauma soil | Bilateral | Multifocal coarse pinpoint<br>Epithelial keratitis | 36 days | Topical Gatifloxacin + fluconazole<br>After 10 days replaced with Voriconazole<br>After 26 daysoral Albendazole added | Complete resolution |
| 2 | 20 M | Nil | Right eye | Multifocal coarse pinpoint<br>Epithelial keratitis | 60 days | Topical Voriconazole + moxifloxacin | Complete resolution |
| 3 | 25 M | Nil | Left eye | Initial sinusitis<br>Later keratitis<br>Multifocal coarse pinpoint<br>Epithelial keratitis | 22 days | Oral azithromycin<br>Topical moxifloxacin | Complete resolution |
| 4 | 26 M | Nil | Bilateral<br>Recurrence of keratitis | Multifocal coarse pinpoint<br>Epithelial keratitis | Recurrence<br>9 months | Topical Voriconazole + moxifloxacin + oral albendazole<br>Superficial keratectomy | Complete resolution |
| 5 | 23 M | Topical steroid use for 1 week | Left eye | Multifocal coarse pinpoint<br>Epithelial keratitis | 28 days | Topical Voriconazole | Complete resolution |
| 6 | 65 F | Trauma/dust particle | Left eye | Multifocal coarse pinhead<br>Epithelial keratitis | 56 days months | Topical Voriconazole + moxifloxacin | Complete resolution |
| 7 | 70 F | Trauma/ vegetative matter | Right eye | Multifocal coarse pinhead<br>epithelial keratitis | 45 days | Topical Fluconazole + moxifloxacin | Complete resolution |
| 8 | 12 M | Type 1 diabetes mellitus | Both eyes | Multifocal coarse pinhead<br>Epithelial keratitis | 30 days | Topical moxifloxacin | Complete resolution |

gatifloxacin 0.5% or moxifloxacin 0.5% either alone or in combination 5–6 times a day after surgical debridement. The duration of medical treatment varied from 7 days to as long as 9 months in one patient. One patient showed recurrence of symptoms after initial resolution and he was then given adjunct treatment with oral albendazole 400 mg BD along with topical voriconazole and even underwent superficial keratectomy. The mean follow-up period was 1 month (range, 2 weeks–4 months). Two patients received nonspecific broad-spectrum antibiotic treatment with natamycin and ciprofloxacin. All of the patients clinical symptoms and signs resolved completely to treatment as documented on subsequent follow-up with no or little residual corneal scarring.

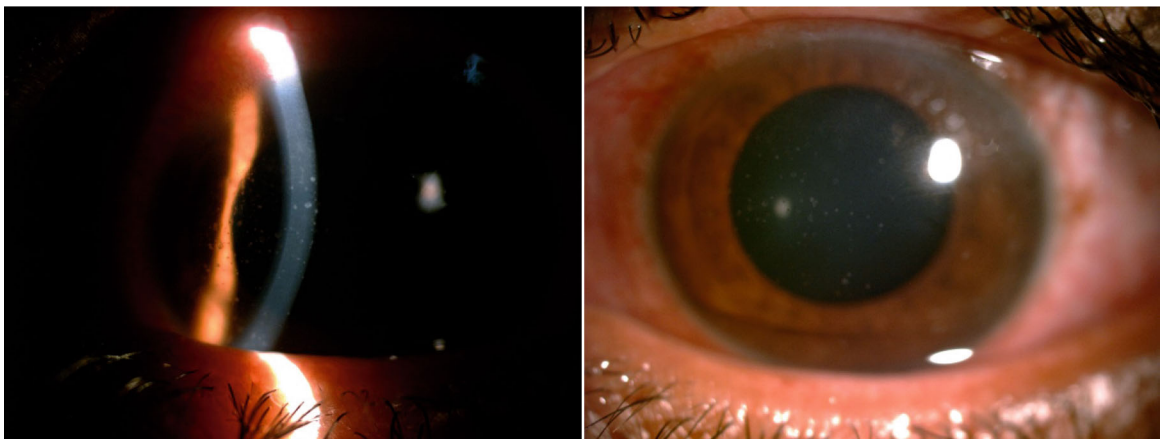

**Fig. 1** Slit-lamp biomicroscopy features showing infiltrate with epithelial defect with surrounding stromal edema

**Table 2** Results of microbiological investigations on corneal scrapings of 8 patients with microsporidial keratoconjunctivitis

| S. no. | Specimen | Calcofluor white | Modified trichrome | Gram stain | PCR |
|---|---|---|---|---|---|
| 1 | Corneal scraping | Positive | Negative | Positive | Positive |
| 2 | Corneal scraping | Positive | Negative | Positive | Positive |
| 3 | Corneal scraping | Positive | Positive | Negative | Positive |
| 4 | Corneal scraping | Positive | Negative | Positive | Positive |
| 5 | Corneal scraping | Positive | Negative | Negative | Positive |
| 6 | Corneal scraping | Positive | Positive | Negative | Positive |
| 7 | Corneal scraping | Positive | Positive | Positive | Positive |
| 8 | Corneal scraping | Positive | Positive | Positive | Positive |

## Discussion

Due to recent increase in the awareness of the diseases, reporting of ocular microsporidiosis have risen worldwide (Sabhapandit et al. 2016). Still, a majority of these infections goes underdiagnosed or misdiagnosed as viral keratoconjunctivitis. However, there is limited information regarding prevalence, clinical and diagnostic profile of ocular microsporidiosis and only handful of studies are available from India. The exact prevalence of ocular microsporidiosis is not known, however, Joseph et al. (2006b) reported 0.4% prevalence rate in South India. One hundred thirty four cases of microsporidial keratoconjunctivitis were reported from Singapore by Loh et al. (2009) in a period of 4 years. We describe eight cases from our region. The previous reports described ocular microsporidiosis in the 16- to 46-year age group (Lewis et al. 2003; Metcalfe et al. 1992). Similar, finding was observed in our study. The disease was unilateral, with a male preponderance in our study, similar to the existing reports (Joseph et al. 2006a). So, far as exposure to foreign body, use of contact lens, soil exposure, ocular trauma and exposure to swimming pool have been identified as predisposing risk factors for ocular microsporidiosis (Tham and Sanjay 2012). Majority (3 patients) of our cases had history of ocular trauma and none of patients in our study used contact lens.

All the patients presented to us with diffuse, multiple, pin-point to pin head lesions, with epithelial or subepithelial infiltrates which were consistent to clinical signs of microsporidial keratoconjunctivitis described in literature (Das et al. 2012). High prevalence of microsporidial keratoconjunctivitis has been reported during rainy season by Das et al. (2012) and Loh et al. (2009) however, we did not observe any seasonal variation in our study. The interesting feature of our study was that all patients were immunocompetent, however one patient had diabetes mellitus.

One of our patients presented with sinusitis followed one week later by keratoconjunctivitis. Keratoconjunctivitis and sinusitis caused by *Encephalitozoon* spp. have been reported (Lewis et al. 2003). The onset of ocular symptoms after the worsening of chronic sinusitis suggests primary upper respiratory tract infection and its subsequent spread to the eye (Rossi et al. 1999).

Microbiological analysis plays an important role in differentiating microsporidial infection from other

infective keratitis. The various techniques available for the diagnosis of ocular microsporidiosis include microscopic examination of microsporidial spores using various staining techniques, culture and PCR. Although, microscopic examination of microsporidial spores has been shown to have high sensitivity but requires expertise (Malhotra et al. 2017; Saigal et al. 2013). On the other hand, role of culture is limited as these pathogen requires tissue culture facilities. Several published studies describe PCR-based methods for diagnosis and species differentiation of microsporidia (Saigal et al. 2013; Reddy et al. 2011). We used microscopic examination using staining techniques and PCR for clinically suspectedmicrosporidial keratoconjunctivitis cases to enhance diagnostic capabilities.

Infectious keratitis is the fourth-leading cause of blindness worldwide and delay in management can lead to ocular morbidity (Reddy et al. 2011; Khor et al. 2018). The management of ocular microsporidiosis poses difficulty because no definitive therapy exists. Various oral or topical agents have been suggested to be useful in the treatment of this pathogen namely fumagillin, PHMB, itraconazole, albendazole but their efficacy remains controversial and long duration of treatment increases the toxicity profile (Agashe et al. 2017). Loh et al. (2009) have found good clinical response to topical fluoroquinolone monotherapy. Khandelwal et al. (2011) advocated use of topical Voriconazole for successful treatment of microsporidial keratitis.

In clinical trial of 0.02% polyhexamethylene biguanide versus placebo in the treatment of microsporidial keratoconjunctivitis, it has been observed that microsporidia is a self-limited disease in immunocompetent patients. However, it may depend on clinician choice to wait and see or administer some drug (Das et al. 2010).

Surgical debridement that therapeutically debulks the organism load from the corneal epithelium may prove to be most helpful in lesion resolution. We noticed a good clinical response with debridement followed by topical 1% voriconazole eye drops in our patients without any significant side effects. The visual prognosis was good but took a long time to resolve completely and one patient showed recurrence of infection who was ultimately treated by superficial keratectomy and addition of oral albendazole therapy. Reported visual outcome after resolution of microsporidial keratitis is satisfactory usually and residual subepithelial scars usually do not affect visual acuity.

## Conclusion

In conclusion, this study is a reminder to clinicians that when multifocal diffuse epithelial keratitis and culture-negative keratoconjunctivitis not responding to medical therapy, regardless of immune status are found in patients with symptoms suggesting an infectious keratitis, microsporidial keratoconjunctivitis should be included in the differential diagnosis. Close association between the ophthalmologist and the microbiologist is essential to make diagnosis and prevent further complications. Our case series shows that careful observation of clinical findings supplemented by a comprehensive microbiological workup aided in pathogen identification and specific antimicrobial administration resulting in the successful outcome of our patient.

**Authors' contribution** SK guided the study and checked the paper, SKA wrote the paper and also performed the experiment, KM did the staining and pcr, NJ collected the sample, SD and AG helped in providing the samples.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no competing interest.

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