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Case Report: Ocular Microsporidiosis: Case in a Patient Returning from India and Review of the Literature

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Abstract. Microsporidia are protists close to the kingdom of fungi that may cause eye infections. Most cases are reported in Asia and affect both immunocompromised and immunocompetent patients. Here, we report a rare case of microsporidial keratoconjunctivitis in an immunocompetent French patient 3 weeks after returning from India. In our patient, Weber trichrome staining of conjunctival scrapings revealed rounded elements approximately 1–3 μm in size. Conventional polymerase chain reaction analysis by ribosomal RNA subunit sequencing showed 100% identity with *Vittaforma corneae*. Treatment by corneal debridement combined with fluoroquinolone eye drops allowed complete resolution of the lesions. Although rare, ocular microsporidiosis should be investigated in a patient who is native to Asia or has returned from an endemic area and presents with keratoconjunctivitis of undetermined etiology.

INTRODUCTION

Microsporidia are eukaryotic, obligate microorganisms with intracellular tropism, closely related to the kingdom of fungi.[1] Since their first discovery in 1857, approximately 1,300 species belonging to nearly 200 genera have been described.[2,3] The organisms are ubiquitous in distribution and cause a wide spectrum of clinical infections in humans, including digestive, ocular, sinus, pulmonary, muscular, and renal infections, mainly involving immunosuppressed patients.[4–6] Ocular disease is the second most common type of infection after digestive infection.[7] Early published studies described two distinct clinical entities affecting the cornea which appear to be correlated to the immune status of the patient: superficial keratoconjunctivitis in immunosuppressed patients and punctate stromal keratitis in immunocompetent patients.[8–10] However, over the past few years, the incidence of microsporidial keratoconjunctivitis has increased significantly in immunocompetent individuals in Asia.[11]

We report a case of ocular microsporidiosis diagnosed in France in an immunocompetent patient who had returned from a holiday in India. We also present a mini-review of the literature dealing with this subject.

CASE REPORT

A 53-year-old male patient, living in France and without any previous medical history, presented at the end of September 2015 at the ophthalmology emergency department of Lille University Hospital with unilateral redness of the left eye associated with a decrease in visual acuity, despite the use of norfloxacin-based eye lotion (Chibroxine®; Théa Laboratoires, Clermont Ferrand, France) for several days. The patient reported that he had been in Rajasthan and Uttar Pradesh (India) for 3 weeks during the monsoon season (August) and had bathed in hotel swimming pools in Jaisalmer and New Delhi. Scraping of the eye lesion was performed. Gram's stain did not reveal any bacteria. Different culture media (blood agar, PolyVitex chocolate agar, heart/brain broth, Lowenstein–Jensen medium, and Sabouraud agar) were sterile. Investigations for *Acanthamoeba* spp. were also negative. Many oval elements, 1–3 μm in size (Figure 1), were observed after staining with Weber's modified trichrome. Investigations for *Microsporidium* spp. in corneal scrapings by conventional PCR targeting the small subunit rRNA gene (forward primer 5′-CAGGTTGATTCTGCCTGACG-3′ and reverse primer 5′-ATCTCTCAGGCTCCCTCTCC-3′) followed by sequencing of the PCR product showed 100% identity (GenBank accession number MG206087) with *Vittaforma corneae* sequences.[12] The disease resolved within 15 days after corneal debridement and local treatment with eye lotion (Norfloxacin 0.3%).

DISCUSSION

Microsporidiosis was first described in 1857 in silkworms by Carl Wihelm von Nägeli.[2] The eukaryotic, unicellular, obligate intracellular parasites involved are ubiquitous and have a worldwide distribution.[4,13] The term "microsporidia" is a designation without any taxonomic justification, currently used to designate organisms belonging to the phylum of Microspora included in the subkingdom of Protista.[14] Phylogenetic analysis suggests that the Microsporidia are related to the Cryptomycota as a sister group to the fungi.[15,17]

Currently, 14 species of microsporidia have been implicated in human diseases.[18] The species described in human ocular infections belong to seven distinct genera: *Enterocytozoon*, *Encephalitozoon*, *Pleistophora*, *Traphipleistophora*, *Brachiola*, *Nosema*, and *Vittaforma*.[19] Cases have been described anecdotally in Europe, Africa, South America, and the United States.[20,21] Although rare, most cases of ocular microsporidiosis are found in Asia. They have been reported in China, Malaysia, and Thailand, and an increasing number of cases are being reported in India and Singapore.[22–24] Their prevalence varies depending on the region and the diagnostic methods available.[21] In Asia, the prevalence of microsporidial keratoconjunctivitis varies between 0.4% and 19.7%,[23,25] whereas the prevalence of stromal keratitis is lower.[26] To

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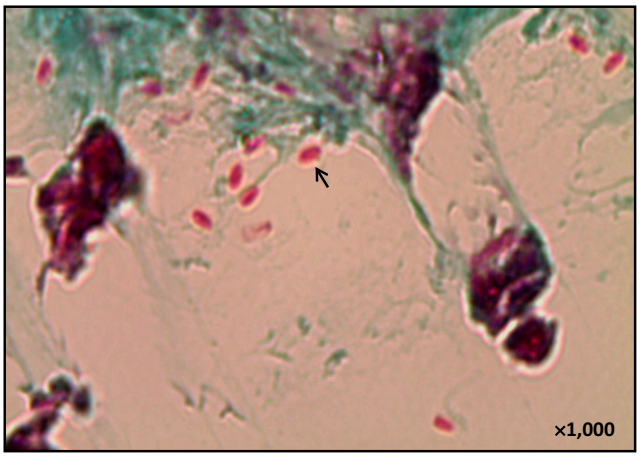

FIGURE 1. Corneal scraping stained with Weber's modified trichrome (×1,000).

date, 56 cases of *Vittaforma corneae* corneal lesions were reported in the Southeast Asia (Table 1).[27–30]

The sources and routes of ocular infection are less clear than those for digestive diseases.[31] Although cases of zoonotic, aerial, alimentary, and interhuman infection have been described, water sources are probably the most important route of infection because most potentially pathogenic species to humans have been identified in water and are resistant to chlorine.[2,32,33] Predisposing factors include the wearing of contact lenses and misuse of lens cleaning solutions, surgery or corneal transplant, corticosteroid eye lotion, trauma, bathing in thermal baths, and exposure to dirty water or soil.[23,34–36] An epidemic has been reported in rugby players.[10] Seasonal variation in infection has been observed in Singapore, India, and Thailand, with a peak in incidence of around 60% in the monsoon period, from July to October.[11,23,37]

Microsporidia are characterized by their spore. This structure is responsible for resistance to environmental changes and allows for dissemination.[38] The spore length varies depending on the species and is generally between 2 and 8 µm, with variations from 1 to 30 µm.[32] Microsporidia do not possess a peroxisome or mitochondria but have an organelle known as a mitosome.[2] The cell wall of the spore is composed of a double envelope with an outer part (exospore) containing glycoproteins and an inner part (endospore) rich in chitin.[5,39] The endospore conceals the plasma membrane delimiting the sporoplasm, the infectious element formed by the cytoplasm and one or two nuclei.[40] Microsporidia can be distinguished from other unicellular organisms by the presence of an ejection apparatus known as the polar filament, which is involved in host cell invasion.[13] Different stimuli can induce quasi-instantaneous extrusion of the polar filament, which ejects the sporoplasm into the cytoplasm of the host cell.[39]

Ocular microsporidiosis affects the cornea, with deep or superficial lesions, and/or the conjunctiva. The most frequently reported forms include punctate stromal keratitis and superficial keratoconjunctivitis. Scleritis and endophthalmia are rare (Table 2).[19,21,41–43] The first case of ocular microsporidiosis was described in a child in Sri Lanka in 1973.[44] It was only in the 1990s, during the acquired immune deficiency syndrome epidemic, that a much larger number of cases of keratoconjunctivitis were reported.[45] Since early 2000, an increase in stromal keratitis and keratoconjunctivitis cases has been described in immunocompetent patients.[21,46] As reported previously for our immunocompetent case, clinical symptoms include unilateral pain, conjunctival redness, photophobia, the sensation of a foreign body in the eye, and blurred vision.[35,45] Progressive and significant decrease in visual acuity complicated by a perforated corneal ulcer in stromal keratitis was also reported.[47] The clinical features of our case may mimic adenovirus or herpes keratoconjunctivitis. However, it appears that the infection is deeper and more extensive in ocular microsporidiosis.[21,45,47] Recently, *Herpes* virus and microsporidia have been reported to coexist in granulomatous inflammation.[48]

Electron microscopy was formerly the reference technique for the identification of species based on studying the structural characteristics of the spore.[49,50] However, this tedious and time-consuming technique has low sensitivity and is not available routinely.[5] The diagnosis of microsporidiosis is generally made by optical microscopy. Different stains are used and a combination of stains can increase the sensitivity of microscopic examination.[51] Using Weber's modified trichrome stain,[52] spores of microsporidia appear a brilliant pink against a blue or pale green background (Figure 1).[51] Other stains used routinely, such as Gram or Giemsa stain, have very limited usefulness for ocular specimens. The spores stain strongly and oval, non-budding forms can be seen which can easily be confused with bacteria or yeasts.[51,53] Fluorochromes Calcofluor white 2M (Merck, Darmstadt, Germany) and Uvitex 2B (Polysciences Inc. Warrington, PA) electively stain chitin in the cell wall of microsporidia spores. This technique has the advantage of being easy, rapid, and sensitive.[49] Nevertheless, reading of slides is time consuming, requires an experienced eye, and does not allow species identification.[14] Precise identification of the species involved is essential for determining the most effective treatment and risk of dissemination.[25]

Molecular identification has the advantage of being rapid and helps to identify most of the species implicated in ocular

TABLE 1
Cases of *Vittaforma corneae* corneal lesions reported in the Southeast Asia

Country	Clinical form	Identification methods	Contamination	Number of cases	Treatment	Reference
India	Keratoconjunctivitis stromal keratitis	PCR (SSU-rRNA)	Water, insect, dust, trauma	15	NR	57
Singapore	Keratoconjunctivitis	PCR (SSU-rRNA)	Mud/soil	11	NR	27
Vietnam	Keratoconjunctivitis stromal keratitis	EM and PCR (SSU-rRNA)	Water	1	Penetrating keratoplasty	28
Taiwan	Keratoconjunctivitis	PCR (16S rRNA)	Water (hot springs)	6	Debridement, topical antibiotics	36
Taiwan	Keratoconjunctivitis	PCR (16S rRNA)	NR	10	Swabbing, topical antibiotics	29
Singapore	Keratoconjunctivitis	PCR (SSU-rRNA)	Mud/soil	4	Fluoroquinolone, topical antibiotics	30
India	Stromal keratitis	PCR (SSU-rRNA)	NR	9	NR	9

EM = electronmicroscopy; NR = not reported; SSU = small subunit.

TABLE 2
Clinical details and isolated species of reported human cases with microsporidosis of the eye, according to the location of lesion

Location	Characteristics of lesion	Immune status of patient most often reported	Main species	Treatment reported to be effective	References
Conjunctiva and cornea	Superficial lesions; keratoconjunctivitis	Immunosuppressed/ immunocompetent	*Encephalitozoon*	Albendazole and/or topical fumidil B alternative: Fluoroquinolone	10, 18, 56, 58, 61
	Deep lesions; punctate stromal keratitis	Immunocompetent	*Nosema* and *Microsporidium*	Penetrating keratoplasty	10, 18, 62
Sclera	Scleritis	Immunocompetent ($N = 1$)	NR	NR	41
Interior eye	Endophthalmia	Immunosuppressed ($N = 2$) Immunocompetent ($N = 1$)	NR	NR	42, 43

NR = not reported.

infections, including *V. corneae*, *Encephalitozoon hellem*, *Encephalitozoon cuniculi*, and *Encephalitozoon intestinalis*.[14] PCR techniques specific for these species have been developed and described.[12,14,54–56] A pan-species PCR, based on amplification of a fragment of 16S rRNA, was developed by an Indian group using conjunctival specimens and had a sensitivity of 83% and specificity of 98%.[57] In our patient, *V. corneae* was identified using a similar molecular approach.

Medical treatment of ocular microsporidiosis has variable success. Its efficacy depends on the species involved and immune status of the patient.[26] Albendazole and fumagillin have superior in vitro activity to other drugs used in the treatment of keratoconjunctivitis, whereas stromal keratitis appears to be refractory to a number of drugs.[26,35,58] Albendazole has good activity against *Encephalitozoon* spp. but only weak activity against *Enterocytozoon bieneusi*.[58] Fumagillin has been studied in in vitro and in vivo models and has been shown to be active against infections caused by *E. hellem*, *E. cuniculi*, *E. intestinalis*, *E. bieneusi*, and *V. corneae*.[58–60] Topical use of fumidil B is recommended for the treatment of keratoconjunctivitis.[35] Fluoroquinolone eyewashes have been used as an alternative in human cases.[35,53,61] Surgical debridement does not appear to be superior to medical treatment and is not required in most of the patients with keratoconjunctivitis.[62,63] Furthermore, a randomized study demonstrated complete cure, without recurrence, in the absence of medical treatment, reinforcing the idea that keratoconjunctivitis is an anatomically limited infection.[26,64] Corticosteroids appear to be ineffective and may even make the disease worse.[10] In our patient, corneal debridement and administration of fluoroquinolones resulted in definitive treatment. By contrast, stromal keratitis requires early surgical treatment by penetrating or deep anterior lamellar keratoplasty.[65]

Although rare, microsporidia are an emerging cause of ocular infection in immunocompetent patients. This etiology should be evoked in a patient with keratitis of undetermined origin originating or returning from Asia, particularly during the rainy season.

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