OCULAR MICROSPORIDIOSIS: AN EMERGING EYE INFECTION

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I. Introduction

The microsporidia are a group of ubiquitous, obligate intracellular parasites. They were first described in Germany by Carl Wilhelm von Nägeli in association with European silkworm disease on September 21, 1857 in a meeting at Bonn.1 Thereafter the parasites became well known as pathogens of invertebrates and fish. They have also been shown to be normal flora in the intestine of immunocopetent individuals2 but the drive to investigate these organisms increased by the more recent discovery of microsporidian infections, especially gastrointestinal, in immunocompromised humans, such as acquired immunodeficiency syndrome (AIDS), organ transplant and cancer patients in the 1980s and 1990s.3 The organisms are well known to veterinarians and general physicians but the awareness about this organism among ophthalmologists is relatively recent. In 1990s several cases of microsporidial keratoconjunctivitis associated with human immunodeficiency virus (HIV) infection appeared from the United States of America. Thereafter, cases have been reported in both immunocompetent and immunocompromised patients from many other parts of the world. Inability to grow the organism in culture and difficulty in diagnosis by conventional laboratory methods has been responsible for the low awareness. The first report from India was from L V Prasad Eye Institute, Hyderabad in 2003 in an immunocompetent individual.4 Thereafter, many reports from India have appeared in peer reviewed journals. With a brief background on the biology and systemic infections, this article focuses on the ocular infections associated with microsporidia, with emphasis on its epidemiology, clinical features, diagnosis and treatment.

Biology of Microsporidia

Microsporidia are unicellular, obligate intracellular parasites and exist in nature as highly organized spores that range in size from 1 - 40 m.5 Two rigid extracellular walls bind the spore. The sporoplasm inside the spore is the infective material of microsporidia. The sporoplasm contains one or two nuclei arranged as a diplokaryon, cytoplasm enriched with ribosomes, golgi apparatus and the polaroplast.6 Mitochondria are characteristically absent. The organelle associated with infection is the polar filament or the polar tube (diameter 0.1-0.2?m). Under appropriate conditions inside a suitable host, the polar filament is discharged. Inside the host cell there are two distinct phases in the development of microsporidia: a proliferative phase (merogony) and a sporogonic phase (sporogony). In merogony, the sporoplasms are released from the spores and become meronts by repeated binary fissions. Meronts develop into sporonts, which are characterized by a dense surface coat. Sporonts divide into sporoblasts that finally develop into mature spores.7

Systemic Infections: Epidemiology, Source and Clinical Profile

Microsporidiosis in humans occurs worldwide and the reported prevalence varies from 0 and 50% depending on the region, diagnostic method, and the population.8 In regions (Africa, Asia, South America) where antiretroviral treatment is not readily available, systemic microsporidiosis has been seen in human immunodeficiency virus (HIV) infected patients with AIDS. Additional risk factors include poor sanitation and exposure to animals.9 Microsporidiosis has also been

seen in immunocompetent individuals especially travellers, children, the elderly, and organ transplant recipients.10 The genotypes of microsporidia known to infect humans have been identified in animals, thus making microsporidiosis a zoonotic disease.11 Association between microsporidia and food/water borne transmission due to contaminated irrigation water has been reported. The organisms have been identified on lettuce, parsley and strawberries in Costa Rica.12 Dairy cows' milk was shown to be positive for microsporidia suggesting transmission through milk.13

The first of human microsporidiosis was reported in 19591 in a 9-year-old Japanese boy with fever, headache, vomiting and spastic convulsions. The parasite was isolated by mice inoculation of cerebrospinal fluid and urine. Less than 10 human cases were reported by 1985, two of which were corneal infections.14

These parasites cause a severe, non-bloody, non-mucoid diarrhoea, progressive weight loss, and malabsorption of fat. Prevalence rates for microsporidiosis in patients with chronic diarrhoea vary depending on the method of diagnosis but an average prevalence is 30%. Most of the AIDS patients who present with microsporidiosis as an enteric pathogen are severely immunosuppressed, with CD4+ counts below 100. Cholangitis ,15 hepatitis,16 chronic sinusitis, myositis and peritonitis17 are other infections that have been described in AIDS patients. E. cuniculi has been reported to cause reversible renal failure in an HIV positive patient.18 A disseminated microsporidiosis in a pancreas and kidney transplant patient has been described.19 Urethritis and prostatitis in the setting of disseminated microsporidiosis has also been described.20 Microsporidia have also been associated with skin infections21 in immunocompromised hosts. Both E. cuniculi 22 and Trachipleistophora spp.21 have presented as encephalitis with mass lesions mimicking central nervous system Toxoplasmosis.

Transmission of microsporidia has been established to be both vertical (transovarial) and horizontal. Although vertical transmission in humans has not been reported, the presence of the parasite in the respiratory tract and in intestinal tract of infected individuals and excretion of the spores in urine and stool suggests that horizontal transmission is possible in humans. The routes may include fecal-oral, oral-oral, inhalation, ingestion or direct inoculation. Direct evidence of zoonotic transmission of microsporidiosis has been demonstrated in a child exposed to puppies infected with E. cuniculi.23 Microsporidian spores are viable in water and also after desiccation at various temperatures; therefore, indirect zoonotic transmission between animals and humans is possible through contaminated water, food, or aerosols. In addition, evidence exists about vector borne transmission of Anncaliia (Brachiola/Nosema) algerae, a natural pathogen of mosquitoes. Reported risk factors of microsporidiosis in HIV- infected individuals have also included bee, wasp or hornet sting. Several species of human microsporidia (E. bieneusi, E. intestinalis, V. corneae) from environmental water (ditch, surface water) samples have been isolated24 which suggests water as source of infection and there is at least one report of presumed waterborne outbreak.25

Ocular Microsporidiosis

Ocular microsporidiosis was first described by Ashton and Wirasinha in 1973 in a 11-year old boy from Sri Lanka with a history of trauma.14 The organisms were initially confused with Leishman-Donovan bodies in the histopathological examination of the corneal sections. However, they were later confirmed to be microsporidia. The organisms stained weakly positive with hematoxylene, periodic acid-Schiff and Gram stain but stained well with Giemsa stain and methylene blue. They were described to be well seen by phase contrast microscopy. The second case of corneal microsporidial infection was an acute necrotizing keratitis.26 The

ultrastructural electron microscopic studies confirmed the organism. Thereafter, in early 1990s several cases of superficial keratitis in patients with acquired immunodeficiency syndrome (AIDS) were reported which were very different from the earlier cases in immunocompetent individuals. 27,28,29 The clinical picture in these patients was that of keratoconjunctivitis and the organisms were present in conjunctiva as well as cornea. In late 1990s and early part of 21st century, superficial epithelial keratoconjunctivitis caused by microsporidia were also reported from immunocompetent individuals.30,4

Risk factors of Ocular Microsporidiosis

Although fecal-oral transmission is the likely route of infection with intestinal microsporidiosis, the source of ocular infection is not clear. Systemic immunosupression including HIV infection has been reported to be associated with microsporidial keratoconjunctivitis.30 Contact lens wear is an important risk factor for corneal infection, which may result from trauma induced by the contact lens on the cornea resulting in a breach of epithelium. The contact lens may act as a vehicle for organisms to reach the cornea. Theng et al have described a case of microsporidial keratoconjunctivitis in an otherwise healthy contact lens wearer.31Topical corticiosteroid32 may predispose to microsporidial superinfection especially in the context of other risk factors.31Occurrence of microsporidial keratoconjunctivitis in a corneal graft33 may be related to local immunosuppression with corticosteroids. Ocular trauma is found to be associated with microsporidial keratoconjunctivitis. Trauma with dust and insect bite has been reported in the literature.34 Out of 152 cases seen over a period of 20 months at L V Prasad Eye Institute, Bhubaneswar, India, 40 (25.3%) cases had a history of trauma (unpublished data). History of mild trauma may be co-incidental. Clinical picture of one of our patients is shown in Figure 1.

Association of keratoconjunctivitis and sinusitis has been reported in the literature3,32 suggesting upper respiratory tract as a primary route of the infection with subsequent spread to the eye.35 Close contact with domestic animals (cats) and birds, may be important source of ocular infection.27,28 Microsporidia are generally transmitted via direct human-to-human contact, but it can also survive in water and food and has been found in surface water used as drinking source.36 A seasonal (rainy season) outbreak of microsporidial keratoconjunctivitis mimicking adenoviral keratoconjunctivitis has been reported by us.37 Large number of patients (46-70%) have had exposure to muddy water in two series of microsporidial keratoconjunctivitis reported recently from Singapore (poster presentations- P0071, P0137, American Academy of Ophthalmology, Atlanta 2008, abstract book, p201, 225).

Clinical features of Ocular Microsporidiosis

Lowder et al reported the first case of microspridial keratoconjunctivitis in 1990.38 The patient had a history of red eyes which did not respond to topical antibiotics. The conjunctiva had a mixed follicular-papillary reaction, and the cornea had a diffuse coarse punctate epithelial keratopathy. In the same year, three additional cases of AIDS associated microsporidial keratoconjunctivitis from New York were reported.39The symptoms in these three cases were photophobia, blurred vision, and foreign body sensation. Slit lamp findings showed a superficial, corneal epithelial keratopathy characterized by coarse fluorescein staining and non-staining epithelial lesion, minimal conjunctival involvement and decreased conjunctival lustre. Microsporidial spores were demonstrated in conjunctival biopsy. Many cases in immunocompetent individuals have been reported in the literature.40,32,41,4,34 Corneal graft infection with microsporidia has also been reported.33 Recently, there has been an upsurge in reporting of microsporidial

keratoconjunctivitis from Singapore (55 cases per year) . The authors described clinical features and management in a retrospective series of 134 patients (poster presentation- P0071, American Academy of Ophthalmology, Atlanta 2008, abstract book, p 201).

Although a number of cases have been reported, microsporidial stromal keratitis is rarer than superficial keratoconjunctivitis and occurs mostly in immunocompetent patients. After the first report in 1973, there have been occasional reports of stromal keratitis caused by microsporidia until 1991.14,26,42,43The most recent reports of stromal keratitis are two cases from UK44 and one each from Bangkok 45and India.46 Both cases from UK were immunocompetent and were originally thought to be herpetic or adenoviral disease. Vemuganti et al from L V Prasad Eye Institute, Hyderabad have reported a larger case series of five cases of stromal keratitis.47 As is evident from these reports, microsporidia stromal keratitis is a slowly progressive disease affecting individuals of any age. The duration of symptoms ranges from months to years suggesting slow indolent nature.47Clinically it may mimic a suppurative or non-suppurative inflammation. No definitive risk factor has been identified, although trauma has been associated in some of the cases.14,47 Clinically, it may present like progressive herpes disciform keratitis with recurrent stromal infiltration and uveitis.44,46,47,48,49 It should be considered in the differential diagnosis of culture-negative stromal keratitis which do not respond to standard medical therapy in immunocompetent individuals.

Although cellular reaction in the anterior chamber is common (poster presentation- P0137, American Academy of Ophthalmology, Atlanta 2008, abstract book, p225), posterior segment involvement due to microsporidial infection is unusual. Meitz et al had reported a case of sclerouveitis with retinal detachment.50 There is a single report of endophthalmitis due to microsporida infection.51 Intraocular microsporidiosis in a patient with idiopathic CD4+ T-lymphocytopenia has also been reported.52

Diagnosis of Ocular Microsporidiosis

The in vivo non-invasive technique of confocal microscopy was found to be useful in the diagnosis of microsporidial keratitis.53,54 High contrast intraepithelial opacities within surface corneal epithelial cells were observed, and diagnosis was confirmed on chromotrope-based Weber stain. This technique offers the potential for instantaneous diagnosis of microsporidial keratitis in the clinic and the ability to monitor the effectiveness of treatment in deep seated infections.

Small, oval spores within the epithelial cells or as extracellular structures can be seen in conjunctival or corneal scrapings. These spores have a uniform oval shape and are non budding, which help to differentiate them from bacteria and yeasts. The spores stain well with calcofluor white, Gram, Giemsa and many other stains. Comparison of commonly used staining techniques for the demonstration of microsporidial spores in corneal scrapings has determined calcofluor white and modified acid fast (1%) stain to be the most rewarding stains.55 We regularly examine smears of corneal scrapings using, 1) potassium hydroxide with calcofluor white stain (KOH+CFW) under fluorescence microscope, 2) Gram stain under light microscope and 3) modified acid fast (1% H2SO4) stain under light microscope. Figure 2 shows the microsporidial spores in corneal scraping smears stained with some of these methods.

The classification of microsporidia is dependent on transmission electron microscopy (TEM). The presence of polar tubule is diagnostic and helps classify an organism as a member of the phylum Microspora.56Although this technique remains the gold standard till date for confirmation and species identification of microsporidia, molecular-based techniques are taking over the role rapidly.

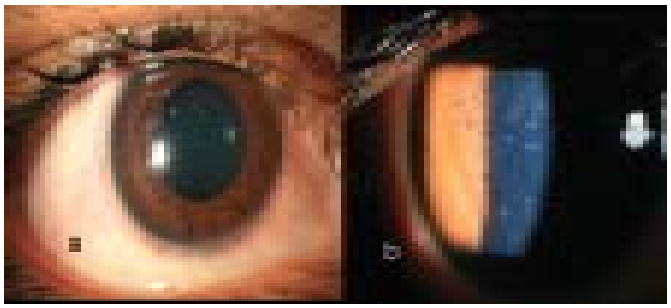

Figure 1: Slit-lamp picture of a patient with microsporidial keratoconjunctivitis showing (a) conjunctival congestion and multiple, coarse, small epithelial lesions involving central cornea, and (b) slit section showing raised, well defined lesions with clear surrounding cornea.

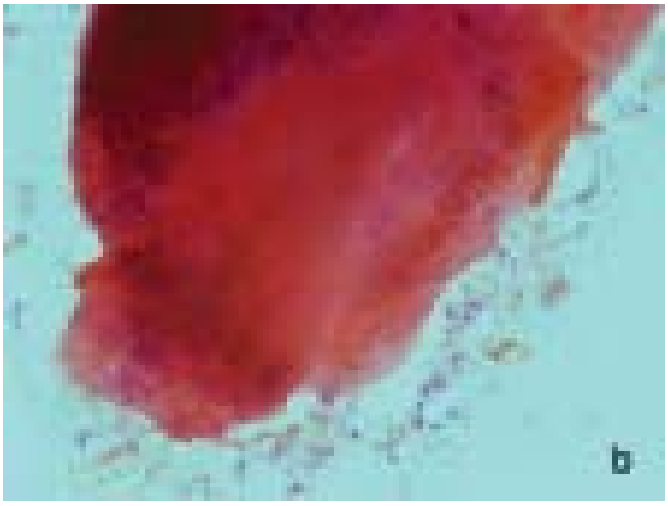

Figure 2: Corneal scraping from a patient with keratoconjunctivitis showing, (a) numerous brightly fluorescent oval spores of microsporidia in calcofluor white stain (fluorescence microscopy, x1000) and gram positive intracellular and extracellular oval spores of microsporidia in Gram stain (light microscopy, x1000)

Being an obligate intracellular parasite, microsporidia can not be cultured in conventional culture media. They require tissue culture for growth. Didier et al28 established three isolates of microsporidia from corneal tissue and conjunctival scrapings in MDCK cells. The other cell lines used to culture these isolates include E6, HLF, MRC-5, MDCK, rabbit kidney (RK-13), and foetal bovine lung fibroblasts. We compared Vero, HeLa and SIRC cell lines for the growth of four species of microsporidia and vero cell line supported the growth the best.57 However, the time taken for growth was long and species identification was not possible.

Use of immunological methods for the diagnosis of ocular microsporidiosis has been limited. Schwartz et al. reported success with immunofluorescent antibody (IFA) techniques against microsporidia for species identification with species-specific antisera.58 Antiserum to E. hellem has been produced by the Division of Parasitic Disease Control, CDC, Atlanta, however, it is not available commercially.

Similarly, molecular studies of the microsporidia have been limited. Conners et al59 have described a polymerase chain reaction (PCR) methodology that employs specific primers based on rRNA followed by sequencing, for the identification of microsporidia from corneal scrapings. PCR for microsporidia has been performed for identifying several Enterocytozoon and Encephalitozoon species of microsporidia. A single 270-base pair fragment was observed on agarose gel electrophoresis which was directly sequenced. Although several nucleic acid-based methods have been developed, commercial kits for clinical application are not yet available. Joseph et al60 were the first to apply a panmicrosporidian 16S rRNA gene based PCR assay on corneal scrapings (positive in direct miscroscopy for microsporidial spore) and obtained a sensitivity of 83% and a specificity of 98%.

Treatment and Outcome

While the treatment of microsporidial keratoconjunctivitis is essentially medical, the stromal variety requires surgical treatment. Currently there are no defined guidelines for the treatment of ocular microsporidial infections. Anecdotal reports of specific drug treatment for microsporidiosis include albendazole, thiabendazole, itraconazole, propamidine isethionate, fumagilin, chlorhexidine, metronidazole, polyhexamethylene biguanide and benzimidazoles. 29,34,35,61,62There is no consistency in clinical

response in vivo and parasitologic response in vitro, to various therapeutic agents. Albendazole is a broad-spectrum antihelminthic that has been shown to be effective in the treatment of microsporidiosis.61 Three of six patients in the series by Chan et al41 were given albendazole together with topical Fumidil B, whereas one was given albendazole alone. All four of these patients responded well to the treatment prescribed, with resolution of the keratitis within one month. Interestingly, the last two patients in that series did not receive any specific microsporidial therapy, but had a spontaneous resolution of the keratitis within two weeks of discontinuation of the topical steroids. Itraconazole has been used in the treatment of ocular microsporidiosis in the dosage of 100mg 2-3 times daily for six weeks and has shown complete disappearance of symptoms.63 Thiabendazole is an antihelminthic benzimidazole with larvicidal activity and some suggestion of activity against protozoan parasites. Nevertheless, a 0.4% suspension applied topically had no effect in the management of at least one case of microsporidial keratoconjunctivitis.29 Propamidine isethionate 0.1% (brolene), when given six times daily for three weeks led to resolution of microsporidial keratoconjunctivitis in a patient reported by Metcalfe et al.64On the other hand, the patients reported by Lowder et al failed to resolve on either brolene or itraconazole.65

Fumagillin has been used extensively by several ophthalmologists.29,66 It is an anti-angiogenesis factor consisting of a water-insoluble antibiotic extracted from Aspergillius fumigatus and inhibits RNA synthesis. Fumidil B (equivalent to 70 µg/mL of fumagillin) used topically as drops applied every hour was found very effective.3 Fumagillin and albendazole exhibit superior in vitro activity as well.67 Oral adjuvant therapy is unnecessary in healthy individuals. In healthy adults, the microsporidial keratoconjunctivitis tends to be self limiting. In a randomized clinical trial conducted by us, comparing the efficacy of 0.02% polyhexamethylene biguanide and placebo in cases of microsporidial keratoconjunctivitis, no significant difference was found in the time taken for healing of the corneal lesions in both the groups (Sharma et al, meeting presentation entitled "A prospective, randomized, double-masked, placebo-controlled trial of topical polyhexamethylene biguanide (0.02%) in patients with microsporidial keratoconjunctivitis" at the annual meeting of Ocular Microbiology and Immunology Group, Atlanta, USA, 2008, unpublished data).

The only surgical intervention that may be used in microsporidial keratoconjunctivitis is therapeutic epithelial debridement.4Debridement debulks the load of organisms from the corneal epithelium. Theoretically it may increase the risk of penetration of organism into the deep stromal layers, or increase the risk of secondary infection, however, despite regular debridement done in our patients, such a complication was not seen. Surgical intervention has been reported commonly with microsporidial stromal keratitis. There is no report of successful medical treatment of stromal microsporidial keratitis. Good outcome is achieved after penetrating keratoplasty.47 Font et al describe a case of stromal keratitis which was initially treated with oral albendazole and topical fumagillin with no improvement.48 Initial lamellar keratoplasty was followed by penetrating keratoplasty as the infiltrates reappeared in the interface within one week. However, lamellar keratoplasty may be done if the infection is restricted to the anterior or mid stroma. Topical fumagillin, which has been shown to be effective in epithelial disease, can be used postoperatively with no significant adverse side effects. 48

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