Introduction

Microsporidia are eukaryotic, spore forming obligate intracellular parasites, first recognized over 100 years ago. Microsporidia are becoming increasingly recognized as infectious pathogens causing intestinal, ocular, sinus, pulmonary, muscular and renal diseases, in both immunocompetent and immunosuppressed patients (1).

Ocular microsporidiosis, though uncommon, could be isolated or part of systemic infections. It occurs mainly in two forms: keratoconjunctivitis form, mostly seen in immunocompromised individuals; stromal keratitis form seen in immunocompetent individuals. are being increasingly recognized as emerging opportunistic infectious pathogen in immunocompromised patients(1,2).

The first case report of corneal microsporidiosis was published in 1973 (3). Two other case reports involving the corneal stroma were reported in 1981 and 1990 by Pinnolis et al (4) and Davis et al (5), respectively. A case series of five patients of Microsporidial stromal keratitis has recently been reported from south India (6). Microsporidial keratoconjunctivitis in a HIV-seronegative patient treated with debridement and oral itraconazole (7) and even in healthy individuals has been reported recently (8). A case of bilateral microsporidial keratitis from Bangladesh--infection by an insect parasite from the genus Nosema also has been reported (9).

Microscopic Features

Microsporidia are small (3.5-5.0 μm in length by 2.0-3.0μm in width), oval obligate intracellular eukaryotic protozoan parasites that belong to phylum Microspora. More than 100 species have classified into approximately 100 genera, and atleast 13 species have been reported to infect mammals (10-13). Phylogenetically Microsporidia are early eukaryotic organisms because they have a true nucleus, possess prokaryotic like ribosome and lack mitochondria(12). Seven genera (Enterocytozoon species, Brachiola species, Encephalitozoon species, Pleistophora species, Nosema species, Vittaforma species and Trachipleistophora species) as well as unclassified microsporidia (collectively refered to as Microsporidium) have been associated with human disease involving immunocompromised patients (10-13).

Epidemiology and Risk Factors

Microsporidia are increasingly recognized as opportunistic infectious pathogen in immunocompromised patients. The source of infection for human and routes of transmission are unknown ; however they are thought to be either orofaecal, resulting from direct inoculation, or occurring after trauma (1). Direct inoculation may occur with close contacts with domestic animals such as cats and birds (9,10); it may also spread from other infected persons (11,12). The normal life cycle of microsporidia comprises of invasion of the spore into the human host cell followed by discharge of the contents into the cytoplasm. Within the cell the sporoblast divides by binary fission to form scizont with 2-6 nuclei, which split into unicellular meronts. The meronts then secrete a rigid capsule and the fully formed spore measures about 2.5x1.5 microns. The cell eventually ruptures to continue the cycle and further destruction of the host tissue.

Clinical Features

Only two species Nosema and Encephalitozoon , are known to cause ocular infections. Two clinical presentations of ocular Microsporidiosis are observed as Corneal Stromal Keratitis, which occurs in immunocompetent patients and is commonly caused by Nosema corneum (13) and a Superficial Punctuate Keratoconjunctivitis occurring in acquired immune deficiency syndrome (AIDS) patients or in contact lens wearers mostly caused by genus Encephalitozoon . However, recent reports suggest that keratoconjunctivitis can also occur in immunocompetent individuals. Ocular Microsporidiosis in immunocompetent patients can mimic as herpes simplex virus keratitis. The patients usually present with history of recurrent episodes of pain, redness, watering, photophobia and dimunition of vision.
Some patient may be misdiagnosed as case of herpes stromal keratitis and get treated with topical steroids and antivirals. On examination patient may present with lid edema, conjunctival congestion. Corneal lesions are usually mid to deep stromal infiltrates with surrounding stromal edema. The overlying epithelium may be intact with edema or in some cases there can be an epithelial defect. There can be exudates on the endothelium.

**Diagnosis**

Microsporidia are small, obligate intracellular parasites that produce infective spores. They are fastidious organisms that are difficult to culture. The organism can be isolated using special tissue culture techniques, which are available only in a few specialized laboratories, and hence routine microbiological diagnosis might be difficult (16,19). Alcohol- fixed cytologic samples of scrapings from the conjunctiva, corneal epithelium, or both or biopsy specimens have proven very useful for demonstrating microsporidial blastospores (5, 20). Often conjunctival scrapings alone provide a satisfactory specimen for cytologic diagnosis in microsporidial epithelial punctuate keratoconjunctivitis. Cytologic findings demonstrate small, oval organisms within the epithelial cells, stromal keratocytes, and histiocytes as well as free extracellular structures (21-23). These spores have a uniform oval shape and are nonbudding, which helps to differentiate them from bacteria and yeasts (24). The spores stains strongly with gram stain (25,26). Giemsa stains have also been successfully used to demonstrate microsporidial spores (21,22). Occasionally, these spores stain poorly or not at all with routine stains (hematoxylin-eosin or the Papanicolaou methods), with the organism being easily overlooked in biopsy specimens or cytologic preparations (24,27).

Definitive genus identification of microsporidial ocular infections requires examination of corneal or conjunctival biopsy specimens, or both, by electron microscopy to demonstrate the number of coils of the filament (21-23, 25-27). The differentiation of *Nosema* species from *Encephalitozoon* is based on several electron microscopic features. First *Nosema* are larger than *Encephalitozoon* ( *Nosema* organisms measures approximately 3.5-5.0μm in length versus 2.0-3.0μm in length for *Encephalitozoon* organisms); second, the absence of a parasitophorous vacuole surrounding the organism within the host cell is more consistent with *Nosema*. Third, the coils of the filament range from 11 to 13 in *Nosema* versus 4 to 7 in *Encephalitozoon*.

**Treatment**

At present there is no known definitive medical treatment of deep microsporidial corneal stromal infections. Some previous reports have suggested that treatment with topical propamidine isethionate 0.1% (Brolene) (27-28) or systemic itraconazole (16) may be effective against microsporidial superficial keratoconjunctivitis. Yee et al (21) reported subjective improvement with debulking and a combination of topical neomycin, bacitracin, and polymyxin B antibiotics in a patient with bilateral epithelial keratopathy caused by *Encephalitozoon*, however complete resolution was achieved only after administration of oral itraconazole.

Friedberg et al (22) warned against corneal scrapings as well as the use of topical steroids or bandage contact lenses in such patients because they may result in secondary infection and penetration of the organisms into the deeper stroma. Recent reports have documented the successful treatment of Microsporidial superficial keratoconjunctivitis with topical fumagillin (29-33). Fumagillin is a naturally secreted water- insoluble antibiotic of *Aspergillus fumigatus* and is noted to possess an inhibitory effect on some intestinal protozoa (32). Fumagillin bicyclohexylammonium salt is water soluble form of fumagillin used commercially. The mechanism of action of fumagillin are not clearly understood but the preliminary data suggest that the drug may alter DNA content or inhibit RNA synthesis in the organism (34,35). Some authors have also suggested the role of oral Albendazole in combination with topical fumagillin. In case where the medical treatment fails penetrating keratoplasty is recommended rather than lamellar graft to treat deep stromal microsporidiosis to avoid any chance of recurrence in the lamellar bed.

**Conclusion**

Microsporidial ocular infections should be considered in the differential diagnosis of culture-negative stromal keratitis or keratoconjunctivitis refractory to conventional medical treatment.

**References**
