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Microsporidial Keratitis

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Abstract

Hundreds of genera and thousands of species of obligate intracellular spore forming eukaryotes constitute the phylum Microspora. Currently these organisms are known to cause infections in the immunocompromised individuals in whom they exhibit diverse clinical manifestations including pulmonary, ocular, intestinal, muscular and renal disease. Ocular microsporidiosis can present as an isolated entity or as a part of systemic infection. Infection is acquired by traumatic inoculation or by contact with contaminated soil or water. Clinically this infection can present as stromal keratitis, scleritis, keratoconjuntivitis and endophthalmitis. Seven genera have been found to be associated with human ocular infections. Infections by these pathogenic organisms involve unique pathogenic mechanism- injection of the sporoplasm in the host cell via polar tube. Protean clinical manifestations and resemblance to herpetic keratitis poses as a challenge in clinical diagnosis of this condition. Diagnosis therefore mainly depends on morphological demonstration of these organisms. Electron microscopy is the 'gold standard' diagnostic method to detect microsporidial spores. Molecular methods like PCR, real time PCR are sensitive tests and aids in identification till the species level. Drugs like albendazole and fumagillin are widely used to treat microsporidial keratitis. Penetrating keratoplasty is useful in treating medically non responding cases.

Keywords: Microsporidia; Keratitis; Spore; PCR; Diagnosis

Introduction

Microsporidia are small eukaryotic, spore forming obligate intracellular parasites belonging to the phylum Microsporidia. They were first recognised 100 years ago from the silk worm *Bombyx mori* as an etiological agent of the pebrine disease-a destructive disease in these worms [1]. Though initially believed to be early diverging eukaryotes, they are currently known to be closely related to fungi [2,3]. These infectious pathogens can exhibit diverse clinical manifestations such as ocular, sinus, renal, intestinal, pulmonary, and muscular diseases. Ocular microsporidiosis can present as stromal keratitis, scleritis, keratoconjuntivitis and endophthalmitis [4]. High index of suspicion is required for the diagnosis of ocular microsporidiosis.

Problem Statement

In 1973 the first case of corneal microsporidiosis was published by Ashton [5]. Later in 1981 Pinnolis et al. reported corneal ulcer due to genus *Nosema* and it was confirmed by electron microscopy [6]. In 1990 Davis et al. reported a case of stromal keratitis caused by genus *Nosema* [7]. Though initially it was believed to be a pathogen of the immunocompromised it has now been reported in a number of immunocompetent cases in various countries [8,9]. Increasing number of cases microsporidial keratoconjunctivitis has been reported mostly in Singapore [8,10,11] and India [12], among healthy, immunocompetent persons since the early 2000s.

Etiology

Microsporidia are small unicellular parasites and are considered as true eukaryotes because they have a nucleus with a nuclear envelope, an intracytoplasmic membrane system, and chromosome separation on mitotic spindles [13]. Joseph et al. reviewed the disease and detected four genera that infect humans *Encephalitozoon*, *Nosema*, *Pleistophora* and *Enterocytozoon* [5]. Seven genera (*Enterocytozoon* species, *Brachiola* species, *Encephalitozoon* species, *Pleistophora* species, *Nosema* species, *Vittaforma* species and *Trachipleistophora* species) as well as unclassified *microsporidia* (collectively referred to as *Microsporidium*) have been found to be associated with human disease involving immunocompromised patients. *Nosema* sp. and *Nosema* like organisms of the nontaxonomic group *Microsporidium* are the parasites involved in ocular microsporidiosis in immunocompetent individuals whereas in immunocompromised individuals *Encephalitozoon hellem* or other *Encephalitozoon*-like organisms were involved [13].

Pathogenesis

Life cycle of these organisms is completed within the human host. There is no role of an intermediate host or a vector in transmitting the developmental stages of microsporidia [13]. The spore is the infective and the resistant stage. In microsporidial keratitis spores are believed to enter the eye either by direct contact or trauma [14]. The spore has a unique means of inoculation of the infective sporoplasm. It extrudes the sporoplasm via the everting and unwinding polar filament into the host cell. Further after invading the cell the sporoplast division takes place by binary fission forming schizont with 2-6 nuclei, which split into unicellular merons. The merons secrete a rigid capsule thus attaining its final size measuring about 2.5 × 1.5 microns. The merons further differentiates into sporonts and spores. The cell finally ruptures

to continue the cycle and further destruction of the host tissue eventually occurs [9]. The spores of the genus Encephalitozoon reside in a parasitophorus vacuole which is a membrane bound vacuole. Members belonging to other genera complete their intracellular life cycle in direct contact with the cell cytosol [15].

Risk Factors

The prevalence of microsporidia is significantly increasing in immunocompetent people. Several predisposing factors for microsporidial keratitis in immunocompetent individuals have been reviewed. Exposure to soil/mud or dirty water is an important risk factor. Drinking water, can act as a reservoir for microsporidia since chlorination does not inactivate spores. Recent studies indicate a high prevalence of microsporidial keratoconjunctivitis in the rainy season, especially in India [12]. Other risk factors are trauma, dust particles, contact lens, insect bites, bathing in unclean river waters and LASIK surgery [16-18]. Exposure to the hot springs has also been reported as a risk factor [19]. Topical steroid therapy can cause localized immunosuppression and thus act as a risk factor [20]. Microsporidial keratoconjunctivitis transmitted by the donor corneal graft have also been reported [21].

Clinical Presentations

Ocular microsporidiosis can present in isolated form or can occur as a part of systemic infection. It follows a slow indolent course with duration of symptoms series ranging from one month to 2 years. Clinically ocular features of microsporidiosis can either represent as corneal stromal keratitis affecting immunocompetent patients caused mostly by *Nosema* and *Microsporidium* or superficial punctuate keratoconjunctivitis which mainly affects immunocompromised patients and contact lens wearers caused mostly by genus *Encephalitozoon* [22,23]. Immunocompetent individuals presenting with multifocal coarse punctate epithelial keratitis have been reported recently [16]. The patients present usually with clinical symptoms such as pain, redness, watering, photophobia and diminution of vision [24].

A close differential diagnosis of microsporidial keratitis is herpetic keratitis which also gives a similar clinical picture such as deep stromal infiltrates and surrounding stromal edema and keratitic precipitates thus it is very often misdiagnosed as viral keratitis [25].

Diagnosis

Isolation of Microsporidia is difficult, as they are obligate intracellular parasites which are fastidious requiring cell culture systems for growth. The diagnosis therefore mainly depends on morphological demonstration of the organisms themselves in scrapings or tissues.

Light Microscopy

Chromotrope staining

Weber's modification of trichrome stain: Here the chromotrope concentration of the staining solution is 10-fold higher, and the exposure time of the smear to the staining solution is prolonged to facilitate the penetration of the dye within the spore wall. Spore wall stains bright pinkish-red, showing sometimes a belt like stripe that girds the spores diagonally or equatorially. The background in the specimens counterstains faint green [26]. A modification of Weber's have been proposed by Ryan and colleagues wherein they substituted aniline blue for fast green and also reduced the level of phosphotungstic acid in the chromotrope staining solution [27]. Another modification was proposed by Kokoskin, where the temperature of staining was increased to 50°C thereby, reducing the time of staining to 10 min [28].

Gram-chromotrope

New staining procedure combining the traditional Gram staining for bacteria and the Weber's chromotrope staining method have been described by Hercules et al. [29].

Other stains used for light microscopy

Warthin starry stain can be used which stains the spore as brownish black. Modified Grams stains (Brown-Brenn, Brown-Hopps are sensitive and generally recommended. Mature spores stain Gram-positive, while immature ones stains red [30]. Haematoxylin and eosin can also be used but its sensitivity is uncertain if the parasites in the specimen are small in number. Giemsa stained smears are poorly sensitive but they have an advantage of revealing developmental stages and making the nuclei visible. Results are excellent on resin embedded sections stained with toluidine blue [31]. Sensitivity of detection by Gram's stain was 90.3%; by Giemsa stain was 64.52% and by modified Ziehl Neelsen stain was 87% as observed by Joseph et al, in their study [32].

Chemofluorescent agents

Because of the chitin present in the spore wall these agents can readily be stained by chemofluorescent agents like calcoflour, Fungi-Flour, or Uvitex 2B. Microsporidial spores appear bright when stained with these agents. Though this is a rapid and sensitive method it is not specific and many other structures like fungal spores will also fluoresce and may give false positive results [31]. Studies show sensitivity of potassium hydroxide with calcoflour white to be 93.5% [32].

Electron microscopy

It is the "gold standard" method for detection of spores of Microsporidia. It also helps in differentiation of species based on their ultrastructural characteristics. Species *Nosema* can be differentiated from *Encephalitozoon* based on electron microscopic features such as size (Nosema species measures approximately 3.5-5.0 µm in length versus *Encephalitozoon* species which measures 2.0-3.0 µm in length) , the absence of a parasitophorous vacuole surrounding the organism within the host cell , number of coils (11 to13 in *Nosema* versus 4 to 7 in *Encephalitozoon*).

Confocal microscopy

Confocal laser scanning microscopy is a rapid non-invasive method to detect Microsporidial spores in the corresponding affected tissue layer. It doesn't subject the patient to the increase risk of secondary infection which is caused by other diagnostic modalities requiring superficial tissue debridement [33]. Microsporidial spores are visualized as high contrast intraepithelial or intrastromal bright dots aligned along keratocytes. It is also an important tool for monitoring the treatment response [34].

Molecular Methods

PCR

Small-subunit rRNA sequences are used for species-specific detection of organisms [13]. Apart from small-subunit rRNA, large subunit rRNA and the intergenic spacer region can also be used as a target sequence [32]. Panmicrosporidial primers (V1 and PMP2) and species specific primers (V1-EB450 andV1-SI500) can be used for detecting microsporidia in clinical specimen [35]. Using panmicrosporidial primers sensitivity and specificity was demonstrated to be 83% of 98% respectively [32]. Utility of PCR for diagnosis of microsporidial keratitis giving a higher sensitivity than traditional cytological and histological detection methods have been studied [36].

Novel molecular methods

Spencer et al. described the use of SYBR Green real-time PCR assay for simultaneously detecting and identifying species of the disease causing Microsporidia using a new MsRTf1/MsRTr1 primer set which showed much greater sensitivity than that achieved by light microscopy and was equivalent to (or better than) that achieved by conventional PCR [37].

Immunofluoresence techniques

Fluorescein-tagged polyclonal antibodies have been used for histological and cytological detection of Microsporidia in human specimens and to visualize different Microsporidial developmental stages in cell cultures. Species-specific polyclonal antibodies have enabled to distinguish between morphologically identical species. Monoclonal antibodies for use in immunofluorescence staining are under development [13]. Though immunofluoresent antibody technique is less convenient compared to chromotrope and flurochromes it may prove to be a very useful method for species differentiation. Reagents for immunofluroscence staining of Microsporidial spores have been developed but they are not yet available commercially and most have been limited to *Encephalitozoon* species spores. The sensitivity is further decreased due to disadvantages such as background staining, and cross-reactions with yeast and bacteria [30].

Cell culture

Cell culture is one of the methods to provide confirmatory as well as diagnostic information, as cell culture is too cumbersome and laborious and requires a specialized laboratory. In clinical setting culture does have a role if the organisms are present in a very small number in the specimen and can be easily missed during examination by routine methods [38]. Monkey and rabbit kidney cell lines (Vero and RK-13), human fetal lung fibroblasts cell line (MRC-5) and Madin-Derby canine kidney cell line (MDCK) are some of the cell lines which can be used for the in vitro culture of Microsporidia. *E. bieneusi*, has been propagated only in short-term cultures [30]. The sensitivity of Vero cell line (1×10^4 spores/ml) is more sensitive than that of rabbit corneal cell line SIRC (1×10^5 spores/ml) and HeLa cells (1×10^7 spores/ml) [38].

Treatment

There is no known definitive medical treatment of deep microsporidial corneal stromal infections. Albendazole-a benzimidazole prevents microtubule formation and acts effectively against *Encephalitozoon* species but not against *E. bieneusi* [39]. Fumagillin-a derivative of *Aspergillus fumigatus* is widely used as an effective antimicrosporidial drug [40-43]. Newer drugs like TNP-470 (a semisynthetic fumagillin analogue), ovalicin (a structurally related antibiotic that is synthesized by *Pseudorotium ovalis*) are being studied for their antimicrosporidial activity [44]. CDC recommends topical fumagillin bicylohexylammonium (Fumidil B) 3 mg/ml in saline (fumagillin 70 µg/ml) eye drops and albendazole 400 mg orally twice a day for systemic management [45]. Topical steroids and bandaged contact lens should be avoided as they could result in corneal perforation followed by secondary infection and deep stromal infection [46]. In patients not responding to medical treatment, penetrating keratoplasty is efficacious in eradicating infection and preventing recurrences [9]. DALK may be considered an option for visual rehabilitation in severe stromal keratitis [47,48].

Conclusion

Once a rare entity, incidence of microsporidial keratitis is rapidly increasing due to the AIDS pandemic. Clinical diagnosis of microsporidial keratitis is challenging and newer advanced diagnostic modalities are now employed to diagnose these infections. Timely diagnosis prevents chronicity and complications. There is huge scope of research in the field of pathogenesis, diagnosis and treatment of microsporidial keratitis.

References

1. Keeling PJ, McFadden GI (1998) Origins of microsporidia. Trends Microbiol 6: 19-23.
2. Hirt RP, Logsdon JM Jr, Healy B, Dorey MW, Doolittle WF, et al. (1999) Microsporidia are related to Fungi: evidence from the largest subunit of RNA polymerase II and other proteins. Proc Natl Acad Sci USA 96: 580-585.
3. Keeling PJ, Luker MA, Palmer JD (2000) Evidence from beta-tubulin phylogeny that microsporidia evolved from within the fungi. Mol Biol Evol 17: 23-31.
4. Garg P (2013) Microsporidia infection of the cornea--a unique and challenging disease. Cornea 32: S33-38.
5. Joseph J, Vemuganti GK, Sharma S (2005) Microsporidia: emerging ocular pathogens. Indian J Med Microbiol 23: 80-91.
6. Pinnolis M, Egbert PR, Font RL, Winter FC (1981) Nosematosis of the cornea. Case report, including electron microscopic studies. Arch Ophthalmol 99: 1044-1047.
7. Davis RM, Font RL, Keisler MS, Shadduck JA (1990) Corneal microsporidiosis. A case report including ultrastructural observations. Ophthalmology 97: 953-957.
8. Chan CM, Theng JT, Li L, Tan DT (2003) Microsporidial keratoconjunctivitis in healthy individuals: a case series. Ophthalmology 110: 1420-1425.
9. Vemuganti GK, Garg P, Sharma S, Joseph J, Gopinathan U, et al. (2005) Is microsporidial keratitis an emerging cause of stromal keratitis? A case series study. BMC Ophthalmol 5: 19.
10. Tung-Lien Quek D, Pan JC, Krishnan PU, Zhao PS, Teoh SC (2011) Microsporidial keratoconjunctivitis in the tropics: a case series. Open Ophthalmol J 5: 42-47.

11. Loh RS, Chan CML, Ti SE, Lim L, Chan KS, et al. (2009) Emerging prevalence of microsporidial keratitis in Singapore: epidemiology, clinical features, and management. Ophthalmology 116: 2348-53.
12. Sengupta J, Saha S, Khetan A, Pal D, Gangopadhyay N, et al. (2011) Characteristics of microsporidial keratoconjunctivitis in an eastern Indian cohort: a case series. Indian J Pathol Microbiol 54: 565-568.
13. Weber R, Bryan RT, Schwartz DA, Owen RL (1994) Human microsporidial infections. Clin Microbiol Rev 7: 426-461.
14. Didier ES, Weiss LM (2008) Overview of microsporidia and microsporidiosis. Protistology 5: 243-255.
15. Rönnebäumer K, Gross U, Bohne W (2008) The nascent parasitophorous vacuole membrane of Encephalitozoon cuniculi is formed by host cell lipids and contains pores which allow nutrient uptake. Eukaryot Cell 7: 1001-1008.
16. Joseph J, Sridhar MS, Murthy S, Sharma S (2006) Clinical and microbiological profile of microsporidial keratoconjunctivitis in southern India. Ophthalmology 113: 531-537.
17. Theng J, Chan C, Ling ML, Tan D (2001) Microsporidial keratoconjunctivitis in a healthy contact lens wearer without human immunodeficiency virus infection. Ophthalmology 108: 976-978.
18. Bommala ML, Nalamada S, Sharma S, Garg P (2011) Microsporidial keratoconjunctivitis in an immunocompetent patient with a past history of laser in situ keratomilieusis surgery. Indian J Med Microbiol 29: 428-430.
19. Fan NW, Wu CC, Chen TL, Yu WK, Chen CP, et al. (2012) Microsporidial keratitis in patients with hot springs exposure. J Clin Microbiol 50: 414-418.
20. Chan CM, Theng JT, Li L, Tan DT (2003) Microsporidial keratoconjunctivitis in healthy individuals: a case series. Ophthalmology 110: 1420-1425.
21. Kakrania R, Joseph J, Vaddavalli PK, Gangopadhyay N, Sharma S (2006) Microsporidia keratoconjunctivitis in a corneal graft. Eye (Lond) 20: 1314-1315.
22. Vemuganti GK, Garg P, Sharma S, Joseph J, Gopinathan U, et al. (2005) Is microsporidial keratitis an emerging cause of stromal keratitis? A case series study. BMC Ophthalmol 5: 19.
23. Ashton N, Wirasinha PA (1973) Encephalitozoonosis (nosematosis) of the cornea. Br J Ophthalmol 57: 669-674.
24. Kai S, Vanathi M, Panda A (2009) Corneal Microsporidiosis. JK science 11: 59-61.
25. Garg P (2013) Microsporidia infection of the cornea--a unique and challenging disease. Cornea 32: S33-38.
26. Weber R, Bryan RT, Owen RL, Wilcox CM, Gorelkin L, et al. (1992) Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. The Enteric Opportunistic Infections Working Group. N Engl J Med 326: 161-166.
27. Ryan NJ, Sutherland G, Coughlan K, Globan M, Doultree J, et al. (1993) A new trichrome-blue stain for detection of microsporidial species in urine, stool, and nasopharyngeal specimens. J Clin Microbiol 31: 3264-3269.
28. Kokoskin E, Gyorkos TW, Camus A, Cedilotte L, Purtill T, et al. (1994) Modified technique for efficient detection of microsporidia. J Clin Microbiol 32: 1074-1075.
29. Moura H, Da Silva JL, Sodré FC, Brasil P, Wallmo K, et al. (1996) Gram-chromotrope: a new technique that enhances detection of microsporidial spores in clinical samples. J Eukaryot Microbiol 43: 94S-95S.
30. Garcia LS (2002) Laboratory identification of the microsporidia. J Clin Microbiol 40: 1892-1901.
31. Gillespie SH, Pearson RD (eds.) (2001) Principles and practice of clinical parasitology. New York, pp. 670.
32. Joseph J, Sharma S, Murthy SI, Krishna PV, Garg P, et al. (2006) Microsporidial keratitis in India: 16S rRNA gene-based PCR assay for diagnosis and species identification of microsporidia in clinical samples. Invest Ophthalmol Vis Sci 47: 4468-4473.
33. Hsiao YC, Tsai IL, Kuo CT, Yang TL (2013) Diagnosis of microsporidial keratitis with in vivo confocal microscopy. J Xray Sci Technol 21: 103-110.
34. Sagoo MS, Mehta JS, Hau S, Irion LD, Curry A, et al. (2007) Microsporidium stromal keratitis: in vivo confocal findings. Cornea 26: 870-873.
35. Müller A, Stellermann K, Hartmann P, Schrappe M, Fätkenheuer G, et al. (1999) A powerful DNA extraction method and PCR for detection of microsporidia in clinical stool specimens. Clin Diagn Lab Immunol 6: 243-246.
36. Conners MS, Gibler TS, Van Gelder RN (2004) Diagnosis of microsporidia keratitis by polymerase chain reaction. Arch Ophthalmol 122: 283-284.
37. Polley SD, Boadi S, Watson J, Curry A, Chiodini PL (2011) Detection and species identification of microsporidial infections using SYBR Green real-time PCR. J Med Microbiol 60: 459-466.
38. Joseph J, Sharma S (2009) In vitro culture of various species of microsporidia causing keratitis: evaluation of three immortalized cell lines. Indian J Med Microbiol 27: 35-39.
39. Didier ES, Maddry JA, Brindley PJ, Stovall ME, Didier PJ (2005) Therapeutic strategies for human microsporidia infections. Expert Rev Anti Infect Ther 3: 419-434.
40. Diesenhouse MC, Wilson LA, Corrent GF, Visvesvara GS, Grossniklaus HE, et al. (1993) Treatment of microsporidial keratoconjunctivitis with topical fumagillin. Am J Ophthalmol 115: 293-298.
41. Garvey MJ, Ambrose PG, Ulmer JL (1995) Topical fumagillin in the treatment of microsporidial keratoconjunctivitis in AIDS. Ann Pharmacother 29: 872-874.
42. Diesenhouse MC, Wilson LA, Corrent GF, Visvesvara GS, Grossniklaus HE, et al. (1993) Treatment of microsporidial keratoconjunctivitis with topical fumagillin. Am J Ophthalmol 115: 293-298.
43. Rosberger DF, Serdarevic ON, Erlandson RA, Bryan RT, Schwartz DA, et al. (1993) Successful treatment of microsporidial keratoconjunctivitis with topical fumagillin in a patient with AIDS. Cornea 12: 261-265.
44. Didier PJ, Phillips JN, Kuebler DJ, Nasr M, Brindley PJ, et al. (2006) Antimicrosporidial activities of fumagillin, TNP-470, ovalicin, and ovalicin derivatives in vitro and in vivo. Antimicrob Agents Chemother 50: 2146-2155.
45. CDC-DPDx-Microsporidiosis -Treatment Information.
46. McCLUSKEY PJ, Goonan PV, Marriott DJE, Field AS (1993) Microsporidial Keratoconjunctivitis in Aids. Eye 7: 80-83.
47. Ang M, Mehta JS, Mantoo S, Tan D (2009) Deep anterior lamellar keratoplasty to treat microsporidial stromal keratitis. Cornea 28: 832-835.
48. Müller A, Stellermann K, Hartmann P, Schrappe M, Fätkenheuer G, et al. (1999) A powerful DNA extraction method and PCR for detection of microsporidia in clinical stool specimens. Clin Diagn Lab Immunol 6: 243-246.