Review Article

Human Microsporidiosis: A Narrative Review of Serodiagnostic Tests

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Abstract

Context: Microsporidiosis is an emerging disease in both immunocompetent and immunocompromised patients. Diagnosis of human microsporidiosis is challenging. Parasitological tests using staining methods have limitations for detecting spores in stool samples. The performance of molecular methods for diagnosing human microsporidiosis is satisfactory, but these methods are expensive and time-consuming. Serodiagnosis tests for the detection of antibodies have insufficient specificity. Detecting microsporidia antigens seems to be an appropriate method for diagnosing human microsporidiosis. Therefore, the present study reviewed serological approaches to assess the current status of these methods for diagnosing human microsporidiosis.

Evidence Acquisition: All related published articles were searched. The search terms were "microsporidiosis", "microsporidiasis", "human microsporidiosis", and "human microsporidiasis", combined with the search terms "diagnosis", "serodiagnosis", "immunodiagnosis", "antigen detection", and "antibody detection". Data were extracted from the articles that met our eligibility criteria. Immunodiagnosis studies in experimentally infected animals were excluded.

Results: Few studies have evaluated the performance of serological tests for diagnosing human microsporidiosis. The IgG response against spore wall protein or polar tube antigens remains at least three years after infection and shows more cross-reactivity with other species. Therefore, the western blot should be conducted as a confirmatory method. Serodiagnosis tests using monoclonal antibodies against microsporidia showed less cross-reactivity and more efficacy compared to polyclonal antibodies.

Conclusions: Indirect fluorescent antibody test based on the monoclonal antibody is highly effective in diagnosing microsporidia species in clinical samples.

Keywords: Antibody, Antigen, Human, Immunodiagnosis, Microsporidiosis, *Microsporidia* spp., Serodiagnosis

1. Context

Microsporidia is a forcible intracellular pathogen that infects a wide range of vertebrates and invertebrates. This microorganism, with more than 200 genera and 1,400 species, belongs to the fungal kingdom (1, 2). Microsporidia can infect most invertebrates, such as bees and silkworms. The first microsporidia that caused pebrine disease was *Nosema bombycis*, which nearly destroyed the European silkworm industry. Since then, microsporidia have caused many economic losses in the bee and fishery industries (3).

To date, 17 species in eight genera of microsporidia have been detected in humans, namely *Anncaliia*, *Encephalitozoon*, *Enterocytozoon*, *Microsporidium*, *Nosema*, *Pleistophora*, *Trachipleistophora*, *Tubulonosema*, and *Vittaforma* (4). The most frequent one in human infections is *Enterocytozoon bieneusi*. Microsporidia can infect both immunocompetent and immunodeficient humans,

especially transplant candidates and patients with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) (5). Clinical manifestations of microsporidiosis are gastroenteritis, hepatitis, peritonitis, keratitis, myositis, cholecystitis, sinusitis, encephalitis, and other cerebral infections (2, 6). Furthermore, this disease has been reported in urinary tract infections, skin ulcers, and bone infections in immunocompetent patients (7).

Microsporidiosis is known as an emerging disease (8). Accurate estimation of the prevalence of human infection has not been possible due to difficult detection. Human infections have been reported from all continents except Antarctica (9). Microsporidia infections had been rarely identified before the AIDS pandemic (10). The prevalence rate of this disease in humans based on antibody detection tests had a range of 0% - 42% (11, 12). In a meta-analysis study in 2018, pooled seroprevalence rate in HIV-positive

patients was reported as 11.8% ([12](#)). Although valuable staining methods, such as Weber's chromotrope-based stain and its modifications (e.g., Gram-chromotrope (GC) and Calcofluor) have been developed, it is challenging to detect spores in stool samples because of its small size ([6](#), [13](#), [14](#)). Microscopic and staining methods are time-consuming and need experienced microscopists. These fungi may not be detected in low-parasite burden specimens or misdiagnosed as yeasts and cocci. It is impossible to identify the spores of different species based on morphology due to their similarity ([13](#), [14](#)). On the other hand, although molecular methods for detecting microsporidia DNA are more sensitive and specific, they require time and equipment and are expensive ([15-17](#)).

Diagnosis and treatment of microsporidiosis become possible with adequate information on the host's immune responses ([18](#)). The innate and adaptive immune systems have principal roles in immune responses against microsporidiosis. The innate immune cells, such as gamma-delta T cells, natural killer cells, macrophages, and dendritic cells can eliminate infection and enhance adaptive immune responses. Activating cytotoxic T lymphocytes and humoral immune response by secreting specific antibodies are vital in clearing the microsporidia infection ([18-20](#)). Immunodiagnosis based on antigen or antibody detection is the appropriate approach for diagnosing human microsporidiosis. Most serodiagnosis studies on human microsporidiosis are based on antigen detection using produced murine monoclonal antibodies. Serological investigations showed antibody detection tests unsuitable for accurately diagnosing human infectious diseases because of cross-reaction and persistent post-treatment. Also, antibodies may remain for several months after successful treatment and cannot differentiate between present and past infections ([21](#)). The present study reviewed serological approaches to evaluate the current status of these methods for diagnosing human microsporidiosis. Moreover, the current narrative review highlights the achievements in designing and improving immunodiagnostic studies on human microsporidiosis.

2. Evidence Acquisition

In this narrative review, all related published articles in PubMed, ISI Web of Science, Google Scholar, and Scopus were searched for one month, from 1 to 30 March 2022. The inclusion criteria were all articles found using the terms microsporidiosis, microsporidiasis, human microsporidiosis, and human microsporidiasis, combined with the search terms of diagnosis, serodiagnosis, immunodiagnosis, antigen detection, and antibody detection. Data

were extracted from articles that met our eligibility criteria. Only immunodiagnostic articles about human microsporidiasis were included. Immunodiagnosis research in experimentally infected animals was excluded.

3. Results

3.1. Antigen Detection Approaches for the Diagnosis of Microsporidiosis

Antigen detection tests are one of the basic immunodiagnostic approaches. These tests use polyclonal or monoclonal antibodies produced against specific antigens or epitopes ([21-23](#)). Antigen detection methods using polyclonal antibodies for diagnosing human microsporidiosis have disadvantages and pitfalls, such as the lack of specificity due to cross-reaction with other species, background signals, and the ability to differentiate between specific species. In contrast, monoclonal antibodies are specifically capable of identifying microsporidia species. In addition, monoclonal antibodies minimize cross-reactivity and background in various immunological tests ([24](#), [25](#)). The use of monoclonal antibodies to determine the species is necessary to treat microsporidia-infected patients ([25](#)). Immunodiagnostic assays, such as enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence antibody test (IFAT), and immunoblot using polyclonal or monoclonal antibodies have been utilized for microsporidia antigens detection in different specimens ([26-29](#)). These tests have diverse sensitivity and specificity. The high sensitivity and specificity of IFAT have been proven to detect microsporidia antigens. Moreover, in published studies, IFAT using monoclonal antibodies has been used to detect microsporidia antigens more than polyclonal antibodies ([29](#), [30](#)). In various studies, several monoclonal and polyclonal antibodies against the polar tube and spore wall proteins (SWPs) of microsporidia species have been designed and produced ([31-34](#)). In previous investigations, five exosporial proteins (EcSWP1, EiSWP1, EiSWP2, EcExP1, and EhSWP1) and three endosporial proteins (EnP2 or SWP3, EnP1, and EcCDA) of the Encephalitozoonidae family have been identified ([35-38](#)). Spores of microsporidia species consist of a thick, electron-dense, and proteinaceous outer layer and an electron-lucent endospore containing chitin and protein. To date, most of the produced antibodies were specific against the SWPs of the Encephalitozoonidae family ([34](#), [39](#)).

In earlier studies, IFAT using polyclonal antibodies raised against several species of microsporidia showed cross-reactivity ([40](#)). More recently, rabbit polyclonal antibodies were produced against *Encephalitozoon cuniculi* and

Encephalitozoon hellem to identify ocular and systemic infections, and polyclonal antiserum was raised against *Encephalitozoon bieneusi* to detect this microsporidia species in deparaffinized tissue sections showed low specificity (41, 42). In the previous study, three monoclonal and polyclonal antibodies produced against *E. hellem* were evaluated by ELISA and western blotting. The ELISA results demonstrated high sensitivity for diagnosing *E. hellem* and revealed cross-reactivity with *E. cuniculi* and *Nosema corneum*. Furthermore, in western blotting, 52, 60, and 62 KD protein bands were detected (43).

Mo and Drancourt produced 24 monoclonal antibodies (IgM and IgG subclasses) against *E. hellem* and evaluated them for antigen detection using IFAT and immunoblotting assays (24). These monoclonal antibodies showed high specificity, and no cross-reaction was detected with *Encephalitozoon intestinalis*, *E. cuniculi*, and bacteria including *Escherichia coli*, *Enterococcus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Proteus vulgaris*, *Salmonella enterica*, *Cryptosporidium parvum*, *Candida albicans*, and *Aspergillus fitmigatus*. In addition, in the western blotting assay, 39, 60, and 68 KD protein bands specifically reacted with produced monoclonal antibodies. These specific bands can be helpful for the diagnosis of microsporidia infection (24). In another research, two produced monoclonal antibodies (by fusion method) against *E. bieneusi* showed high specificity using IFAT. Two monoclonal antibodies were specific for the spore wall of *E. bieneusi*, and no cross-reaction was reported with the spore of other microsporidia, yeast, and bacteria. The IFAT indicated high performance compared to Chromotrope 2R, Uvitex 2B staining method, and polymerase chain reaction (PCR) (44).

In a study from Portugal, an IFA assay to detect the spores of microsporidia in the 166 feces and 43 urine samples of patients with AIDS showed high sensitivity and good agreement in comparison with modified trichrome (MT) stain as a gold-standard method. However, it revealed moderate sensitivity for detecting spores in 71 pulmonary specimens (45). In a study performed by Beckers et al., the combination of two specific monoclonal antibodies against the polar filament and surface of *E. intestinalis* spore was evaluated by IFAT to detect the *E. intestinalis* spores in stool samples (46). Some cross-reaction with fecal bacteria and fungi was observed, and no cross-reaction was reported with the spores of *E. hellem* (46).

Al-mekhlafi et al. conducted a study using the IFAT-monoclonal (Mab) antibody to detect microsporidia spores (47). This study evaluated 50 positive and 50 negative stool samples confirmed by Weber modified trichrome. *Enterocytozoon bieneusi*, *E. intestinalis*, and mixed infections were detected by IFAT in 75%, 12.5%, and

12.5% of fecal samples, respectively. The IFAT showed a specificity of 86% and sensitivity of 98% in comparison with Weber modified trichrome staining method (47). In another study, Ghoshal et al. used IFAT-Mab to detect *E. bieneusi* and *E. intestinalis* spores in the fecal samples of 19 immunocompromised patients infected with microsporidia (confirmed by MT staining) and 181 negative stool samples (29). The *E. bieneusi* was detected in all positive fecal samples using IFAT. This assay showed sensitivity, specificity, positive predictive value, and negative predictive value of 100%, 99.4%, 95.5%, and 100%, respectively, compared to MT staining as the gold-standard method. Furthermore, a high agreement was reported between MT staining and IFAT (K = 0.915, P = 0.049). When PCR was taken as the gold standard, sensitivity, specificity, and positive and negative predictive values of the IFA assay were 95.2%, 100%, 100%, and 99.4%, respectively. Moreover, PCR and IFAT showed a high degree of agreement (K = 0.973, P = 0.027) (29). Therefore, IFAT-based monoclonal antibody is an efficient assay with high sensitivity and specificity for diagnosing common microsporidia compared to gold-standard techniques, such as PCR and MT staining microscopy. Furthermore, IFA-Mab decreased cross-reactions and background noise with yeast, bacteria, and common antigens between different species and genera of microsporidia, including *E. hellem*, *E. cuniculi*, *E. intestinalis*, and *E. bieneusi*. In another study, seven monoclonal antibodies were produced against the SWP1 of E. intestinalis and other Encephalitozoon species. In IFAT, four monoclonal antibodies (isotype IgG2a) reacted with the exo and endosporial proteins of *Encephalitozoon* spp. The results showed that three monoclonal antibodies (isotype IgG3) reacted with the endoplasmic content of the *E. intestinalis* spore. Four monoclonal antibodies using IFAT revealed cross-reaction with *E. cuniculi* and *E. hellem*. In this study, *E. intestinalis*-positive human stool samples reacted strongly with monoclonal antibodies produced against exospores antigens. These monoclonal antibodies showed an adverse reaction with *E. bieneusi*-positive human stool samples. In the western blot test, four monoclonal antibodies reacted with *E. intestinalis* protein fractions of 36.4-201 KDa, and the dominant reacting protein band was 45 KDa (48). Furthermore, Alfa Cisse et al. evaluated two monoclonal probes specific for *E. bieneusi* and *E. intestinalis* using IFAT (30). This study collected 61 and 71 stool samples from seropositive patients with HIV and immunocompetent children. *E. bieneusi* was diagnosed in 13.1% of seropositive cases with HIV (30). The IFAT showed specificity and sensitivity of 100% compared to PCR. Previous results using western blot were consistent with the findings of this study by Izquierdo et al. (48).

3.2. Antibody Detection Approaches for the Diagnosis of Microsporidiosis

Scanty studies have evaluated antibodies against microsporidia antigens in clinical samples. Antibody detection tests have pitfalls and problems, such as low specificity because of cross-reactivity, and are unsuitable for the follow-up of parasitic infection after treatment (49-51). In earlier antibody detection studies using IFAT and ELISA, spore wall antigens were exclusively targeted (30, 32). Recently, polar tubs and spore wall antigens have been used to evaluate antibody response against microsporidia infection (48).

3.2.1. Spore Wall Antigens

Microsporidia spp. spore walls have three layers, the plasma membrane layer, electron-lucent endospore, and proteinaceous exospore. Most of the proteins in the exospore are involved in invasion and triggering immune responses. Five SWPs are known, two of which include SWP1 as a serine glycine-rich protein and SWP2 as a glycoprotein antigen with a molecular weight of 150 kDa (31-34). This antigen is present in the mature spore of *E. intestinalis*. The SWP3 was reported in all microsporidia based on proteomic studies. Another protein in the exospore wall of microsporidia is SWP4. It plays a role in spore adhesion to carbohydrate compounds. In *E. cuniculi* and *E. hellem*, SWP5 was found by proteomic evaluations (35-38).

3.2.2. Polar Tube Proteins

The polar tube is essential in invading and transporting the nucleus and sporoplasm into host cells. Six polar tube proteins (PTPs) are present in *Microsporidia* spp. Mannosylated protein PTP1 has a vital role in host-cell interaction. In addition, PTP2 and PTP3 are identified by immunological and proteomic studies. The PTP4 exists at the tip of the polar tube and binds this part to the host transferrin receptor 1. The monoclonal antibody or recombinant antibody can prevent invasion by blocking this receptor. The PTP4 and PTP5 are present in *Anncaliia algerae*, *E. intestinalis*, and *E. cuniculi*. Recently, PTP6, a histidine-serine-rich antigen with multiple glycosylation sites, has been identified in *Nosema bombycis* and *E. hellem* (52).

Recombinant antigens are the main target antigens used in antibody detection tests. The IFAT using produced recombinant antigens of SWP1, PTP1, PTP2, and PTP3 were evaluated for IgG antibody response in HIV-negative immunocompetent patients infected with *E. cuniculi*. One month after infection, only IgG response against recombinant SWP1 was strong. At this time, no reaction was observed using the PTP1 recombinant antigen. Consistently, one month after infection, polar tube antigen showed a

negative result in IFAT. This study showed that only spore wall antigens are detectable after one month of disease. In addition, three times IgG response to recombinant polar tube antigens was observed 20 months after infection. This research demonstrated that the spore wall of *E. intestinalis* and *E. hellem* had cross-reaction more than recombinant polar tube antigens. However, the spore wall or polar tube of *V. corneae* showed no cross-reactivity. These serum samples in western blot analysis using *E. cuniculi* type I strain as antigen showed a strong reaction with 28 KDa bands one month after infection. Moreover, 20 months after infection, eight protein bands were detected, including 17, 20, 28, 30, 32, 34 - 38, 42, and 47 kDa. The sharpness of these bands decreased slowly 32 months after infection. Western blot using *E. intestinalis* antigen displayed only one protein of 26 kDa. In IFAT using *E. intestinalis* antigen, serum samples had a titer of 1: 640 to the spore wall and 1: 320 to the polar tube. In addition, serum samples showed cross-reactivity with the *E. hellem* spore wall (titer of 1: 640) and polar tube (titer of 1: 160) (52).

Humoral response against *E. cuniculi* in HIV- negative immunocompetent patients showed a strong IgG antibody titer to both spore wall and polar tube antigens. Therefore, in addition to spore wall antigens, polar tube antigens are important in immune responses against *Enterocytozoon* spp. In the western blot, 17 - 150 KDa protein bands of *E. cuniculi*, SWP1, and PTP1 recombinant antigens were strongly detected. Consequently, two recombinant antigens are more useful for microsporidia serodiagnosis. The IFAT and western blot showed IgG response at least three years after infection. A low titer of specific antibodies was detected six years after infection (52). In a case report study, *Encephalitozoon* species spore was detected in the urine sample of a child with convulsive seizures and strong IgG and IgM antibody response. Unfortunately, the details of the serological test have not been specified (53). It is imperative to determine microsporidia species for treatment. As a result, a western blot assay can help reduce cross-reactions between species. The previous study used western blot as a confirmatory method for microsporidia species diagnosis (10). Hollister et al. observed a high reaction using the whole antigen of *Encephalitozoon* spore in ELISA (10). However, in the western blot assay, these sera do not react with spore wall antigens. This highly positive reaction may be related to cross-reaction with nonspecific antibodies. Due to cross-reactions, positive samples must be confirmed by western blot or recombinant antigens when spore wall antigens are used in tests, such as ELISA (10).

4. Conclusions

Although microscopic examinations using specific staining methods, namely Weber's chromotrope-based stain, GC, and Calcofluor are gold standard methods for microsporidia spore detection, these methods have limitations, including cross-reactivity with fungi and some bacteria, especially in stool samples. Furthermore, these methods are time-consuming, need experienced microscopists, and cannot identify microsporidia species. Different molecular methods for determining microsporidia species in clinical samples have great importance, especially for treating microsporidiosis patients. Although this method showed high specificity and sensitivity, it had weaknesses, such as being more expensive, time-consuming, and requiring equipment. Immunological diagnostic methods based on antigen and antibody detection are other assays for microsporidiosis diagnosis. Detection of anti-microsporidia antibodies has disadvantages, including low specificity and cross-reactivity between microsporidia species. In contrast, antigen detection tests are more reliable and valuable because of more negligible cross-reactivity and high specificity. Convincing evidence has demonstrated that antigen detection tests, such as IFAT, are more sensitive and specific for microsporidia serodiagnosis. This method identifies and differentiates common microsporidia in humans, including *E. bieneusi* and *E. intestinalis*. The IFAT using monoclonal antibodies can identify microsporidia spores easier and faster than a microscopic assay with specific staining. In developing and low-income countries, the use of IFAT is more cost-effective compared to the molecular method. However, the duration of the IFAT test is shorter than the molecular approach. Finally, species-specific monoclonal antibody-based IFAT is highly effective in diagnosing microsporidia species in clinical samples and the proper treatment.

Footnotes

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