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Intestinal Microsporidiosis: a New Entity in Malaysia?

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ABSTRACT

Objective: Intestinal microsporidia is an emerging human disease caused by microsporidia. A study was conducted to determine the prevalence of microsporidia in patients with gastro-intestinal symptoms and to examine the clinical manifestations associated with intestinal microsporidiosis. **Methods:** A descriptive cross-sectional study using a well-structured questionnaire; a review of medical records was also undertaken. Positive stool samples were defined as presence of one or more pinkish-violet ovoid structures with a belt-like stripe under high power field (100x) using modified gram-chromotrope stain (MGC). **Results:** A total of 353 faecal specimens of patients was examined and 100 patients were found to have positive stool samples for microsporidia. The overall prevalence of microsporidia was 28.3%. Acute and chronic diarrhoea were seen in 49.0% and 36.0% patients, respectively. The commonest clinical presentations were diarrhoea (85.0%) with 83.0 % of patients having loose or watery stools, vomiting (75.0%), foul-smelling stools (60.0%), nausea (59.0%) and cramping abdominal pain (39.0%). The least common symptoms were fever (15.0%), mucous in stool (5.0%) and blood in stool (4.0%). **Conclusion:** This study concludes that the prevalence of microsporidia is still high (28.3%) and the majority of patients (93.0%) are symptomatic; the most common gastro-intestinal symptom is diarrhoea with loose or watery stools. Hence, it is recommended that a stool screening for microsporidia be done in selected patients presented with gastrointestinal symptoms.

Keywords: Intestinal microsporidia, gastrointestinal symptom, modified gram-chromotrope stain (MGC), diarrhoea, loose or watery stool

INTRODUCTION

Numerous 'new' gastrointestinal pathogens have emerged in recent years, including one of the important types of intestinal sporozoa: microsporidia. Microsporidia are an unusual group of eukaryotic, obligate intracellular protozoan that affect both vertebrate and

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invertebrate hosts. The first documented case of microsporidiosis in humans was published in 1959 but did not gain much attention until the acquired immunodeficiency syndrome (AIDS) pandemic developed in 1985.[1,2] Since then, microsporidia have emerged as the aetiologic agents of opportunistic infections in persons with AIDS, other immuno-compromised patients and also in immuno-competent individuals.[3,4,5,6,7,8]

Before the era of AIDS, prevalence data for microsporidia in humans was done via serology and it was inconclusive due to several uncertainties in interpreting the results.[8] Recently, it has been estimated that the prevalences in AIDS patients approximately ranged from 5 to 50% or even higher and varied according to geographic location and diagnostic techniques used.[9,10] Furthermore, the epidemiology of microsporidia remains largely unknown, and routes of transmission and sources of human microsporidial infections have also been difficult to ascertain.[1]

These parasites are now known to cause a wide spectrum of clinical diseases in humans. The species involved are *Encephalitozoon cuniculi, E. hellem, E. (Septata) intestinalis, Enterocytozoon bieneusi, Trachipleistophora hominis, Trachipleistophora anthropopthera, Pleistophora* species, *Vittaforma (Nosema) corneae, Microsporidium spp., Nosema ocularum, Brachiola (Nosema) connori, Brachiola vesiculatum* and *Brachiola (Nosema) algerae*. The diseases produced by microsporidia are commonly seen in AIDS patients. The parasites cause a severe, non-bloody, non-mucoid diarrhoea, with up to 10 or even more bowel movements per day, nausea, vomiting, anorexia, fever, cramping abdominal pain, slow progressive weight loss and malabsorption of fat, D-xylose, and vitamin B12.[11] However, in immuno-competent individuals, these parasites frequently cause acute but self-limiting diarrhoea.[1]

Little is known about the magnitude and epidemiology of this newly emerging parasite in Malaysia. The first description of microsporidia in two HIV patients was reported by the Institute for Medical Research (IMR) in the 1980s but the data was found to be inconclusive as the role of these parasites was not properly examined due to the small number of cases (pers. comm).

In Malaysia, a prevalence of 10.8% and 13.0% was reported in an observational study done in 818 patients, using gram chromotrope (GC) and modified gram chromotrope (MGC), respectively (Norhayati *et al.* pers. comm.). Another community study done by Norhayati *et al.* (pers. comm.) on asymptomatic aborigines (Orang Asli) children in Selangor revealed a prevalence of 20.7%. Notwithstanding, the clinical manifestations of intestinal microsporidiosis were not clearly examined.

This study was conducted to ascertain the current status on the epidemiology and clinical manifestations of this infection in warded patients and also those managed by the Outpatient Department of HUKM.

METHODS

Study Area and Study Population

This is a cross-sectional study on the relationship between the clinical manifestations of intestinal microsporidia and the socio-demographic data of patients in Hospital Universiti Kebangsaan Malaysia (HUKM), Cheras. Both outpatients and inpatients were enrolled

into this study which was conducted from March until December 2004. Each stool sample was sent to the Laboratory of Medical Microbiology and Immunology in HUKM and was given a special code without prior knowledge of the study subjects' clinical diagnosis. Patients were informed of the purpose of this study and if they agreed to participate, a signed consent form was obtained. The study was approved by the Research and Ethical Committee, Faculty of Medicine, Universiti Kebangsaan Malaysia. Socio-demographic and clinical data were obtained from the medical records and subsequently recorded into the well-structured questionnaire that was modified from Molina *et al.*[19]

Structured Questionnaire

The data was collected for each stool sample sent from a patient containing the following information as shown in Appendix 1: age, sex, clinical symptoms (fever, diarrhoea, abdominal pain, etc.), the underlying medical conditions, type of diarrhoea, description of stool and medical treatment.

Faecal Examination

Fresh stool samples were collected into wide-mouth screw-cap 100 ml clean containers. All procedures were carried out in a biohazard cabinet.

Parasite Detection

A smear of faeces in 0.9% saline was examined for the presence of trophozoites, ova and larvae of the protozoa and helminths. In addition, iodine preparation was also done for the detection of protozoan cysts. The method of parasitic detection was performed according to the procedure previously described by Brasil *et al.* [3] and Gumbo *et al.* [4]

Approximately 10 gm of faeces was mixed thoroughly and a thin smear fixed in methanol was made for the detection of microsporidian spores using modified chromotrope stain. All slides made for the detection of parasites from the respective samples were given the respective laboratory number and examined without knowledge of the patient's biological data or the clinical diagnosis or HIV status.

Criteria used for the identification of the spore was the presence of one or more pinkish-violet ovoid structures with a spore wall and a belt-like stripe, over an examination of at least 100 fields/100x, confirmed by two parasitologists.[3]

Bacterial Detection

Faecal specimens were inoculated onto the common solid media (Oxoid, Oxoid Ltd, UK) used in the laboratory, that is, blood, deoxycholate, Camphylobacter, *Clostridium difficile* agar and Thiosulphate Citrate Bile Salt (TCBs). Growths of suspected enteric pathogens were processed further for identification. Enrichment broths, that is, alkaline peptone water (Oxoid, Oxoid Ltd, UK) and selenite broth (Oxoid, Oxoid Ltd, UK) were used for the recovery of *V. cholerae*, *Salmonella* and *Shigella spp*. All specimens were incubated at 37°C for 24 hours except *Campylobacter jejuni* specimens which were incubated at 42°C under micro-

aerophilic conditions for 48 hours. Final identification of the significant isolates was done using Analytical Profile Index (API 20E, 20 NE [bioMerieux, Inc] North Carolina).

Detection of toxins produced by *Clostridium difficile* was done using *C. difficile* Toxin A test (Oxoid, Oxoid Ltd, UK). *Escherichia coli* serogroup O157 was identified by latex agglutination test (Oxoid, Oxoid Ltd, UK). However, detection of toxins produced by enterotoxigenic *E. coli* (ETEC) via PCR was not carried out as the facility was not available during the study period.

Viral Detection

Latex agglutination test (Rotalex, Orion Diagnostica, Finland) for rotavirus antigen detection in stool specimens was used selectively for paediatric patients less than 5 years old. This procedure has already been established following the standard operating procedure (SOP) for rotavirus gastroenteritis in HUKM.

Negative Stool

A negative stool evaluation was defined according to Bini *et al.*[12] as a specimen that was negative for stool culture for common enteric pathogens. It also included the absence of ova and parasites, *Clostridium difficile* toxin and the latex agglutination test for rotavirus.

Statistical Analysis

Statistical analysis of data was performed using Statistical Package for Social Sciences for Windows SPSS 11.5 (SPSS Inc., Chicago, IL, USA). For descriptive data, rate (percentage) was used to assess the prevalence of illness. Chi-square test was used to test for associations between variables. Observed differences in data were considered significant if $p<0.05$ was obtained.

RESULTS

General Characteristics of Study Population

Three hundred and fifty-three patients (160 males; 193 females) participated in this study. Among which, 71(20.1%) were from the age group of 0-6; 22 (6.2%) in 7-12 age group; 9 (2.5%) in 13-17 age group; 60 (17.0%) in 18-30 age group; 94 (26.6%) in 31-55 age group; and 97(27.5%) aged 56 and over. All stool samples were examined for enteric pathogens within the 90-month study period and overall prevalence of microorganisms detected was 33.7%, while 66.3% was negative for microorganisms.

Enteric Pathogens Isolated from Faecal Samples of All Patients

Table 1 shows the prevalence of microorganisms isolated from positive faecal samples. Amongst the 119 microorganisms isolated, microsporidia alone was the most common pathogen detected in 28.3% patients, followed by helminths and bacteria in 1.1% and 2.0% patients, respectively. Multiple pathogens were identified which consisted of microsporidia and bacteria in 2.3% patients. Figure 1 shows microsporidian spores in faecal smear using modified chromotrope stain.

Presenting Complaints of Patients in Relation to Isolation of Enteric Pathogens

Table 2 shows the presence of enteric pathogens in faecal specimens and presenting complaints of patients. The most presenting symptom amongst the 109 patients with enteric pathogens was diarrhoea at 61.8%; followed by cramping abdominal pain (24.9%), vomiting (50.7%), nausea (41.3%), foul smelling stool (19.8%), and mucus in stool (25.8%). Blood in stool was the least common, 4.2%. Interestingly, of the 119 subjects studied, the overall prevalence of enteric pathogens in patients with gastro-intestinal symptoms (91.6%) was more frequent in patients without symptoms (8.4%). The difference was statistically significant (χ^2=39.127; p=0.000).

Demographic Profile of Patients with Microsporidia

In total, 100(28.3%) stool samples were positive for microsporidia; patients aged 6 and below (23.0%) and 31-55 (29.0%) were predominant age groups. The male : female ratio was 1.1:1, and Malays (57%) were the predominant race group followed by Chinese (30.0%), Indians (7.0%) and (6.0%) others. Table 3 shows the general characteristics of 100 patients with microsporidia.

Table 1. The prevalence of specific enteric pathogens detected in faecal samples of 353 patients

Enteropathogen	Number of cases	Prevalence (%)
Microsporidia	100	28.3
Ascaris lumbricoides	4	1.1
Salmonella spp†*Escherichia coli*†	52	1.40.6
Microsporidia and bacteria*	8	2.3
No microorganism	234	66.3
Total	353	100

* Mixed with bacteria and identified as non-pathogenic *E. coli*, *Enterobacter spp.* and *Salmonella spp.*

† Significant isolates as patients presented with bacteraemia

Table 2. The presence of enteric pathogens isolated from faecal specimens and gastro-intestinal symptoms

Gastro-intestinal symptoms	Enteric pathogens isolated		
	Yes (%)	No (%)	Total (%)
Yes	109 (91.6) †	139 (59.4)	248 (70.3)
No	10 (8.4)	95 (40.6)	105 (29.7)
Total	119 (100)	234 (100)	353 (100)

†Significant (p<0.05) via chi-square test.

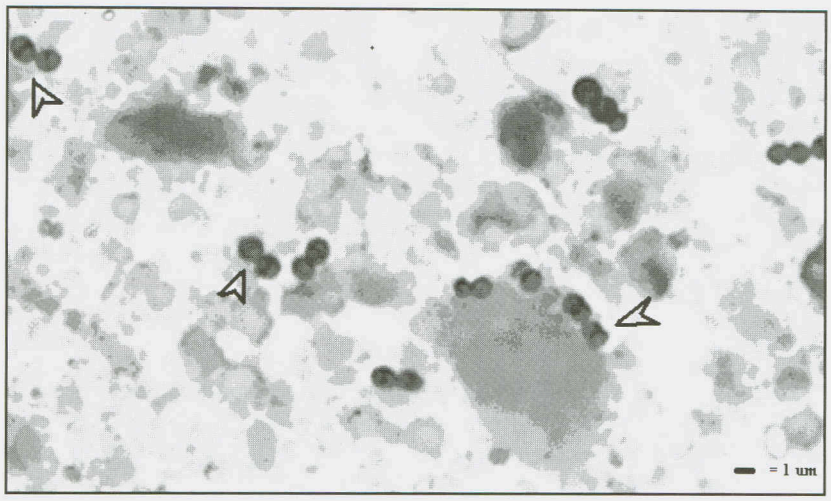

Figure 1. Spores of microsporidia in a stool specimen stained with modified chromotrope. Note the characteristic ovoid structures (arrow heads) with a belt-like stripe or equitorial line. Original magnification, x1,000.

Clinical Features of Patients with Microsporidia

Amongst the 100 patients who were positive for microsporidia, the majority (85) had diarrhoea (85.0%). Furthermore, 83 patients complained of loose or watery stools (83.0%). Forty-nine (49.0%) of the 85 patients had acute episodes of diarrhoea. Meanwhile, 36 patients (36.0%) had chronic episodes of diarrhoea. Acute diarrhoea is defined as a diarrhoeal episode lasting less than four weeks while chronic diarrhoea is defined as an increase in frequency of defecation more of than three motions per day for more than four weeks.[13]

The second most common presentation was vomiting, that is in 75 cases (75.0%) followed by foul smelling stool and nausea in 60 (60.0%), and 59 patients (59.0%), respectively. Thirty-nine patients (39.0%) had cramping abdominal pain. Meanwhile, only 15 patients had fever (15.0%). The least common presentations found in the 100 patients with microsporidia were mucus and blood in their stools, in 5 (5.0%) and 4 (4.0%), patients, respectively. Table 4 shows the signs and symtoms presented by 100 patients with microsporidia.

DISCUSSION

This study showed a high prevalence (28.3%) of intestinal microsporidia among patients managed in HUKM compared with two previous local studies in Malaysia. Nonetheless, the prevalence that has been reported worlwide ranges from 7.0 to 50.0%. This percentage varies due to various reasons as reported by others.[11,13,14]

In general, detection methods have been shown to influence the prevalence data as well as the selection of studied population and geographical variation. The method used in this study was a modified Gram-Chromotrope stain that has a comparable level of sensitivity

Table 3. Demographic profile of 100 patients with microsporidia in stool samples in HUKM, Kuala Lumpur

Demography data	No. of patients	%
Sex		
Male	52	52
Female	48	48
Age		
6 years and below	23	23
7-12 years	8	8
13-17 years	3	3
18-30 years	15	15
31-55 years	29	29
≥ 56 years	22	22
Ethnic groups		
Malay	57	57
Chinese	30	30
Indians	7	7
Others	6	6

Table 4. Presenting clinical features of 100 patients with microsporidia in their stools.

Clinical features	No. of patients	%
Diarrhoea	85	85
Acute	49	49
Chronic	36	36
Nausea	59	59
Vomiting	75	75
Cramping abdominal pain	39	39
Fever	15	15
Nature of stool		
Loose or watery	83	83
Foul-smelling	60	60
Mucus	5	5
Blood	4	4

ranging from 80 to 100%. It is also cheaper than other advanced techniques, e.g. transmission electron microscopy (TEM), and also can detect low numbers of microsporidian spores. Thus, it can be used as a screening tool for clinical specimens in our country. However, the staining procedure is quite lengthy; it takes ninety minutes to complete before the slide can be examined. Moreover, it is operator-dependent, and may give false-negative results.[1,15,16]

Nevertheless, several modifications have been made to shorten the time and to increase the contrast between spores and background.[1,11,17]

This study also found that intestinal microsporidiosis is neither associated with gender or race differences, as the ratio is almost equal in both males and females (1.1 : 1). Malays were the most predominant race that had microsporidia, followed by Chinese, Indians and others. However, one study reported that these two variables have no influence in the acquisition of microsporidia.[18] There were also three main age groups that were most affected such as young children less than 6 years old and the middle age group aged 31 and above. These findings may not reflect the true age group prevalence in the population, as HUKM is a tertiary centre where cases are selectively referred for further management. Also, the number of patients did not represent the entire population of Malaysia.

Patients with microsporida commonly presented with acute diarrhoea compared to chronic diarrhoea in this study. This finding was in contrast with another study reported by a researcher that chronic diarrhoea is the commonest presentation.[9] Whereas, acute self-limiting diarrhoea is common in healthy individuals.[1] As patients in this study constituted a combination of healthy and non-HIV immuno-compromised individuals, the clinical manifestations seen reflected both groups. Nonetheless, clinical manifestations of microsporidiosis in non-HIV immuno-compromised individuals are almost similar to HIV patients (pers. comm.).

Other clinical presentations found in this study were loose or watery stool, nausea, vomiting, cramping abdominal pain and foul smelling stool. These findings are consistent with the fact that microsporidia generally causes malabsorption even though it is not specific, and are in agreement with a cohort study done in HIV patients.[19,20] Nevertheless, parameters for malabsorption were not specifically examined such as D-xylose absorption test and vitamin B12 level [19] in this study.

This study also showed that patients with intestinal microsporidia had little mucus or blood in their stools which was in agreement with another study.[1] In this study, there was a small percentage of patients presented with mucous and blood in intestinal microsporidiosis. However, the significance of this association was not examined. It has been reported that patients with intestinal microsporidia usually have no fever.[19] In this study, we found that only 15 (15.0%) patients with intestinal microsporidia had fever at presentation. However, we have not demonstrated the significance of association of fever with microsporidia. Other clinical manifestations such as severe weight loss, wasting, and malabsorption reported in previous studies, were not observed in this study due to the limitations mentioned before.

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APPENDIX 1

MICROSPORIDIA STUDY

Bahagian A: Profil pesakit

Section A: Patient's profile

1. Nombor Siri / *Serial No:*

2. Tarikh lahir / *Date of Birth*:

3. Jantina / *Gender:* ☐ Lelaki / Male ☐ Perempuan / *Female*

4. Bangsa / *Ethnic:*
   ☐ Melayu / *Malay* ☐ Cina / *Chinese*
   ☐ India / *Indian* ☐ Lain-lain (Sila nyatakan) / *Others (Please specify):*
   _____

Bahagian B: Perihal Pesakit

Section B: *Information on Patient*

1. Tarikh kemasukan / *Date of Admission:*

2. Tarik keluar wad (jika keluar) / *Date of Discharge (if discharged):*

3. Nyatakan masalah utama anda dimasukkan ke dalam wad sekarang (boleh lebih dari satu masalah):
   Main problem for your current admission to the ward (can be more than one problem):
   _____
   _____

4. Sejarah penyakit yang lepas sebelum kemasukan (boleh lebih dari satu)
   History of underlying medical illness prior to admission:
   ☐ Ya / Yes ☐ Tidak / *No* ☐ Tidak pasti / Not sure

   a. Jika ya, sila nyatakan/ *If yes, please specify:*
   _____

5. Sejarah rawatan penyakit sebelum kemasukan:
History of medical treatment prior to admission:

☐ Ya / *Yes* ☐ Tidak / *No* ☐ Tidak pasti / *Not sure*

a. Jika ya, sila nyatakan/ *If yes, please specify:*

_____

Bahagian C: Tanda-tanda penyakit Microsporidia Usus
Section C: Signs and Symptoms of Intestinal Microsporidia

Pernahkah anda mengalami tanda-tanda seperti berikut?
Have you ever had the following signs and symptoms?

1. Demam (lebih dari 38.5°C) / *Fever (more than 38.5°C)*

☐ Ya / *Yes* ☐ Tidak / *No* ☐ Tidak pasti / *Not sure*

2. Cirit-birit / Diarrhoea

☐ Ya / *Yes* ☐ Tidak / *No* ☐ Tidak pasti / *Not sure*

Jika ya, sila jawab soalan yang berikutnya
If yes, please answer the following question

a. Jangka masa cirit-birit (tarikh di kira dari hari pertama kemasukan):
Duration of diarrhoea (date is counted from the first day of admission):

☐ Kurang dari empat minggu lepas / *Less than four weeks ago*

☐ Lebih atau sama dengan empat minggu lepas / *More and four weeks ago or longer*

☐ Tidak pasti / *Not sure*

b. Kekerapan cirit-birit (berdasarkan bilangan kekerapan membuang najis lembik atau cairan dalam masa satu hari):
Frequency of diarrhoea (based on the no. of episodes of passing loose or watery bowel movements per day):

☐ Dua atau lebih cairan najis dalam sehari / *Two or more watery stools or fluid per day*

☐ Tiga atau lebih najis lembik dalam sehari / T*hree or more soft stools per day*

3. Najis cair atau lembik / *Watery or Loose stool:*

☐ Ya / *Yes* ☐ Tidak / *No* ☐ Tidak pasti / *Not sure*

4. Najis berbau sangat busuk / Foul smelling stools:

☐ Ya / *Yes* ☐ Tidak / *No* ☐ Tidak pasti / *Not sure*

5. Muntah-muntah / vomiting:

☐ Ya / *Yes* ☐ Tidak / *No* ☐ Tidak pasti / *Not sure*

6. Rasa mual / nausea:

☐ Ya / *Yes* ☐ Tidak / *No* ☐ Tidak pasti / *Not sure*

7. Najis bercampur dengan lendir/ Stools mixed with mucus:

☐ Ya / *Yes* ☐ Tidak / *No* ☐ Tidak pasti / *Not sure*

8. Najis bercampur dengan darah / Stools mixed with blood:

☐ Ya / *Yes* ☐ Tidak / *No* ☐ Tidak pasti / *Not sure*

9. Sakit kekejangan di bahagian perut / Cramping abdominal pain:

☐ Ya / *Yes* ☐ Tidak / *No* ☐ Tidak pasti / *Not sure*

10. Pernahkah anda diberi rawatan yang spesifik semasa menpunyai tanda-tanda tersebut?
Have you ever been specifically treated for those signs and symptoms?

☐ Ya / *Yes* ☐ Tidak / *No* ☐ Tidak pasti / *Not sure*

a. Jika ya, sila nyatakan jenis rawatan (boleh nyatakan lebih dari satu jenis):
If yes, please state the type(s) of treatment given (can be more than one type):

☐ Cecair intravena / *Intravenous fluid*

☐ Garam rehidrasi oral / *Oral Rehydration Salts (ORS)*

☐ Antibiotik / *Antibiotic*

☐ Lain-lain / Others (Sila nyatakan / *Please specify*):_____

Bahagian D: Maklumat organisma di dalam najis
Section D: Information on organism found in the stool

11. Tarikh specimen dihantar / *Date of specimen sent:*

12. Tarikh specimen diterima / *Date of specimen received:*

13. Jenis patogen enteric yang dijumpai:
Type of enteric pathogen found:

☐ Microsporidia sahaja / *Microsporidia only*

☐ Microsporidia dan lain-lain (sila nyatakan nama spesis) / *Microsporidia and others (please specify name of species):*

_____

☐ Cacing (sila nyatakan nama spesis) / H*elminths (please specify name of species):*_____

☐ Protozoa lain (sila nyatakan nama spesis) / *Other protozoa (please specify name of species):*
_____

☐ Bakteria (sila nyatakan nama spesis) / *Bacteria (please specify name of species):*
_____

☐ Virus (sila nyatakan nama spesis) / Viral *(please specify name of species):*
_____

☐ Tidak dapat dikenalpasti / *Not identified*