A MIXED INFECTION OF INTESTINAL MICROSPORIDIOSIS AND SALMONELLOSIS IN A 2-YEAR-OLD BOY WITH INHERITED IMMUNODEFICIENCY SYNDROME

'Azlin MY[1], Mekadina B[1], Rozliana HMH[2], and Wathiqah W[1].
[1]Department of Parasitology & Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Wilayah Persekutuan Kuala Lumpur, Malaysia
[2]Bacterial section, Disease Division, National Public Health Laboratory, Lot 1853, Sungai Buloh, Selangor, Malaysia

Correspondence:
'Azlin Mohd Yasin
Department of Parasitology & Medical Entomology,
Level 16, Preclinical Building,
Faculty of Medicine,
Universiti Kebangsaan Malaysia,
Hospital Canselor Tuanku Muhriz,
Jalan Yaacob Latif,
Bandar Tun Razak, 56000 Cheras,
Wilayah Persekutuan Kuala Lumpur, Malaysia
Email: azlinomy@ukm.edu.my

Abstract

Gastrointestinal microsporidiosis is a major cause of chronic diarrhea in people with acquired immune deficiency syndrome. However, it can also affect individuals with inherited or congenital immunodeficiency. We reported a case of intestinal microsporidiosis and salmonellosis in a young boy with Hyper IgM syndrome. He presented with severe diarrhea and fever for 5 days. Stool examination showed heavy infection of *Microsporidia* spp. PCR confirmed the species as *Enterocytozoon bieneusi.* He was treated with albendazole for 25 days which abated the symptoms. To our knowledge, this is the first reported case of intestinal microsporidiosis in inherited immunodeficiency, X-linked Hyper IgM (XHIGM).

Keywords: Intestinal Microsporidiosis, Hyper IgM Syndrome, Immunodeficiency

Introduction

Microsporidiosis is caused by infection of *Microsporidia* spp. which is an obligate intracellular spore-forming protozoa. Clinical features of microsporidiosis vary from gastrointestinal involvement to distance dissemination to various organs (1). Gastrointestinal microsporidiosis is globally known as a major cause of chronic diarrhea and wasting in people with acquired immune deficiency syndrome (AIDS) (2). However, it can also affect any immunosuppressed individuals which includes individuals with inherited or congenital immunodeficiency (1) and one of them is X-linked Hyper IgM (XHIGM). This is a rare form of primary immunodeficiency disease and it is caused by mutations in the gene that codes for CD40 ligand (CD40L) (3). Impaired CD40 ligand expression will lead to defective T-cell interactions with monocytes and dendritic cells, resulting in abnormal cell-mediated immune functions and increased susceptibility to opportunistic infections, malignancy and autoimmune diseases (3, 4).

In this report, we present a 2-year-old boy with Hyper IgM syndrome who suffered from fever, severe diarrhea, upper respiratory infections and rashes. To our knowledge, this is the first reported case of intestinal microsporidiosis in inherited immunodeficiency, X-linked Hyper IgM (XHIGM).

Case report

A 2-year-old boy was admitted to the Hospital Universiti Kebangsaan Malaysia's pediatric ward in June 2016 with a history of severe diarrhea and high-grade fever of five days' duration. The stool was watery, passing of motion occurring more than three times per day and with foul-smelling stool. No dysentery was noted. He was also noted to have a mild runny nose, productive cough and sore throat. Medical history includes diagnosis of Hyper IgM Syndrome (CD40 ligand mutation: IV SI-2A>G) at the age of 6 months old following recurrent epiglottitis and prolonged persistent pyrexia.

On admission, he looked lethargic with signs of moderate dehydration. He was febrile with a temperature of 38°C but other vital signs were stable. Lung examination revealed that it was clear. Skin examination revealed multiple small

skin rashes on the extensor surface of extremities, cheek and scalp. The rash was erythematous plaques, papules punctuated with vesicular lesions and excoriations. Parents claimed the rash had developed over the past one week. The perianal region was noted to have minimal reddish appearance with no rash, no fissure and no abnormal lesions seen. Other system examination was unremarkable.

The full blood test showed severe neutropenia (absolute neutrophil count (ANC) 600/µL) and thrombocytopenia (platelet count 100,000/µL). Stool culture was reported to be negative, but blood culture sent on the second day of illness yielded *Salmonella* sp. (3+). Two specimens of bronchoalveolar lavage were sent for culture and sensitivity, and the result was stated as 'No growth'. Chest radiography showed no abnormality.

He was diagnosed with non-typhoidal salmonella (NTS) infection and infected atopic dermatitis. He was treated with fluid support, intravenous ceftriaxone 100 mg/kg/day and metronidazole (7.5 mg/kg) 8 hourly. His skin lesions were treated with topical corticosteroid and emollients. However, most of the clinical problems did not abate and diarrhea became more frequent. A stool sample was sent on the third day of hospitalization for a special request to look for oocysts of *Cryptosporidium* spp. Modified Ziehl-Neelsen staining was negative for oocysts of *Cryptosporidium* spp. but a few oval organisms were seen, as shown in Figure 1. Gram-Chromotrope Kinyoun (GCK) staining was done and it confirmed that the oval organisms were spores of *Microsporidia* spp. with severity infection of 3+ as shown in Figure 2. Numerous spore sizes of about 0.75 – 0.8 µm × 1.15 – 1.25 µm were seen. Polymerase chain reaction (PCR) was done and confirmed the presence of *Enterocytozoon bieneusi* species (Figure 3).

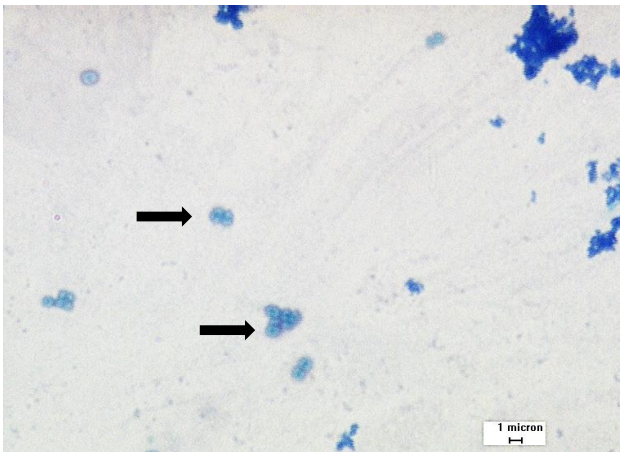

Figure 1: Scanty bluish oval structures (indicated by arrows) seen in modified Ziehl-Neelsen stain under light microscopy (x1000 magnification)

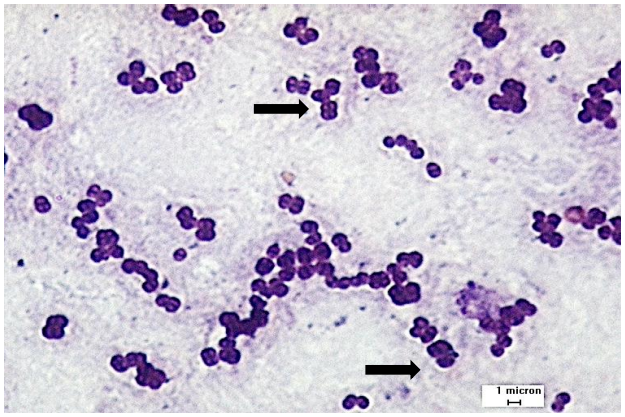

Figure 2: Heavy infection of *Microsporidia* spp. seen in GCK stain under light microscopy (x1000 magnification). The purplish ovoid spores measured 0.5 – 2.5 µm in diameter with horizontal stripe (belt) seen clearly at the centre (indicated by arrows)

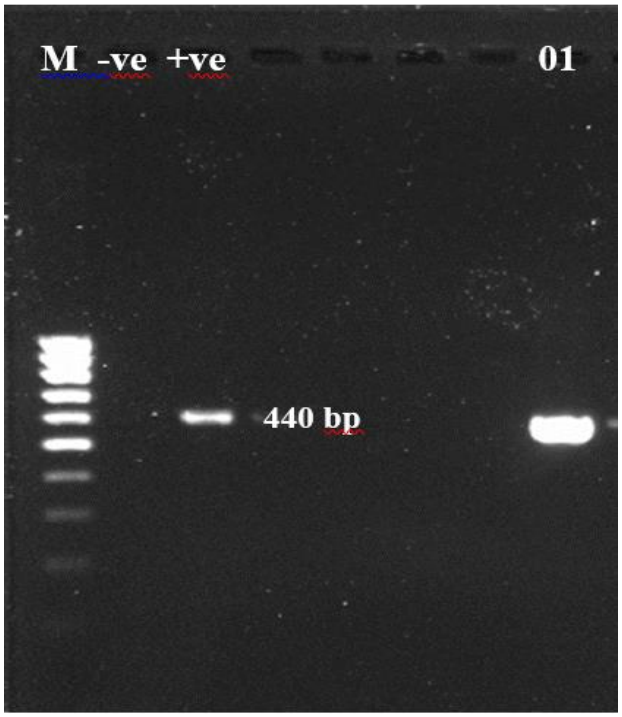

Figure 3: Gel electrophoresis of 440 bp fragment *of E. bieneusi* using PCR

Notes:
M: 100bp DNA ladder
-ve: negative control (distilled water)
+ve: positive control
01: patient's specimen

The patient was diagnosed with mixed infections of severe microsporidiosis and salmonellosis. He was treated with albendazole 400 mg twice daily and continued with ceftriaxone for a total of 14 days. His diarrhea and

temperature subsided slowly and the rashes on his skin were also noted to be much improved.

After 10 days of admission, all symptoms were gone, and he was able to tolerate soft diets. Repeated stool smear performed every two days showed reducing number of spores. On day 25th of admission, only 2 spores were noted in 100 high-power fields and treatment was then stopped. The patient was discharged with an increase of weight by 1 kg. Upon follow-up at one month and 6 months post-discharge, the child was well, actively playful and asymptomatic. Stool examination for *Microsporidia* was negative.

Discussion

A first hospital-based study on intestinal microsporidiosis among patients with and without gastrointestinal symptoms in Malaysia reported a prevalence of 13.0% (2); about one-third was observed in the immunocompetent group and another two-thirds were detected in immunosuppressive patients especially those with hematological malignancy or a combination of malignancy and diabetes mellitus (2). To our knowledge, this is the first report of severe intestinal microsporidiosis co-infected with salmonellosis in a 2-year-old boy who suffers from a very rare inherited immunodeficiency disorder, Hyper IgM Syndrome (CD40 ligand mutation: IV SI-2A>G).

Disseminated infection to the subcutaneous tissue has never been reported in any *E. bieneusi* infection so far, thus the skin problems in this patient were most likely due to his inherited immunodeficiency syndrome causing atopic dermatitis or it could also be caused by Non typhoidal salmonellosis (NTS). This infection usually manifests as self-limited acute gastroenteritis but may also cause severe invasive infections, exclusively among children or immunosuppressed individuals. A study in Bintulu Sarawak has reported a very high incidence of invasive NTS which widely differed from other Asian countries (5). Another study done in Malaysian patients who attended tertiary care hospitals reported that 81.8% of NTS were community-acquired. *Salmonella enterica* serovar Enteritidis had the highest blood invasiveness. However, it reported that extra-intestinal focus of infection noted in 30.9% of the patients were mostly in the lungs and soft tissue (6). Another study reported that 34% of NTS infections had infections of subcutaneous tissue or lymphadenitis and these patients recovered with antimicrobial therapy alone (7). In this case, his skin conditions improved with a mix of treatment which includes topical corticosteroid, emollients and antibiotics. Gastrointestinal symptoms in this patient arguably may also be caused by salmonellosis and his pre-existing conditions. However, as stated earlier, NTS infection usually presented with self-limiting gastroenteritis and it is very rarely presented in invasive NTS (8). In this case, diarrhea did not resolve after antibiotic treatment and in fact became more frequent. It is most likely that this child's gastrointestinal problem was caused by heavy infection of *E. bieneusi* spores because the symptoms abated after albendazole was added to the management. *Cryptosporidium* spp. and *Microsporidia* spp. are well known for their importance in causing severe chronic diarrhea in immunocompromised individuals; thus they are amongst other intestinal parasites that need to be ruled out in AIDS or other immunodeficiency diseases patients who presents with chronic diarrhea (9). The staining used to differentiate between these two organisms were different and it is possible that many microsporidiosis cases may be overlooked if the physician did not order the specific tests, but in this case, the physician did. Ziehl-Neelsen staining is used mainly to stain the wall of acid-fast organisms such as *Mycobacterium* spp. and *Cryptosporidium* spp. It is also useful as narrow spectrum fungal stain; however, it cannot detect microsporidia spores (10). The GCK staining method first introduced in 2011 (11) used in this case was able to demonstrate the spores clearly and alerted the physicians to add necessary treatment. This patient has been diagnosed with such heavy infection of intestinal microsporidiosis, thus it was possible to consider respiratory microsporidiosis as a differential diagnosis because some cases of respiratory microsporidiosis due to *Enterocytozoon* spp. has been reported (12, 13). Detection of spores in bronchoalveolar lavage would be helpful in the future to mitigate this problem.

Albendazole has been reported to reduce the frequency and volume of diarrhea and stabilize the weight of patients with *E. bieneusi* infection in some studies (14) but this is not associated with clearance of the organism on stool specimens or biopsy specimens (15). Other drugs have been mentioned as being more efficient in the treatment of *E. bieneusi* infection such as fumagillin, nitazoxanide, metronidazole or their combination with albendazole (16, 17). Other studies use antiretroviral therapy for microsporidiosis with great effect (18). Oral fumagillin is active against *E. bieneusi*; however, its use is limited due to significant bone marrow toxicity. In this case, albendazole treatment has definitely abated the diarrhea and upper respiratory infection. He responded well and has shown increment in his body weight by a kilogram since his admission. However, it takes more than 20 days to reduce the number of spores significantly.

In conclusion, *Microsporidia* should be included in the differential diagnosis of chronic diarrhea in immunosuppressed hosts; the possibility of this infection causing systemic infection should also be ruled out. Albendazole did treat the symptoms but as in other case reports, it did not eradicate the *E. bieneusi* infection.

Acknowledgement

We would like to thank the patient for kind permission to publish this case report and the Department of Parasitology & Medical Entomology, Faculty of Medicine, UKM for their laboratory technical support.

Competing interests

We declared that we have no significant competing financial, professional or personal interests that might have

influenced the performance or presentation of the work described in this manuscript.

References

1. Stark D, Barratt JLN, Van Hal S, Marriott D, Harkness J, Ellis JT. Clinical significance of enteric protozoa in the immunosuppressed human population. Clin Microbiol Rev. 2009;22:634-50.
2. Norhayati M, Azlin M, Al-Mekhlafi MH, Anisah N, Aini UN, Fatmah MS, *et al.* A preliminary study on the prevalence of intestinal microsporidiosis in patients with and without gastrointestinal symptoms in Malaysia. Trans R Soc Trop Med Hyg. 2008;102(12):1274-8.
3. Schneider LC. X-linked hyper IgM syndrome. Clin Rev Allergy Immunol. 2000;19:205-15.
4. Qamar N, Fuleihan RL. The hyper IgM syndromes. Clin Rev Allergy Immunol. 2014;46:120-30.
5. Mohan A, Munusamy C, Tan YC, Muthuvelu S, Hashim R, Chien SL, *et al.* Invasive *Salmonella* infections among children in Bintulu, Sarawak, Malaysian Borneo: a 6-year retrospective review. BMC Infect Dis. 2019;19:330.
6. Abu NA, Nor FM, Mohamad M, Abidin ASZ, Adnan A, Nor NSM, *et al.* Community-acquired bacteremia in paediatrics: Epidemiology, aetiology and patterns of antimicrobial resistance in a tertiary care centre, Malaysia. Med J Malaysia. 2016;71(3):117-21.
7. Lee WS, Puthucheary SD, Parasakthi N, Choo KE. Antimicrobial susceptibility and distribution of non-typhoidal *Salmonella* serovars isolated in Malaysian children. J Trop Pediatr. 2003;49(1):37-41.
8. Stanaway JD, Parisi A, Sarkar K, Blacker BF, Reiner RC, Hay SI, *et al.* The global burden of non-typhoidal salmonella invasive disease: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis. 2019;19(12):1312-24.
9. Abubakar I, Aliyu SH, Arumugam C, Usman NK, Hunter PR. Treatment of cryptosporidiosis in immunocompromised individuals: Systematic review and meta-analysis. Br J Clin Pharmacol. 2007;63:387-93.
10. Franzen C, Muller A, Hegener P, Salzberger B, Hartmann P, Fatkenheuer G, *et al.* Detection of microsporidia *(Enterocytozoon bieneusi)* in intestinal biopsy specimens from human immunodeficiency virus-infected patients by PCR. J Clin Microbiol. 1995;33(9):2294-6.
11. Salleh FM, Al-Mekhlafi AM, Nordin A, Yasin 'Azlin M, Al-Mekhlafi HM, Moktar N. Evaluation of gram-chromotrope kinyoun staining technique: Its effectiveness in detecting microsporidial spores in fecal specimens. Diagn Microbiol Infect Dis. 2011;69(1):82-5.
12. Weber R, Kuster H, Keller R, Bachi T, Spycher MA, Briner J, *et al.* Pulmonary and intestinal microsporidiosis in a patient with the acquired immunodeficiency syndrome. Am Rev Respir. 1992;146(6):1603-5.
13. Kicia M, Sędzimirska M, Sak B, Kváč M, Wesołowska M, Hendrich AB, *et al.* Respiratory microsporidiosis caused by *Enterocytozoon bieneusi* in an HIV-negative hematopoietic stem cell transplant recipient. Int J Infect Dis. 2018;77:26-8.
14. Dieterich DT, Lew EA, Kotler DP, Poles MA, Orenstein JM. Treatment with albendazole for intestinal disease due to *Enterocytozoon bieneusi* in patients with AIDS. J Infect Dis. 1994;169:178–83.
15. Conteas CN, Berlin OGW, Ash LR, Pruthi JS. Therapy for human gastrointestinal microsporidiosis. Am J Trop Med Hyg. 2000;63(3-4):121-7.
16. Massip P, Massip P, Linas M, Linas M, Bicart-se A, Bicart-se A, *et al.* Successful treatment with nitazoxanide of. Antimicrob Agents Chemother. 2000;44:167-8.
17. Molina JM, Goguel J, Sarfati C, Chastang C, Desportes-Livage I, Michiels JF, *et al.* Potential efficacy of fumagillin in intestinal microsporidiosis due to *Enterocytozoon bieneusi* in patients with HIV infection: results of a drug screening study. AIDS. 1997;11(13):1603-10.
18. Demarchi IG, Cardozo DM, Aristides SMA, Moliterno RA, Silveira TGV, Cardoso RF, *et al.* Activity of antiretroviral drugs in human infections by opportunistic agents. Brazilian J Pharm Sci. 2012;48:171-85.