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Frequency of Microsporidial Infection in Immunocompromised Patients with Staining and Molecular Methods Based on Internal Transcribed Spacer Region Gene in Two Cities of Southwest Iran during 2013-2014

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Abstract

Microsporidiosisis anopportunistic infection frequently diagnosed in immunocompromised patients. Microsporidiosis causes symptomatic or asymptomatic infections immunocompromised/immunosuppressed patients may experience severe and disseminated microsporidiosis. Fecal samples were taken from 186 patients with human immunodeficiency virus (86) and cancer (100). Microsporidia spp. were detected 27.9% (24/86) and 11.6% (10/86) in HIV + patients, 18% (18/100) and 7% (7/100) of cancer patients using polymerase chain reaction (PCR) and trichrome staining respectively. *Enterocytozoon bieneusi* was detected in 28(66.7%) patients, while *Encephalitozoon intestinalis* was detected in 10 (23.8%) patients. *Encephalitozoon cuniculi* was detected in only one patient, while *Encephalitozoon hellem* was detected in three patients. Microsporidia was significantly higher in patients with diarrhea than those without diarrhea (P=0.001). CD4 T cells blood count was significantly lower (<200 cells/µl blood) in HIV+ patients with Microsporidia (P=0.001). Also, there was significant difference between contact with animals with positive results of HIV+ and cancer patients (P< 0.005). This study confirmed that PCR technique was more sensitive than trichrome staining for diagnosis. Moreover, intestinal microsporidiosis caused diarrhea in HIV+ and cancer patients. This influenced treatment. Therefore, early diagnosis of microsporidiosis is effective on treatment strategies.

Keywords: HIV, Immunocompromised Patients, Iran, Microsporidia, PCR

1. Introduction

Opportunistic infections are major causes of morbidity and mortality in AIDS and cancer patients receiving chemotherapy. Microsporidia are a group of obligate intracellular parasites which are ubiquitously distributed

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among invertebrate and vertebrate hosts. Nearly 1200 species of Microsporidiah ave been identified of which14 species cause infections in mammals including humans[1,2]. Since the AIDS pandemic around the world in 1980, species of Microsporidia have been recognized as etiologic causes of opportunistic infections.T he clinical symptoms of microsporidiosis depend on the infected site and immune system. Recently, a large number of microsporidiosis cases have been reported as opportunistic infections in patients with HIV$^+$ and cancer patient receiving chemotherapy, transplant recipients, passengers, children and the elderly[2–7]. In HIV$^+$ patients, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are responsible for many gastrointestinal infections in humans[8]. They may cause persistent diarrhea with mal absorption and weight loss, also asymptomatic infections have been reported in immunocompetent persons[9]. Microsporidia may present urinary, pulmonary or disseminate infections includingsinusitis, encephalitis, tracheobronchitis, nephritis, hepatitis, myositis, ocular and stromal keratitis, increasingly are identified by *E. intestinalis* spp[10].

Up to 2013 in Iran, 27041 HIV$^+$ individuals are identified and 1866 out of them are in Khuzestan Province. The number of registered HIV$^+$ individuals in Abadan and Khorramshahr cities grew approximately to 460 cases, in which 115 individuals were identified up to 2013 among an estimated population of 342162 citizens (www.IranHIV.com). Based on the 2012 report ofthe National Program of Cancer Register, over 84829 cancer cases have been diagnosed in Iran among 77450000 people; statistics show that 90000 Iranians are expected to have cancer diagnosis[11].

Human microsporidiosis occurs worldwide, but data on the prevalence and geographic distribution of microsporidial infections are still incomplete and very diverse due to the use of different diagnostic methods, no specialized personal and the focus of parasitic studies on common parasites[12]. Microsporidia have been less reported among cancer patients worldwide[13]; moreover, no studies have been conducted on the prevalence of Microsporidia in cancer patients in Iran despite its occurrence in HIV$^+$ population. It has been reported that immunosuppressive therapy may be associated with higher risk of severe parasitic infections[13,14]. To date, many papers reported microsporidial infection in HIV$^+$ patients[9], but we did not have any precise information of intestinal microsporidiosis infection in this patient in Iran except rare studies that were carried out by Agholi*et al.*, Mirjalali and Ghorbanzadeh[15–18]. Serological assays are not useful for diagnosis, however, they may be used for epidemiological studies. Routine diagnosis is based on spore observation by light microscopy using modified trichrome staining, Uvitex 2B or Fungi flour, but unable to differentiate between the species. However, for the differentiation of the microsporidian species, PCR methods due to their sensitivity and specificity are recommended for this aim[19–21].To our knowledge, there is no epidemiological study conducted on Microsporidiain Ahvaz area to date. This study determines the frequency of Microsporidia species in fecal isolates of patients with HIV$^+$ and cancer receiving chemotherapy in the southwest of Iran (Khuzestan) by using PCR method in comparison with modified trichrome blue (MTB) staining.

2. Materials and Methods

2.1 Sample

During a period of one year from March 2013 to April 2014, 186 stool samples were collected from patients that were divided into 2 groups on the basis of their cause of illness: Group 1 consisted of 86 HIV$^+$ patients from 2 hygienic centers (Prevention and Control of Diseases Center) of Khorramshahr and Abadan. Patients included men and women in the age range of 9 to 64 years, who referred to laboratory due to periodic checkup or receiving HAART drug with or without diarrhea. Most of these patients had received antiretroviral therapy (ART); HIV$^+$/AIDS stage of the patients was classified according to CD4+ T-cells levels that fell below 200 cell/µl is submitted in AIDS stage. Group 2 consisted of 100 cancer patients undergoing chemotherapy. All specimens were randomly obtained from confirmed cancer patients undergoing chemotherapy and/or radiotherapy at different treatment stages with or without diarrhea such as gastrointestinal cancer (31 patient), blood cancer (25 patient), lung cancer (10 patient), liver cancer (7 patient), lymphoma (7 patient), breast cancer (5 patient), prostate cancer (5 patient), skin cancer (4 patient), cervical cancer (3 patient) and fallopian cancer (3 patient). There were 68 males and 32 females, as all cancer patients referred to the two hospitals in Ahvaz province, Golestan (General Hospital) and Shafa (Thalassemia and Hemoglobinopathy Research Center) of Ahwaz Jundishapur University of Medical Sciences. From each patient one stool sample

was collected, filtered and stored in 2.5% K_2CrO_7 at 4°C or frozen at -20 °C for the tests[8]. Based on the appearance of stool, each sample was considered in one of the grades: formed stool, soft stool or diarrhea stool. CD4+ T cells were counted in blood taken from86 HIV^+ patients by flow cytometer technique.

2.2 Questionnaire

A questionnaire form was designed to collect data related to the presence of microsporidia in during the process of specimen collection for those items (age, sex, HIV/AIDS stage, patient type, location life in a city or rural, contact with animals, CD4 count, with or without diarrhea and drug usage).

2.3 Staining of Stool Smears

A stool specimen was mixed with phosphate buffered saline (1:3); the mixture was filtered through a gauze plug and centrifuged at 2000 rpm for 2 min. A thin smear was taken from 20 µl pellet suspension of stool and applied on a glass slide; the smear was dried in air and fixed in methanol. Then, it was stained with Modified Weber's Chromo tube based trichrome staining[22]. Smears were tested by oil immersion (1000×magnification) to determine negative specimens. Screening was carried out twice. Pinkish-red oval shape measuring <2 µm in length, with a characteristic posterior vacuole and belt like strike in the middle, were identified as microsporidian spores[23].

2.4 DNA Extraction

DNA extraction and purification were carried out by using QIA amp DNA Stool Mini Kit(QIAGEN) based on instructions from the manufacturer. The stool sample was filtered thrice by a 20 µm filter (25 mm in diameters) to remove debris. Stool sediment (100 µl) was vortexed and suspended in 96 µl lysis buffer containing 100 mM ethylene diamine tetra acetic acid (pH=8),100 mM Tris HCL (pH=8), 2% sodium dodecyl sulfate, 150 mM NaCl and 0.4 mg/ml proteinase K enzyme (20 mg/ml).The DNA pellet was centrifuged to ensure sedimentation, then obtained DNA was stored at -20°C[24].

2.5 PCR Amplification

Multiplex nested PCR targeted internal transcribed spacer (ITS), small subunits (SSU) and large subunits (LSU) of ribosomal DNA (rDNA). Three sets of primers were used for PCR amplification. Upstream primers MSP-1 (TGA ATG[G/T]GT CCC TGT) and MSP-3 (GGA ATT CAC ACC GCC CGT C[A/G][C/T]TAT) targeted three SSUs and recognized many microsporidian species, such as *E. Bieneusi* and *Encephalitozoon* spp. Downstream primers MSP-2B (GTT CAT TCG CAC TAC) and MSP-4B (CCA AGC TTA TGC TTA AGT CCA GGG AGT) targeted five LSUs of *E. bieneusi*, while MSP-2A (TCA CTC GCC GCT ACT) and MSP-4A (CCA AGC TTA AGT [C/T][A/C]AA[A/G]G GGT) recognized *Encephalitozoon* spp[8,20,22]. Finally, reactions occurred in 20 µl containing 0.3 mMdNTPs, 2 mM $MgCl_2$, 1x PCR buffer, 1U Taq polymerase (Fermentas), 1 µm primer and 3 or 5 of extracted DNA. The first PCR mix contained 1 µl primer (MSP-1, MSP-2A, MSP-2B) and 3 µl template DNA. The second nested PCR reaction involveda mixture containing 1 µl primer (MSP-3, MSP-4A, MSP-4B) and 2 µl first amplification product mixture. Amplicon size ofMSP-3, MSP-4B product was estimated at500bp for *E. bieneusi*, while amplicon size of MSP-3, MSP-4A product was estimated at300bp for *Encephalitozoon* spp. Amplifications were doneby using the Applied Biosystems™ thermal cyclersfor primary and secondary reactions; these amplifications included denaturation (95°C; 5 min), 36 denaturation cycles(95°C; 30s), primer annealing (55 °C; 1 min) and elongation (72°C; 2 min). The last elongation was extended to 10 min. Each reaction set involved a negative control with ultrapure water and a positive control with the cultured template DNA which was originally isolated from HIV^+ patients. The amplified products were visualized on 1.5% agarose gel at 80 KV for 70 min. The gel was stained with 2 µg/ml ethidium bromide and observed under ultraviolet light (Figure 1).

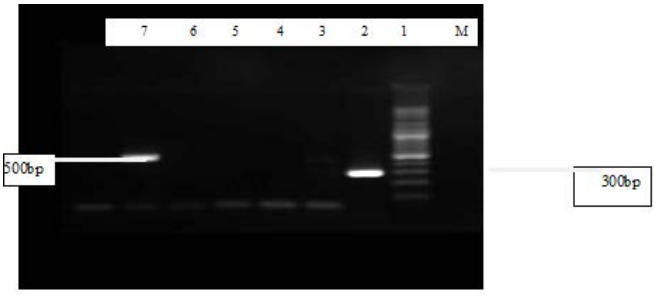

Figure 1. Electrophoresis of Nested PCR product; M: Marker 100bp; 1: Positive sample for Encephalytozoon; 2-3-4-5: Negative samples; 6: Positive sample for Enterocytozoon; 7: Negative control.

2.6 Statistical Analysis

The student t-test, Chi-square and independent t-test were used to compare means of the studied groups; Fisher's exact test was used to determine factors of microsporidiosis in SPSS 16 software.

2.7 Ethical Considerations

This study was approved by the ethics committee (No: ajums.rec.1392.219) of Postgraduate Institute of Medical Education and Research, Jundishapur University of Medical Sciences, Iran.

2.8 Restriction Fragment Length Polymorphism (RFLP)

Based on Katzwinkel-Wladarschet al., the RFLP used involved 30 µl consisting of 20 µl secondary PCR product, 1 µl Mnl1 (http://www.thermoscientificbio.com/restriction-enzymes/mnli)enzyme (Fermentas), 0.1 µl BSA (10 mg/ml), 3 µl buffer (10 concentrated, supplemented with BSA (Fermentas) and 5.9 µl super distilled water22. Subsequent incubation (37 °C, 12 h) was followed by visualization of fragments in 2% agarose gel containing 0.2 µg/ml ethidium bromide. The restriction enzyme (Mnl1) cleaved SSU-rDNA sequence into several fragments with species *E.bieneusi* (500bp) 180, 90, 60bp, *E. intestinalis* (294bp) 160, 60 bp and three bands under 20bp, *E. cuniculi* (307bp) 210 and 90 bp and *E. hellem* (315bp) was expected to produce fragments 180 and 80 bp (Figure 2).

3. Results

3.1 Prevalence of Microsporidia in HIV/AIDs Patients

From 86 HIV-infected patients, 24 (27.9%) patients were positive for microsporidia by PCR and 10 (11.6%) only by modified trichrome blue staining (Table 1). 30 (34.9%) were female, while 56 (65.1%) were male, although there was a preponderance of males, there was no significant difference in infection between genders (male vs female=25% vs 33.3%)(P=0.412). Out of the 24/86 positive specimens, 54.3%(19/35) had diarrhea vs 9.8%(5/51)without diarrhea (P=0.001) (Table 3). Concurrent infection with *E.intestinalis* and *E.bieneusi* was observed in one case. Microsporidia were common in patients aged 28–48 years. The frequency of microsporidia was higher in AIDS stage (51.4%) than HIV stage (15.8%).No significant difference (P=0.246) was found in microsporidia between patients who received treatment (34.2%) and those who did not receive treatment (22.9%). There was no significant difference in age, gender, drug use, occupation, and residency of HIV$^+$ patients with and without microsporidia. There were significance differences in patients type, life level, living location, contact with animals and with or without diarrhea between positive and negative result of HIV$^+$ patients(P=0.001).Out of 24 positive patients 10 (33.3%) were female and 14 (25%) were male, aged 9–64 years (mean 38.2). Table 2 shows the mean of CD4+ count of HIV$^+$ patients with positive results (181 cell/µl) and HIV$^+$ patients with negative result (483 cell/µl). Lower CD4+ counts (<200 µl) were found in HIV$^+$ patients with diarrhea (54.3%) and patients without diarrhea (9.8%). There was a statistically significant difference between CD4+ cell count and HIV$^+$ patients(P=0.001). By sequencing the amplicons, among the 24 positive patients 17(70.8%) were identified with *E. bieneusi* and 5 (20.8%), 1 (4.2%),1(4.2%) were identified with *E. intestinalis*, *E. cuniculi* and *E. hellem*, respectively. Also, 1 patient (4.2%) was diagnosed with concurrent infection of *E.bieneusi* and *E. intestinalis* (Table 4).

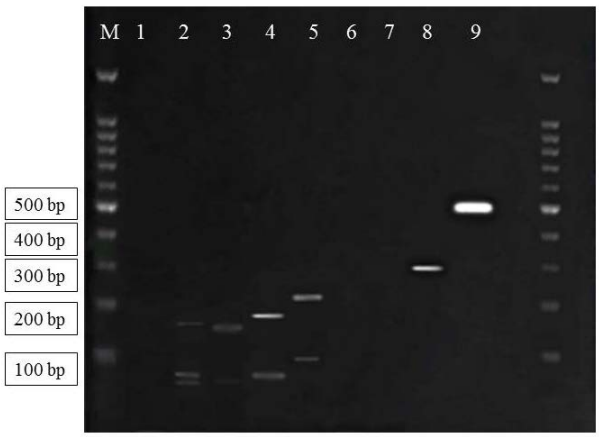

Figure 2. Electrophoresis of RLFP products by MnlI enzyme; M: Molecular Marker 100bp (Fermentas company);1) Negative control; 2) Enterocytozoonbineusi (60, 90, 180 bp); 3) Encephalitozoonintestinalis (60, 160 bp); 4) Encephalitozoonhellem(80, 180 bp) ; 5) Encephalitozooncaniculi (90, 210 bp); 6,7) Negatives samples; 8)Encephalitozoon control without enzyme;9) Enterocytozoonbineusi control without enzyme.

Table 1. Prevalence of microsporidia detected by PCR and staining methods in HIV+ patients

Patient	PCR		Staining		Total
	Negative	Positive	Negative	Positive	
HIV	48 (84.2%)	9 (15.8%)	54 (94.7%)	3 (5.3%)	57 (100%)
AIDS	14 (48.3%)	15 (51.7%)	22 (75.9%)	7 (24.1%)	29 (100%)
Total	**62 (72.1%)**	**24 (27.9%)**	**76 (88.4%)**	**10 (11.6%)**	**86 (100%)**

Table 2. Mean rate of CD4 cells in HIV+ patient with positive and negative results

Cell	PCR	N	Mean	SD*
CD4	Negative	62	483.3065	231.70166
	Positive	24	181.8750	107.11810

*SD = Standard Deviation

3.2 Prevalence of Microsporidial Infections in Cancer Patients

From 100 patient samples, 18/100 (18%) and 7/100 (7%) patients were positive for microsporidia by PCR and trichrome staining, respectively (Table 3). All smears positive by light microscopy were positive by PCR too. Co-infection was not seen in this group. Microsporidial infection was reported more in gastrointestinal patients (32.3%), followed by patients with lymphoma (28.6%), lungcancer (10%) and blood cancer (8%)(Table 4). No significance difference was observed in gender, drug usage, patient type, occupation and life level ofcancer patients with positive microsporidian results and cancer patients with negative results; however, number of male patients was higher (68%). But there were significance differences in age, living location, contact with animals and with or without diarrhea between positive and negative result of cancer patients(P=0.011)(Table 3). By sequencing of the amplicons, from 18 positive patients, 11(61.1%), 5 (27.8%), and 2 (11.1%) were identified with *E. bieneusi*, *E. intestinalis* and *E. hellem*, respectively. No case of *E. cuniculi* was identified (Table 4).

3.3 DNA Sequencing

ABI3730XL sequence analyzer was used for direct sequencing of PCR products. Samples were sequenced in both directions to confirm the species identification. Sequences were aligned and compared with sequences in GenBank. The most prevalent microsporidian species, *E.bieneusi*, was detected in 17 HIV$^+$ patients and 11 cancer patients who exhibited 100% homology with *E.bieneusi*in GenBank, also amplicons sequencing from 10 patients (5 HIV$^+$ and 5 cancer patients) were detected with *E.intestinalis*, exhibited 100% homology with *E.intestinalis* in GenBank. *E. cuniculi*were identified in one patient; these isolates exhibited 97% homology with the *E. cuniculi* sequence reported in GenBank. *E. hellem* was identified in 3 patients; these isolates exhibited 97% homology with the *E. hellem* sequence reported under GenBank.

4. Discussion and Conclusion

Prior to the advent of highly active antiretroviral therapy (HAART) in mid-1990, there was 5–50% reported cases of microsporidiosis in HIV patients worldwide (mean 15%) and even higher depending on methods of diagnosis, geographical area, hygienic and immune condition of study population[25,26]. By spread of HAART in the developed countries, prevalence of the opportunistic infections including microsporidiosis dramatically declined due to improvements inimmune system[2,5,27],while opportunistic infections such as microsporidia remain problematic in developing countries where HAART is not easily available for HIV$^+$ patients[28,29]. In Ahvaz, detection of microsporidia is not routinely carried out in hospital or medical diagnostic centers; therefore, little is known about intestinal microsporidiosis in HIV$^+$ and cancer patients. This can be attributed to the lack of accurate diagnostic methods, lack of experienced personnel and their very small size for diagnosis. This study was the first epidemiological study in Ahvaz on frequency of microsporidia in 2 groups

Table 3. Prevalence of microsporidia in HIV+ and cancer patients with or without diarrhea by PCR

Specimen type	PCR(HIV$^+$ patient)		Total	PCR(cancer patient)		Total
	Negative	Positive		Negative	Positive	
Diarrhea	16 (45.7%)	19 (54.3%)	35(100%)	18 (60%)	12 (40%)	30 (100%)
No diarrhea	46 (90.2%)	5 (9.8%)	51(100%)	64(91.4%)	6 (8.6%)	70(100%)
Total	**62 (72.1%)**	**24 (27.9%)**	**86(100%)**	**82 (82%)**	**18(18%)**	**100**

Table 4. Frequency of microsporidia species detected among HIV+ and cancer patients by sequencing

Patients	Sequencing						Total
	Enterocytozoonbieneusi			Encephalitozoon			
	D	J	M	intestinalis	cuniculi	hellem	
HIV	5(55.6%)	0	0	2(22.2%)	1(11.1%)	1(11.1%)	9
HIV	11(73.3%)	1(6.7%)	0	3(20%)	0	0	15
Lym. C	0	0	0	2(100%)	0	0	2
Gastr. C	6(60%)	1(10%)	1(10%)	1(10%)	0	1(10%)	10
Blood. C	1(50%)	0	0	1(50%)	0	0	2
Lung. C	0	0	1(100%)	0	0	0	1
Liver. C	0	0	0	1(100%)	0	0	1
Prostate. C	1(100%)	0	0	0	0	0	1
Skin.C	0	0	0	0	0	1(100%)	1
Total	**24(57.1%)**	**2(4.8%)**	**2(4.8%)**	**10(23.8%)**	**1(2.4%)**	**3(7.1%)**	**42**

*C=Cancer *Gastr. C=Gastrointestinal Cancer *Lym.C=Lymphatic Cancer

of patients at risk for microsporidiosis; HIV+ and cancer patients by applying PCR and staining methods. In this study, microsporidiawas more frequent in HIV+ group compared to patients receiving chemotherapy (27.9% and 18%, respectively).

Prevalence rates of microsporidiosis in HIV+ individuals in this study are similar with previous reports such as in Iran 22.53%[18], in Nigeria 23.3%[30], 27.5% in Brazil[31], 26.7% in India[32], while higher in reports from Russia 18.9%[8], 17.4% Venezuela[33], 9.5% Hanoi, Vietnam[27], 10.5% Tunisia[34], 18.9%[4], in India15.9%[35],5.2% in Cameroon[36], 10.5% in Niamey, Niger[27], in India 6.5%[37], in Malaysia 8.5%[38], in France 10.5%[34], whereas lower in 77% among HIV-infected children in Uganda[39],42.7% in Portugal[40], 81.2% in Thailand[41]. These results are very similar to the prevalence found in patients with HIV in Ethiopia with 39 (16%) by PCR and 18(7.6%) by staining that *Enterocytozoon bieneusi* were 77% (30/39) and *Encephalitozoon intestinalis* 15.4% (6/39)[29].In developing countries, prevalence of *E.bieneusi*is 2.5% - 51%[9]in HIV+ patients with diarrhea and <5% in patients without diarrhea[36]. In this study *E.bieneusi* prevalence rates for HIV+ patients with diarrhea was 66.7% (16/24) and 29.2% (7/24) for patients without diarrhea, therefore the higher occurrence of *E.bieneusi* is not surprising. In this study, *E.bieneusi* was identified more than *E.intestinalis* and also this study identified *E.cuniculi* and *E.hellem* and our results supported by the previous study[8].

In Iran out of 81 HIV+ patients, 30.86% (25/81) were infected with microsporidia and *E.bieneusi* were identified in all of positive sample and no species of *Encephalitozoon* were found[15]. *E.bieneusi* was the most prevalent microsporidian identified in HIV+ patients with diarrhea[2]. *E.bieneusi* and *E. intestinalis* generally detected in feces and *E. intestinalis* are often disseminated and cause systemic infection[42]. Two other species of Encephalitozoon, *E.cuniculi* and *E.hellem*, which are rarely detected in feces usually cause systemic diseases; these species are primarily shed in urine rather than feces[2]. *E.cuniculi* and *E.hellem* detected in feces of two patients determine the effect of all three *Encephalitozoon* species on diarrhea in immunosuppressive and HIV+ patients and this is in agreement with Sokolova finding[8]. Diarrhea is common among non-HIV+ and immune suppressed patientsin cluding transplant recipients, children and patients with chronic diseases, although the etiology of diarrhea is different in these patients: viral, bacterial, parasitic, oropportunistic intestinal parasites[9,43]. Few cases of microsporidia have been reported in cancer patients with pulmonary infection; this can be attributed to immune suppression caused by treatment in patients with leukemia[44]. In the current study, the majority of infections were caused by *E.bieneusi* leading to gastrointestinal disorders (32.3%).This can be attributed tothe depression of cellular immunity which protects the body against microsporidial infections[44].

In this study, the mean age of cancer patients was 56 years old. It was found that age is a risk factor for infection.

This is consistent with Lores*etal.*[45]Microsporidia was significantly higher in patients with diarrhea (40%) than patients without diarrhea (8.6%); however, these values are lower than those reported in previous studies from Malaysia (30% and22% by PCR and MTB, respectively, instool samples of cancer patients) and more common in patients with diarrhea (78%) than patients without diarrhea (29.3%)[46,47]. This finding is also consistent with a study conducted in Egypt where microsporidia were detected in 17% and 10% by PCR and MTB, respectively, and infection rate was significantly higher in cancer patients with diarrhea than those without diarrhea. Other studies reported lower rate of microsporidial infection (8.6% by MTB and 10.9% by PCR) in cancer patients[14,34]. Coyle *et al*. found a clear relationship between microsporidia and diarrhea; they concluded that microsporidia infected 44% of HIV$^+$ patients with diarrhea, whereas it only infected 2.3% of the patients without diarrhea[26].

Few studies have been conducted on the prevalence of intestinal microsporidiosis in HIV$^-$immuno suppressed patients, such as cancer patients or transplant recipients[6,47]. In this study, PCR could detect a higher number of positive cases than microscopic-based methods; this is consistent with the previous studies[3,22,46]. Moreover, light microscope does not work when the stool contains undetectable levels of spores; this is consistent with Gumbo *et al.*[48] In Zimbabwe, microsporidia was detected in 18%, 51% of diarrhea patients by microscopic and PCR, respectively. These findings were supported by others who reported PCR is more sensitive(100%) compared to microscopic techniques in detecting microsporidial infection in HIV$^+$ patients[34,49,50].The increased sensitivity of PCR can be attributed to lower threshold of microsporidia (10^2 spores/g stool) compared to optical microscopy in which the cut-off point ranges from 10^4 to 10^6 spores[51]. Althoughtrichrome staining is are liable and an available technique for detection of microsporidian spores in vitro, detection process is difficult and time-consuming for staining[46,52].Moreover, higher prevalence of *E. bieneusi* compared to *E. intestinalis* has been reported in some studies[29,36,53]. While in one study in Egypt in cancer patients reported that *E. intestinalis* was the only species identified and the most prevalent in leukemic patients[46].As shown in other studies, PCR could detect *E. intestinalis* (20%) and *E. hellem* (10%) in stool samples taken from cancer patients[20], while Chabchoub et al found equal prevalence of *E. intestinalis* and *E. hellem* (33.3%)[34]. Similarly, higher prevalence of *E. intestinalis* has been reported among HIV patients[9,38,46]. In our study prevalence rate of microsporidia in cancer patients is 18% and this result is similar with 21.9% in Malaysia, 17% in Poland in transplant recipients, 20.77% in Mexico in all patients[3,54,55] and higher than previous report with 4.8% in cancer patients in Egypt, 15.1% in cancer patients in Iraq, and 10% in Turkey[14,56].

Animal contact cancontribute to the transmission of microsporidia to HIV$^+$ and cancer patients. Birds are natural hosts of *E. hellem*[57,58]. Conversely, humans infected with *E. intestinalis* transmit this species[12]. Moreover, *E.bieneusi* infects a wider range of both animals and humans; the genotype identified in currentsamples is M,J,D. This finding is in agreement with previous report[8]. This intestinal parasite istransmitted by contaminated food or water. Therefore, it is more difficult to reduce the risk of transmission of intestinal microsporidial infection in developing countries[59]. Thus, HIV$^+$ and cancer patients need proper personal hygiene; for example, they need to boil the water before drinking. Those HIV$^+$ patients with<100 cells/mm^{3}CD4+ cell counts are highly susceptible to intestinal microsporidiosis[29]. In this study,CD4+ cell count was <200 cells/mm^{3}in more than half of HIV$^+$ patients with intestinal microsporidiosis. Findings of this study are consistent with previous reports on the relationship between microsporidiosis in patients with lower CD4+ T cells[4,46]. Further studies are required o determine the sources of these infections to reduce their prevalence and determine microsporidian infections, which cause disseminated diseases in HIV$^+$ and immune suppressed patients. Inconsistency of the results can be attributed to manners of stool preparation or DNA extraction. DNA extraction from microsporidia spores in stool has many complexities including very small size of spores, rigid doubled-layer wall and also lower counts of stool samples. Moreover, immune suppression, geographical and socioeconomic factors source of infection, and diagnostic tests used can impress the quality and quantity of molecular results[19,21,46].In conclusion, the occurrence of microsporidia in patients with HIV$^+$ and cancer patients at the level of 27.9% and 18% respectively, suggests that these pathogens should be taken into account when other etiological agents cannot be found in those diarrheic patients. Moreover, this study shows that PCR is able to detect and identify species of intestinal microsporidia with the highest sensitivity

than traditionally used staining methods. Therefore, these patients might maintain good personal hygiene for prevention and applying proper treatment.

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