



doi • 10.5578/tt.10207
Tuberk Toraks 2016;64(1):9-16
Geliş Tarihi/Received: 01.06.2015 • Kabul Ediliş Tarihi/Accepted: 08.10.2015

KLİNİK ÇALIŞMA
RESEARCH ARTICLE

Evaluation of pulmonary microsporidiosis in iatrogenically immunosuppressed patients

Soykan ÖZKOÇ¹
Songül BAYRAM DELİBAŞ¹
Çiler AKISÜ¹

¹ Department of Medical Parasitology, Faculty of Medicine, Dokuz Eylül University, Izmir, Turkey

¹ Dokuz Eylül Üniversitesi Tıp Fakültesi, Tıbbi Parazitoloji Anabilim Dalı, İzmir, Türkiye

SUMMARY

Evaluation of pulmonary microsporidiosis in iatrogenically immunosuppressed patients

Introduction: *Microsporidia spp.* are ubiquitous and infect a wide variety of invertebrates and vertebrates, including humans. Pulmonary microsporidiosis, characterized by nonspecific symptoms like fever, cough and dyspnea, is often overlooked in the differential diagnosis of pulmonary infections in immunosuppressed patients. In this study, we aimed to determine the prevalence of pulmonary microsporidiosis in iatrogenically immunosuppressed patients and to evaluate the patient characteristics.

Materials and Methods: Bronchoalveolar lavage (BAL) specimens from 63 iatrogenically immunosuppressed patients and 28 controls were examined with PCR. By using PMP1 and PMP2 common primers specifically designed for *Enterocytozoon bienersi*, *Encephalitozoon intestinalis*, *Encephalitozoon cuniculi* and *Encephalitozoon hellem* small-subunit ribosomal DNA (SSU-rDNA) regions at 250-279 bp were amplified. In addition, PCR positive BAL specimens were examined with modified trichrome staining method for *Microsporidia* spores.

Results: Out of 63 immunosuppressed patients, nine (14.2%) had *Microsporidia spp.*, but none of the control patients had *Microsporidia spp.* on PCR. This difference between two groups was statistically significant ($\chi^2 = 4.439$; $p = 0.035$). On the other hand there was not a statistically significant relationship between PCR positivity and patient characteristics such as gender and age. Of nine patients with *Microsporidia* PCR positive, only one had spores of *Microsporidia sp.* Out of eight patients without spores, one had *Mycobacterium tuberculosis*, one patient had *Klebsiella pneumoniae* and five patients had *Pneumocystis jirovecii* DNA.

Conclusion: This is the first study to evaluate the pulmonary microsporidiosis in immunosuppressed patients in Turkey. The results of the study indicated that *Microsporidia spp.* should be taken into account in the differential diagnosis of pulmonary infections in immunosuppressed patients and it is important to use molecular methods such as PCR in the laboratory diagnosis of the causative agent.

Key words: *Microsporidiosis*, pulmonary, immunosuppressed

ÖZET

İyatrojenik immün baskılanmış hastalarda pulmoner mikrosporidiosisün değerlendirilmesi

Giriş: *Microsporidia* türleri insanın da dahil olduğu geniş bir omurgalı ve omurgasız canlı grubunu infekte edebilen zorunlu hücre içi parazitlerdir. Ateş, öksürük ve dispne gibi nonspesifik semptomlarla

Yazışma Adresi (Address for Correspondence)

Dr. Soykan ÖZKOÇ
Dokuz Eylül Üniversitesi Tıp Fakültesi,
Tıbbi Parazitoloji Anabilim Dalı, İZMİR - TURKEY
e-mail: soykan.ozkoc@deu.edu.tr

karakterize olan pulmoner microsporidiosis, immün baskılanmış hastalardaki pulmoner enfeksiyonların ayırıcı tanısında çoğu zaman düşünülmemektedir. Biz bu çalışmada, iatrojenik olarak immün baskılanmış hastalardaki pulmoner microsporidiosis prevalansını saptamayı ve hasta karakteristiklerini değerlendirmeyi amaçladık.

Materyal ve Metod: Çalışma grubuna alınan 63 immün baskılanmış hasta ve kontrol grubundaki 28 hastanın BAL sıvısı örnekleri, PCR yöntemiyle incelendi. PCR yönteminde *Enterocytozoon bienewsi*, *Encephalitozoon intestinalis*, *Encephalitozoon cuniculi* ve *Encephalitozoon hellem small-subunit ribosomal DNA (SSU-rDNA)* bölgesine özel tasarlanmış PMP1 ve PMP2 primerleri ile 250-279 bp uzunluktaki bölge amplifiye edildi. Ek olarak PCR pozitif bulunan örnekler modifiye trikrom boyası ile *Microsporidia spp.* sporları yönünden incelendi.

Bulgular: Immün baskılanmış hastaların 9 (%14.2)'unda *Microsporidia spp.* PCR pozitifliği saptanırken kontrol grubu hastalarının hiçbirinde pozitiflik gözlenmedi. İki grup arasındaki bu fark istatistiksel olarak anlamlı bulundu ($\chi^2 = 4.439$; $p = 0.035$). Diğer taraftan hastaların yaş ve cinsiyet özellikleri ile PCR pozitifliği arasında istatistiksel açıdan anlamlı bir ilişki saptanmadı. Mikroskopik değerlendirme sonucunda PCR ile pozitif saptanan dokuz hastanın sadece bir tanesinin BAL örneğinde *Microsporidia spp.* sporları saptandı. *Microsporidia spp.* sporları saptanmayan sekiz hastadan birinde *Mycobacterium tuberculosis*, birinde *Klebsiella pneumoniae* pozitif olarak bulunurken, beş hastanın örneğinde *Pneumocystis jirovecii* DNA'sı pozitifliği.

Sonuç: Bu çalışma, immünsüpresif hastalardaki pulmoner mikrosporidiosis değerlendirildiği ülkemizdeki ilk çalışmadır. Çalışmanın sonuçları, immün baskılanmış hastalardaki pulmoner enfeksiyonların ayırıcı tanısında *Microsporidia spp.*'nin de mutlaka değerlendirilmesi gerektiğini göstermiş aynı zamanda etkenin laboratuvar tanısında PCR gibi moleküler yöntemlerin kullanılmasının önemini ortaya koymuştur.

Anahtar kelimeler: *Microsporidiosis*, pulmoner, immün baskılanmış

INTRODUCTION

Microsporidia spp. are obligate intracellular eukaryotic parasites which form small oval spores (1). Although there are about 1200 species of *Microsporidia sp.* which can infect both vertebrate and nonvertebrate hosts, most of the human microsporidiosis infections are caused by *Enterocytozoon sp.* and *Encephalitozoon spp.* (1,2). In recent studies using genetic analyses, the zoonotic potential has clearly been documented about these parasites (3).

The clinical course of microsporidiosis varies with the site of the infection and the host immunity. Asymptomatic or moderate microsporidiosis is likely to remain a local infection in immunocompetent people, while it may present with infections of the hepatobiliary, pulmonary, renal and central nervous systems in addition to severe diarrhea in patients with AIDS and those with iatrogenic immunodeficiency (2,4).

It has been shown that *Microsporidia spp.* can infect the respiratory tract and the lungs (5). Furthermore, there has an increase in the number of reports on pulmonary microsporidiosis in recent years (6). Pulmonary colonization resulting from contamination through inhalation and regurgitation or oral-fecal and hematogenous routes can lead to opportunistic infections presenting with fever, cough and dyspnea especially in patients with AIDS and transplant recipients (2,5-8). There have also been reports

showing that the parasite can be detected in specimens from the respiratory tract in patients with urinary and intestinal microsporidiosis (6,10). Botterel et al. detected *Microsporidia* spores in BAL specimens from an AIDS patient with intestinal microsporidiosis and they concluded that the patient had an asymptomatic colonization since he did not have any pulmonary complaints (6).

In Turkey, it has been observed that both clinicians and laboratory specialists do not take account of microsporidiosis in the differential diagnosis of pulmonary infections. Prior studies on this issue mostly focus on revealing intestinal microsporidiosis and a range of 1.05- 69.9% *Microsporidia spp.* positivities in different patient groups have been reported (11-19). However there have not been any studies on the prevalence of pulmonary microsporidiosis in our country. Therefore, in this study we aimed to investigate the pulmonary microsporidiosis in iatrogenically immunosuppressed patients.

MATERIALS and METHODS

Patients

BAL specimens sent to Parasitology Laboratory of Dokuz Eylül University Hospital between January 2011 and May 2014 were examined in this study. Sixty-three iatrogenically immunosuppressed patients developing pulmonary infection while being followed in hematology-oncology, bone marrow transplantation,

nephrology, rheumatology, chest diseases and intensive care units of the hospital were included into the study group. Twenty-eight immunocompetent patients undergoing bronchoscopy during routine clinical examinations were included into the control group. All patients' records were reviewed and data about underlying diseases, immunosuppressants given, age and gender were collected. Ethical approval was obtained from Non-Invasive Research Ethical Committee of Dokuz Eylül University Medical Faculty (number: 2014/23-04).

BAL Specimens and DNA Extraction

BAL specimens were centrifuged at 1500 rpm for 10 min and 200 µL of obtained pellet was kept at -20°C until DNA extraction. DNA extraction was performed with an extraction kit (Macherey-Nagel, Düren, Germany) in accordance with the manufacturer's guidelines. Ultrapure distilled water was used as negative control and *Enterocytozom cuniculi* isolate obtained from ATCC isolate bank (founded in the framework of a project conducted by TUBITAK) was used as positive control.

Microsporidia PCR

Small-subunit ribosomal DNA (SSU-rDNA) regions at 250-279 bp were amplified with the primers PMP1 (5'-CACCAGGTTGATTCTGCCTGAC-3') and PMP2 (5'-CCT CTCCGGAACCAACCCTG-3') specifically designed for *Enterocytozoon bienersi*, *Encephalitozoon intestinalis*, *E. cuniculi* and *Encephalitozoon hellem* (14,18,20-21). For each reaction, 2.5 µL 10x reaction buffer, 2.5 µL MgCl₂ (25 mM stock solution), 2.5 µL dNTP (2 mM stock solution), 1 µL Hot start Taq DNA polymerase (1 U/µL stock solution), 0.75 µL primer (10 µM stock solution) and 5 µL DNA sample were added and the final volume was completed to 25 µL with distilled water. Amplification was performed under the following conditions: after an initial denaturation at 94°C for 10 minutes, 35 cycles at 94°C for 30 seconds, at 60°C for 30 seconds, at 72°C for 30 seconds and final extension at 72°C for 10 minutes (20,21). In order to enhance sensitivity, a 5-µL specimen taken from the first amplification product was amplified again with the same primers under the same conditions as in the first reaction (20,21). To evaluate amplification of target DNA, 10 µL-amplification products were analysed by electrophoresis on 1.5% agarose gel under 100 volt for one hour. Then gel was stained with

1 µg/mL ethidium bromide and visualized under UV light. PCR was repeated three times for each specimen, for confirmation of results.

Staining

PCR positive BAL specimens were subjected to staining with Weber's modified trichrome after cytocentrifugation and examined under light microscopy at a final magnification of (x) 1000 (14).

Statistical Analysis

Statistical analyses were made with SPSS 15.0. Chi-square test was used for assessing the difference in prevalences. Mann-Whitney U test and Fisher's exact test were used to compare differences between the patients with *Microsporidia* spp. and those without *Microsporidia* spp. in the study group. $p < 0.05$ was considered significant.

RESULTS

In the study group, there were 45 males (71%) and 18 females (29%) with a median age of 58 years (18-71 years). In the control group, there were 19 males (68%) and nine females (32%) with a median age of 63 years (27-66 years). Of 63 patients with iatrogenic immunosuppression, 38 were on anticancer treatment, 14 were on corticosteroid treatment (> 20 mg/day), eight were on anti-rejection treatment (azathioprine, cyclosporine, mycophenolate mofetil etc.) and three were taking other immunosuppressants (methotrexate, TNF-α inhibitors).

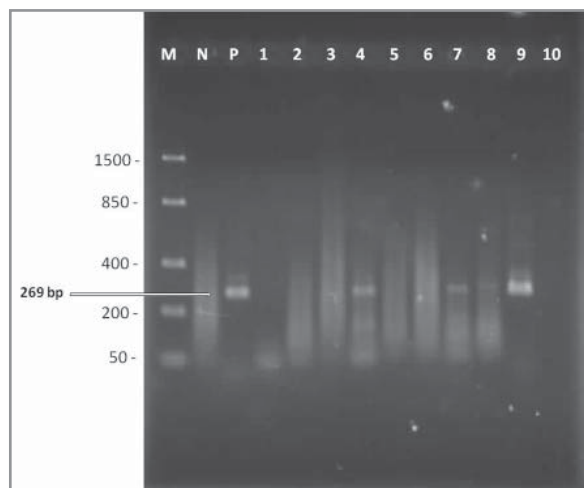


Figure 1. Agarose gel electrophoresis of PCR products of *Microsporidia* spp. SSU-rDNA gene. M: Marker, N: Negative control, P: *E. cuniculi* isolate (positive control), lanes 4, 7, 8, 9: positive patients and lane 1, 2, 3, 5, 6, 10: negative patients.

Microsporidia PCR positivities were detected in nine out of 63 immunosuppressed patients (14.2%), there was no positivity in immunocompetent patients (Figure 1). The prevalence of *Microsporidia* spp. was statistically higher in the immunosuppressed patients ($\chi^2 = 4.439$; $p = 0.035$).

The median age was higher in PCR positive immunosuppressed patients (median age: 66 years; range: 35-78 years) than in those with PCR negative

(median age: 57 years; range: 18-89 years), though the difference was not significant ($p = 0.06$) (Table 1). There was not a significant difference between PCR positivity and gender ($p = 0.255$). Characteristics of study and control groups are shown in Table 1.

Cytological investigation of the PCR positive BAL specimens revealed microsporidian spores only in one patient (Figure 2). This patient with multiple myeloma had no other microbial pathogens. Laboratory results

Table 1. Characteristics of study and control groups

	<i>Microsporidia</i> spp. PCR (+)	<i>Microsporidia</i> spp. PCR (-)	p value
Control Group (n=28)	n= 0	n= 28	-
Age [median (range)]	-	63 (27-66)	-
Female	-	9 (32.1%)	-
Male	-	19 (67.9%)	-
Diagnosis of patients			
Pneumonia		7 (25%)	
Chronic obstructive pulmonary disease (COPD)		6 (21.4%)	
Lung cancer		4 (14.3%)	
Interstitial lung disease (ILD)		2 (7.1%)	
Other		9 (32.2%)	
Study Group (n= 63)	n= 9 (14.2%)	n= 54 (85.8%)	
Age [median (range)]	66 (35-78)	57 (18-89)	0.060
Female	4 (44.4%)	14 (25.9%)	0.255
Male	5 (55.6%)	40 (74.1%)	
Underlying disease			
Hematological disease			
Lymphoma	1 (11.1%)	10 (18.5%)	1.000
Leukemia	1 (11.1%)	10 (18.5%)	1.000
Multiple myeloma	2 (22.2%)	1 (1.85%)	0.051
Hemophagocytic acute respiratory distress	0 (0%)	1 (1.85%)	
Solid organ tumors			
Lung cancer	1 (11.1%)	9 (16.7%)	1.000
Breast cancer	0 (0%)	2 (3.7%)	
Larynx cancer	0 (0%)	1 (1.85%)	
Organ transplantation			
Renal transplantation	2 (22.2%)	4 (7.4%)	0.201
Liver transplantation	0 (0%)	3 (5.6%)	
Inflammatory diseases/vacuities			
Rheumatoid arthritis	0 (0%)	3 (5.6%)	
Wegener's granulomatosis	1 (11.1%)	3 (5.6%)	0.469
Mixed connective tissue disease	0 (0%)	2 (3.7%)	
Other pulmonary diseases			
COPD	1 (11.1%)	2 (3.7%)	0.142
ILD	0 (0%)	2 (3.7%)	
Hypersensitivity pneumonitis	0 (0%)	1 (1.85%)	

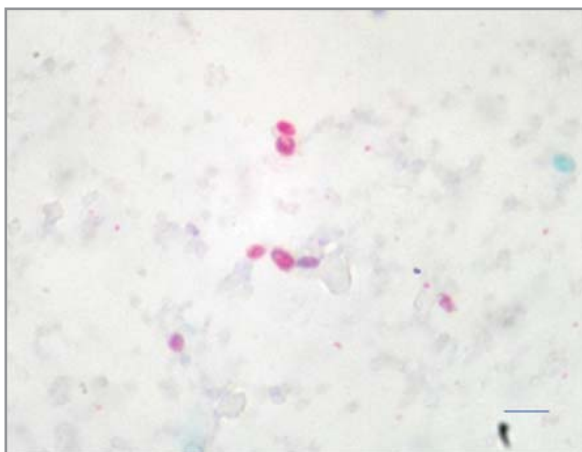


Figure 2. *Microsporidia* spp. spores stained with Weber's modified trichrome stain viewed by light microscopy (magnification x 1000). Bar, 5 µm.

from eight patients without microsporidian spores but PCR positivity revealed *Mycobacterium tuberculosis* in one patient, *Klebsiella pneumonia* in one patient and *Pneumocystis jirovecii* in five patients. One patient did not have any other etiological agents. Demographic/clinical characteristics, radiology and laboratory results of the PCR positive patients are shown in Table 2.

DISCUSSION

It is known that *Microsporidia* spp. cause severe systemic infections in immunosuppressed patients but there is little epidemiological evidence for pulmonary microsporidiosis. It is thought that many possible infections might be overlooked due to not only insufficient focus on microsporidiosis in the differential diagnosis of infections but also difficulties in detection of *Microsporidia* spp. in laboratory investigations (1-4).

Most reports about pulmonary microsporidiosis have been stated as case reports in immunosuppressed patients. It is generally observed in patients with AIDS and transplant recipients, may vary from asymptomatic colonization to death from cardiopulmonary failure (7-10,22-26). On the other hand there has been only one study on the prevalence of pulmonary microsporidiosis. Lobo et al. investigated presence of *Microsporidia* spp. in BAL specimens from 150 HIV patients and 50 non-HIV patients with PCR in Portugal (27). Although there was no positivity in non-HIV patients, they reported that two HIV patients had *Microsporidia* spp. So far, only one article has been published in our country

about pulmonary microsporidiosis. Yazar et al. reported that a myeloblastic leukemia (AML-M3) patient presenting with fever, cough, dyspnea and sputum had microsporidian spores in BAL specimens (26). In this study, for the first time, the prevalence of pulmonary microsporidiosis was investigated in Turkey. As far as we know it is the first report which conducted in immunosuppressed patients without HIV. The results showed that 14.2% of the iatrogenically immunosuppressed patients had pulmonary microsporidiosis.

When the literature data about microsporidiosis prevalence analyzed, it was found that researches in our country had mostly dealt with intestinal microsporidiosis. Intestinal microsporidiosis prevalence have been reported between 1.05-69.9 % in patients with different immune status from different geographic regions (11-19). The results of these studies show that we often come across this zoonotic pathogen. This situation may be one of the reasons of high pulmonary microsporidiosis prevalence that we found in our study.

Pulmonary microsporidiosis has been thought to be due to dissemination of gastrointestinal or urinary tract infections rather than an external transmission (1,2,6). Initiation of pulmonary symptoms following diarrhea due to *E. bienensei* in patients with AIDS and isolation of the same causative agent in the patients' pulmonary specimens contributes to the above mentioned view (1,22). This study was directed towards detection of *E. cuniculi*, *E. bienensei*, *E. hellem* and *E. intestinalis*, most frequently isolated species in human infections. By using common primers, SSU-rDNA gene of the parasites were amplified. Unfortunately, since genotyping could not be performed to confirm the types of species, all PCR positive specimens were considered as having *Microsporidia* spp. in general. However since this is a retrospective study, urine and feces specimens from the patients with *Microsporidia* PCR positivity could not be accessed and could not be evaluated for *Microsporidia* spp.

The symptoms fever, cough, wheezing, dyspnea and imaging signs such as lobar consolidation are not specific for clinical diagnosis of microsporidiosis (1,5-10,22). Researchers had to make their decisions regarding the other pathogens found in BAL specimens. Scaglia et al. reported two AIDS patients

Table 2. Clinic, radiologic and laboratory features of patients found to have *Microsporidia* sp. on PCR

Patient no	Age	Gender	Diagnosis	Symptom	Immunosuppressive treatment	Radiological findings at HRCT*	Weber's trichrome	Other laboratory results	Outcome of patient
1	74	F	COPD/congestive heart failure	Cough, respiratory distress, fever	Prednisolone (> 20 mg/day for 6 weeks)	Bilateral diffuse ground-glass opacities	-	<i>P. jirovecii</i> PCR +	Discharged
2	35	F	Chronic renal failure/renal transplantation	Respiratory distress, fever	Cyclosporine, mycophenolate, mofetil, azathioprine	Bilateral diffuse interstitial opacities	-	<i>P. jirovecii</i> PCR +	Exitus
3	66	M	Lung cancer	Respiratory distress, tachycardia	Cisplatin + etoposide/taxotere/navelbin	Not determined	-	<i>P. jirovecii</i> PCR +	Exitus
4	58	M	Acute myeloblastic leukemia-m7	Cough, hemoptysis	High doses of cytarabine	Cavitational lesions at right upper lung segment	-	Isolation of <i>M. tuberculosis</i>	Discharged
5	73	M	Multiple myeloma	Cough, respiratory distress, fever	Cyclophosphamide, bortezomib and dexamethasone	Bilateral diffuse pulmonary opacities and extensive consolidations areas	+	-	Exitus
6	65	F	Chronic renal failure/renal transplantation	Cough, sputum	Mycophenolate, mofetil, azathioprine, prednisolone	Diffuse centrilobular ground-glass opacities	-	<i>P. jirovecii</i> PCR +	Discharged
7	78	M	Multiple myeloma	Respiratory distress	Cyclophosphamide, bortezomib and dexamethasone	Bilateral lobar consolidation at inferior segments	-	Isolation of <i>K. pneumonia</i>	Exitus
8	71	F	Wegener's granulomatosis/chronic renal failure	Cough, dyspnea	Prednisolone (> 20 mg/day)	Milimetric ground-glass opacities at right inferior lung segment	-	-	Discharged
9	50	M	Non-hodgkin lymphoma	Cough, sputum, fever respiratory distress	Rituximab-cyclophosphamide/vincristine/Prednisolone (R-CHOP)	Bilateral diffuse ground-glass opacities	-	<i>P. jirovecii</i> PCR +	Exitus

* HRCT: High Resolution Computed Tomography.

having pulmonary symptoms and *E. hellem* in their BAL specimens. One of these patient was evaluated as *Pneumocystis carinii* pneumonia (PCP) and the other as tuberculosis. Therefore, the presence of *E. hellem* was concluded as colonization (9). However, Orenstein et al. incriminated *E. cuniculi* for fatal pneumonia developing in an acute myeloid leukemia patient undergoing bone marrow transplantation when they could not isolate any other causative agent (8). del Aguila et al. detected *Mycobacterium avium* complex (MAC) and *E. bienewisi* in BAL and sputum specimens from a patient with AIDS developing fever and productive cough while they were following the patient for intestinal microsporidiosis. The researchers achieved clinical improvement by offering treatment directed towards the two causative agents (23). The patients in the present study were prone to opportunistic infections due to immunosuppressive therapies. The hospital records of PCR positive patients showed that one patient had *M. tuberculosis* and another patient had *K. pneumoniae*. Responses to appropriate antibiotic treatments in these patients suggested that the patients had *Microsporidia* spp. colonization. Five *Microsporidia* PCR positive patients were also included in another study about *P. jirovecii* colonization and nested PCR revealed *P. jirovecii* DNA (unpublished data). Since they haven't received anti-PCP treatment, their responses to treatment could not be evaluated. It remained unclear whether the patients had PCP or *P. jirovecii* colonization. As a result, it could not be revealed whether the symptoms were due to microsporidiosis and/or PCP. However, one of the PCR positive patients had *Microsporidia* spores in BAL specimen. Examination of BAL sediment was negative for *P. jirovecii* and other microbiological pathogens. He had nonspecific symptoms such as fever, cough and respiratory distress. On physical examination, there were widespread crepitan ralles in both lungs and in radiographic examinations, diffuse, bilateral pulmonary oposities and extensive consolidations areas were determined. The patient did not respond to empirical treatments and died. Pulmonary symptoms in this patient might be caused by *Microsporidia* spp. The last *Microsporidia* PCR positive patient who did not have any other microbiological agents in BAL specimen did not receive specific treatments for microsporidiosis. The

patient recovered as a result of empirical and supportive treatment and was discharged. Therefore, it was considered as *Microsporidia* spp. colonization.

CONCLUSION

The present study is the first to investigate the prevalence of pulmonary microsporidiosis in immunosuppressed patients in Turkey. The results of the study showed that *Microsporidia* spp. should be taken into consideration in the differential diagnosis of pulmonary infections in iatrogenically immunosuppressed patients and the diagnosis by conventional staining is inadequate and should be supported by molecular methods.

REFERENCES

1. Didier ES, Weiss LM. Microsporidiosis: current status. *Curr Opin Infect Dis* 2006;19:485-92.
2. Didier ES, Weiss LM. Microsporidiosis: not just in AIDS patients. *Curr Opin Infect Dis* 2011;24:490-5.
3. Mathis A, Weber R, Deplazes P. Zoonotic potential of the microsporidia. *Clin Microbiol Rev* 2005;18:423-45.
4. Sancak B, Akyön Y. Microsporidia: Genel özellikleri, enfeksiyonları ve laboratuvar tanısı. *Mikrobiyol Bul* 2005;39:513-22.
5. Kelkar R, Sastry P, Kulkarni S, Advani S. Pulmonary microsporidial infection in a patient with CML undergoing allogeneic marrow transplantation. *Bone Marrow Transplant* 1997;19:179-82.
6. Botterel F, Minozzi C, Vittecoq D, Bourée P. Pulmonary localization of *Enterocytozoon bienewisi* in an AIDS patient: case report and review. *J Clin Microbiol* 2002;40:4800-1.
7. Scaglia M, Sacchi L, Croppo GP, da Silva A, Gatti S, Corona S, et al. Pulmonary Microsporidiosis due to *Encephalitozoon hellem* in a patient with AIDS. *J Infect* 1997;34:119-26.
8. Orenstein JM, Russo P, Didier ES, Bowers C, Bunin N, Teachey DT. Fatal pulmonary Microsporidiosis due to *encephalitozoon cuniculi* following allogeneic bone marrow transplantation for acute myelogenous leukemia. *Ultrastruct Pathol* 2005;29:269-76.
9. Scaglia M, Gatti S, Sacchi L, Corona S, Chichino G, Bernuzzi AM, et al. Asymptomatic respiratory tract Microsporidiosis due to *Encephalitozoon hellem* in three patients with AIDS. *Clin Infect Dis* 1998;26:174-6.
10. Martínez-Girón R, Esteban JG, Ribas A, Doganci L. Protozoa in respiratory pathology: a review. *Eur Respir J* 2008;32:1354-70.
11. Atambay M, Karaman U, Daldal N, Çolak C. İnönü Üniversitesi Turgut Özal Tıp Merkezi Parazitoloji Laboratuvarına gelen erişkin hastalarda *Microsporidium* görülme sıklığı. *Türkiye Parazit Derg* 2008;32:113-5.
12. Usluca S, Aksoy Ü. İmmün sistemi baskılanmış bir çocukta *Microsporidium* spp. enfeksiyonunun polimeraz zincir reaksiyonu ile tanımlanması. *Mikrobiyol Bul* 2010;44:679-83.

13. Karaman U, Atambay M, Daldal N, Çolak C. Kanser tanısı almış hastalarda *Microsporidium* görülme sıklığı. *Türkiye Parazit Derg* 2008;32:109-12.
14. Karaman Ü, Daldal N, Atambay M, Çolak C. The epidemiology of *Microsporidiasis* in humans (Malatya sample). *Turk J Med Sci* 2009;39:281-8.
15. Karaman Ü, Şener S, Çalık S, Şaşmaz S. Akut ve kronik ürtikerli hastalarda *Microsporidia* pozitiflik oranı. *Mikrobiyol Bul* 2011;45:168-73.
16. Türk S, Doğruman Al F, Karaman U, Kuştimur S. Investigation of *Microsporidia* prevalence by different staining methods in cases of diarrhea. *Mikrobiyol Bul* 2012;46:85-92.
17. Turgay N, Unver-Yolasiğmaz A, Oyur T, Bardak-Özdemir S, Töz S. Monthly distribution of intestinal parasites detected in a part of western Turkey between May 2009-April 2010-results of acid fast and modified trichrome staining methods. *Türkiye Parazit Derg* 2012;36:71-4.
18. Hamamcı B. Farklı hasta gruplarında *Enterocytozoon bienewisi*'nin (Protozoa, *Microsporidia*) araştırılması. Doktora Tezi, Kayseri: Erciyes Üniversitesi Sağlık Bilimleri Enstitüsü; 2013.
19. Hamamcı B, Çetinkaya Ü, Berk V, Kaynar L, Kuk S, Yazar S. Kemoterapi Alan Kanserli Hastalarda *Encephalitozoon intestinalis* ve *Enterocytozoon bienewisi* Prevalansı. *Mikrobiyol Bul* 2015;49:105-13.
20. Samie A, Obi CL, Tzipori S, Weiss LM, Guerrant RL. *Microsporidiasis* in South Africa: PCR detection in stool samples of HIV-positive and HIV-negative individuals and school children in Vhembe district, Limpopo Province. *Trans R Soc Trop Med Hyg* 2007;101:547-54.
21. Fedorko DP, Nelson NA, Cartwright CP. Identification of *microsporidia* in stool specimens by using PCR and restriction endonucleases. *J Clin Microbiol* 1995;33:1739-41.
22. Teachey DT, Russo P, Orenstein JM, Didier ES, Bowers C, Bunin N. Pulmonary infection with *microsporidia* after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2004;33:299-302.
23. del Aguila C, Lopez-Velez R, Fenoy S, Turrientes C, Cobo J, Navajas R, et al. Identification of *Enterocytozoon bienewisi* spores in respiratory samples from an AIDS patient with a 2-year history of intestinal *Microsporidiasis*. *J Clin Microbiol* 1997;35:1862-6.
24. Hocevar SN, Paddock CD, Spak CW, Rosenblatt R, Diaz-Luna H, Castillo I, et al. *Microsporidiasis* acquired through solid organ transplantation: a public health investigation. *Ann Intern Med* 2014;160:213-20.
25. Mohindra AR, Lee MW, Visvesvara G, Moura H, Parasuraman R, Leitch CJ, et al. Disseminated *microsporidiasis* in a renal transplant recipient. *Transpl Infect Dis* 2002;4:102-7.
26. Yazar S, Eser B, Yalçın Ş, Şahin İ, Koç AN. A case of pulmonary *Microsporidiasis* in an acute myeloblastic leukemia (AML)-M3 patient. *Yonsei Med J* 2003;44:146-9.
27. Lobo ML, Xiao L, Antunes F, Matos O. *Microsporidia* as emerging pathogens and the implication for public health: a 10-year study on HIV-positive and -negative patients. *Int J Parasitol* 2012;42:197-205.