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ORIGINAL ARTICLE

Molecular Characterization of Intestinal Microsporidia in Immunocompromised Patient in THI-QAR Province

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ABSTRACT

Introduction: Immunocompromised host has one or more defects in the normal defense mechanisms that protect against infectious agents. Microsporidia are group of protozoa characterized via obligate intracellular and spore formation, detected in many hosts. Up till now, 1300 microsporidia species or more, related to 150 genera, were, microsporidiosis caused by microsporidia.

Objectives: To conduct Molecular characterization of intestinal microsporidia in immunocompromised patient in Thi-Qar province cases.

Patients and Methods: Totally, two hundred stool samples were collected and subdivided into 185 cancer patients and 15 organ transplantation patients. Samples were examined for microsporidiosis by light microscopy smears stained by Chromotrope Kenyon stain and by PCR techniques.

Results: using Polymerase chain reaction technique (PCR), microsporidia were detected in eleven sample out of 116 sample that were positive by microscopic examination and Chromotrope Kenyon stain. Positive sample by PCR were sent for sequencing and the result showed that the samples of the current study were similar to three microsporidia, Enterocytozoon bieneusi, Encephalitozoon cuniculi and Encephalitozoon intestinalis.

Conclusion: The present study confirmed the presence of Microsporidia infection is among immunocompromised patients.

Keyword: Molecular, immunocompromised , microsporidia, PCR

INTRODUCTION

Immunocompromised host has one or more defects in the normal defense mechanisms that protect against infectious agents. These defects predispose the person to increased risk the severe life-threatening infections (1). Protozoa have come to the predominant parasitic infection in immunocompromised patients(2). Among parasitic infections, microsporidiosis caused by microsporidia is now constituted a major disease problem in the seriously immunocompromised population and HIV infection patients (3).

Microsporidia are group of protozoa characterized by obligate intracellular and spore formation, detected in many hosts. Up till now, 1300 microsporidia species or more, related to 150 genera, were detected (4). Species human infection is Enterocytozoon bieneusi together with three species of genus Encephalitozoon, Encephalitozoon hellem, Encephalitozoon intestinalis and Encephalitozoon cuniculi. The spores are quite small, approximately in size ranging from 1-4 µ about the size of Escherichia coli. One unique feature of these spores is a polar tube, which is coiled with the spore and extrudes to attach to human cells on infection.

The organisms are transmitted from human to human via the fecal-oral route and transmitted mainly through contaminated food, water, direct contact with dog feces and sexual contact (5).

Microsporidia parasite can be detected firstly based on the clinical symptoms, including: weight loss, diarrhea, stomach discomfort and fever, in the affects people with immunodeficiency, AIDS, cancer, children and pregnant women (7, 6). Furthermore, using different laboratory methods depends on direct visualization of spores in fecal samples by light microscopy using gram chromo trope Kenyon staining and Fluorescent microscopy using Calcofluor White staining technique. However, both techniques cannot differentiate the species of microsporidia(8).

Polymerase chain reaction (PCR) is a highly sensitive and specific technique successfully used to differentiate the species of microsporidia.

This study was considered the second study carried out in Thi-Qar province in order to molecularly characterize of intestinal microsporidia in immunocompromised patient in Thi-Qar province.

The Aim of Study:

1) To confirm the initial identification of potential microsporidia species using molecular techniques.
2) To sequence the PCR products in order to characterize the recorded Microsporidia.

PATIENTS AND METHODS

Stool Samples Collection: Two hundred stool samples were collected from patients with cancer, Al Haboubi Hospital and Rafi Hospital, the Oncology Cancer Center in AL- Nasiriya city, for period from July 2022 to October 2022. The ages of the patients from whom samples were collected ranged between 5> and 70< years. The samples were transferred using ice box and kept in the Laboratory of Medicine, and placed inside the freezer for preserving the samples to prevent them from spoiling. Isolated samples were taken from 115 women and 60 men patients with cancer. From organ transplantation patients, nine women and six men were examined.

Molecular studies: DNA extraction from stool samples was performed using Presto™ Stool DNA Extraction Kit (Geneaid, Taiwan).

Polymerase Chain Reaction (PCR): The PCR technique was performed for detection Encephalitozoon hellem, Encephalitozoon intestinalis, and Encephalitozoon cuniculi along with Enterocytozoon bieneusi. based on ssrRNA gene from Human stool samples.

Forward Primer (PMP1): 5'-CACCAGGTTGATTCTGCCTGAC-3'
Reverse Primer (PMP2): 5'-CCTCTCCGGAACCAAACCTG-3'

This primer pair (250-350bp) was provided by Scientific Researcher Co. Ltd., Iraq.

RESULTS

Polymerase Chain Reaction (PCR): percentage of infected and non-infected patients with microsporidia by using PCR: The PCR method is reliable and frequently used to confirm parasite infections. In the present study, 116 patients stool samples were examined for the presence of microsporidia parasite using PCR technique and the result showed that 11(9.5%) were infected with microsporidia while, 105(90.5%) stool samples were negative Figure (4-1, 4-2).

In the table (4-6), there was statistically significant differences (P. value= <0.001, OD= 8.00, 95% CI= 3.94-16.6) for Cancer patients to PCR technique was showing more accuracy than microscopic technique (Chromotrope Kenyon stain), whereas to organ transplant patients no statistically significant differences (P. value= 0.999, OD= 0.727, 95% CI= 0.0836-5.06) were detected between PCR technique and microscopic technique (Chromotrope Kenyon stain)

Result of DNA Sequence: The DNA sequencing method was carried out to genetic relationship analysis in small subunit ribosomal RNA gene in local Encephalitozoon spp. isolates.

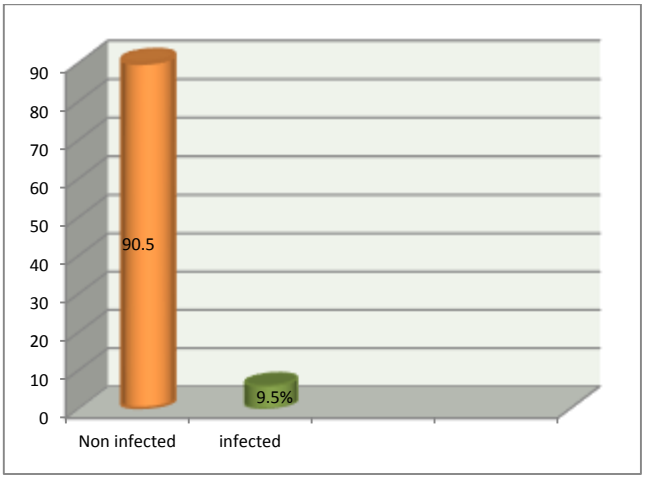

Figure 1: percentage of infected and non-infected patients with microsporidia by PCR.

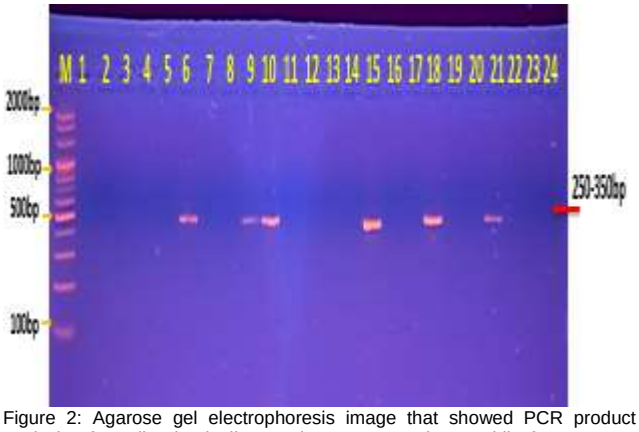

Figure 2: Agarose gel electrophoresis image that showed PCR product analysis of small subunit ribosomal RNA gene Microsporidia from Human stool samples. M (Marker ladder). Lane (1-24) Showed some positive subunit ribosomal RNA gene at (250-350bp) product size.

Table 1: Comparison between microscopic and PCR according infection.

Type of Examination	Cancer patients						Organ transplant patients					
	Total	Positive	Negative	OD	95% CI	P-value	Total	Positive	Negative	OD	95% CI	P-value
Chromo trop Kenyon stain	185	77(41.62%)	108(58.38%)	8.00	3.94 to 16.6	<0.001	15	4(26.67%)	11(73.33%)	0.727	0.0836 to 5.06	0.999
PCR	110	9 (8.18%)	101(91.82%)				6	2 (33.33%)	4(66.67%)			

Table 2: The NCBI-BLAST Homology Sequence identity percentage between local Encephalitozoon sp. isolates and NCBI-BLAST closed genetic related local Encephalitozoon species isolates.

Local isolates	Accession number	Homology sequence identity (%)		
		NCBI related Encephalitozoon spp.	Accession number	Identity (%)
IQN.No.1	OQ023039.1	Enterocytozoon bieneusi	FR729098.1	99.29%
IQN.No.2	OQ023040.1	Enterocytozoon bieneusi	FR729098.1	99.28%
IQN.No.3	OQ023041.1	Encephalitozoon cuniculi	KF169729.1	99.57%
IQN.No.4	OQ023042.1	Enterocytozoon bieneusi	FR729098.1	99.57%
IQN.No.5	OQ023043.1	Encephalitozoon intestinalis	EU436735.1	99.86%
IQN.No.6	OQ023044.1	Enterocytozoon bieneusi	FR729098.1	99.50%

DISCUSSION

Infection with the microsporidia parasite using the PCR technique: In the current study, 200 stool samples were collected from patients with cancerous tumors and organ transplantation, the results of staining showed 81 positive samples infected with microsporidia parasite, after which the positive samples were examined by PCR technique using the diagnostic primer. For the parasite Encephalitozoon intestinalis, 11 positive stool samples appeared during this examination. Results of a study using PCR technique reported a detection rate of microsporidiosis of 17% in cancer patients in Egypt. In Iran, a study was aimed to E. amplify the DNA of bieneusi and E. intestinalis and the result was 10.5% (20/199) that was similar to the current study. In south Africa, 170 stools samples were collected. Microsporidia presence was 56 (32.9 %) by RT-PCR. This studies disagree with the current study. In Iraq, 83 samples were examined, the percentage was 18%. This study was similar with the current study (9).

In a study conducted in Malaysia, a total of 289 stool samples from immunocompromised patients were examined for the presence of microsporidia spores by the microscopy technique. Out of the 93 positive stool samples by microscopy, 45 (15.5%) were successfully amplified by PCR. Enterocytozoon bieneusi DNA was amplified in all 45 samples by PCR. No E. intestinalis was detected by PCR among immunocompromised patients. This study was similar with the present study (10).

A study in Addis Ababa, Ethiopia showed infection with the microsporidia parasite using PCR method, the results were 39(16%) positive out of 243 stool samples. This study in slightly disagreed with present study regarding the rate of infection with microsporidia (11).

CONCLUSION

1) The present study showed that the Microsporidia infection is abundant among immunocompromised patients.
2) Immunocompromised like cancer disease makes patients more susceptible to parasitic infection.
3) The present study showed the presence of Microsporidia infection among organ transplantation kidney patients.

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