MICROSPORIDIOSIS IN MALIGNANCY AFFECTED PATIENTS IN MOSUL, IRAQ

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ABSTRACT

Over nine months period, the incidence of microsporidiosis in patients receiving treatment for malignancy was investigated. A total of 53 patients (27 males and 26 females) were involved in this study. All patients had stool analysis for *microsporidium* spp. stools from 53 control groups (30 males and 32 females) have also been investigated. *Microsporidium* spp. spores were detected in stools of 5 males and 3 females among patients treated with anti-cancer therapy. Three patients particularly affected with leukemia showed positive results. It was concluded that advanced stages of malignancy affected patients who received anti-cancer therapy are at risk from infection with this pathogen, while working physicians showed little awareness concerning this opportunistic infection.

INTRODUCTION

The immunocompromised host has one or more defects in the normal defense mechanisms that protect against infectious agents. These defects predispose the individual to an increased risk of severe life-threatening infections.[1] Protozoa have come to be the predominant parasitic infection in immunocompnomised patients.[2] Among these parasitic infections, microsporidiosis caused by Enterocytozoon *bieneusi* is now constituted a major disease problem in the seriously immunocompromised population and HIV-infected patients.[3] Human microsporidial infections have been documented globally. The sources of *microsporidia* infecting humans and their modes of transmission are uncertain. Ingestion of spores is the most probable source of transmission.[4] Although microsporidiosis appears to occur most frequently in persons infected with HIV, it is emerging as an infection in otherwise immunocompromised hosts as well as immunocompetent individuals.[5,6] Patients with severe cellular immunodeficiency appear to be at the highest risk for developing microsporidial disease.[4] The microsporidium *E. bieneusi* has been associated with diarrhea in recipients of organ transplants and with self-limited watery diarrhea in immuno-competent adults as well as in children.[7] It has also been reported in up to 33% of HIV infected patients with unexplained chronic diarrhea.[8] Diagnosis of microsporidiosis was made by light microscopy[9,10] of stool samples, while definitive species identification of microsporidia is made by electron microscopy, antigenic analysis or molecular analysis.[4,9,11] Microsporidiosis may spread from the gut to the upper respiratory tract and kidneys, but it usually responds well to treatment with albendezole.[12] It can also cause a wide range of illness from diarrhea to involvement of CNS,eyes, viscera and muscles.[13] As far as we know, this is the first study of microsporidiosis in Mosul-Iraq, conducted among malignancy affected patients who were regarded as immuno-suppressed patients due to disease pathogenicity or as a consequence of their anti-cancer regimens like radiation or chemotherapy.

MATERIAL and METHODS

Subjects:

This study was conducted in Mosul city (IRAQ), over a period of nine months from August 2001 to April 2002 in Ibn-Sina teaching hospital, Al-Zahrawi teaching hospital, Hazem Al-Hafeth hospital for nuclear medicine and Al-Razi general hospital. Fifty three malignancy affected patients (27 males and 26 females) were studied. These subjects who were presumably immunocopromised patients received cytotoxic drugs and/or radiation therapy. Their age varied from 6 to > 55 years. The control group was composed of 53 (apparently healthy) individuals (30 males and 23 females), their age range from 5 to > 57 years. None had any past or present history suggesting malignant diseases, nor had they received any type of anti-cancer therapy. They were either patient's relatives or subjects who were randomly selected.

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Specimens:

Stool samples were collected from both patients and controls. The preservatives used for stool samples were sodium acetate, acetic acid formaldehyde and potassium dicromate (K_2CrO_4). Two stool samples were obtained from each subject. The first sample was obtained before receiving the anti-cancer therapy, and the second sample obtained after the patient had received the therapy. Only one stool sample was obtained from each individual of control group. Fecal samples were examined microscopically (wet mounts) and macroscopically by the direct method using normal saline and lugol's iodine. Zinc sulfate flotation technique was also used as a concentration method for processing the stool samples. The Weber technique (Trichrome stain) modified by Deluol and Mahoun for *microsporidia*[6,14] was used to detect microsporidic spores, which appeared small, oval shaped, stained pink in colour with distinct colourless vacuoles of varying coloured spores appeared very well against the Malachite green background.

RESULTS

The types of malignant diseases among tested patients (immunocopromised) are presented in (Table-1). The results showed that breast carcinoma, leukemia and lymphoma were the most prevalent malignant diseases among our patients (35.85%, 26.42% and 13.21% respectively).

Table 1. Types of malignant disease among tested patients (immunocompromised).

Malignancy/type	Patient	
	No.	%
Breast carcinoma	19	35.85
Leukemia	14	26.42
Lymphoma	7	13.21
Laryngeal carcinoma	5	9.43
Metastasis carcinoma	4	7.55
Colon carcinoma	4	7.55
Total	53	100

The clinical symptoms among tested patients before treatment and after treatment showed that all patients suffered from weight loss, while fever and diarrhea were noticed in 79.61% and 45.28% of treated patients respectively.

Table 2. Clinical symptoms among tested patients before treatment and after treatment.

Symptoms	Patients			
	Untreated		Treated	
	No.	%	No.	%
Weight loss	53	100	53	100
Fever	38	79.61	19	35.85
Diarrhoea	24	45.28	5	9.43
Abdominal pain	21	39.62	12	22.64
Vomiting	9	16.98	1	1.87
No symptoms	3	5.66	-	-

Three types of anti-cancer therapy were given to investigated patients. Twenty one patients (39.62%) received cytotoxic drugs only, while 17 patient (32.08%) were subjected to radiation therapy only, and fifteen patients (28.30%) treated with both cytotoxic and radiation therapy (Table-3).

Table 3. Types of anti-cancer therapy among tested patients.

Therapy / type							
Cytotoxic		Radiation		Both		Total	
No.	%	No.	%	No.	%	No.	%
21	39.62	17	32.08	15	28.30	53	100

The diagnosis of microsporidiosis was based on the detection of *microsporidia* spores in stools of both patients and controls. Our results presented in (Table-4) showed that 8 patients (5 males and 3 females) of 53 treated patients were infected with *microsporidia* spores (15.09%). While *microsporidia* spores have been detected in only 5 patients of untreated group (9.43%). No *microsporidia* spores have been observed in stools of control group.

Table 4. Mircrosporidia spp. spores in stools of malignant patients according to the type of anti-cancer therapy.

Therapy/ Regimen	Patient / group (n = 53)					
	Untreated			Treated		
	No.	%	% (fromtotal)	No.	%	% (fromtotal)
Cytotoxic	3	37.5	5.66	1	20	1.89
Radiation	1	12.5	1.89	-	-	-
Both	4	50.0	7.55	4	80	7.55
Total	8	100	15.09	5	100	9.43

According to the type of anti-cancer regimen, the spores of *microsporidia* spp. were detected in stools of 4 patients who had received both type of anti-cancer therapy (cytotoxic and radiation), and in 3 patients who received cytotoxic drugs only. Leukemia affected patients showed the highest rate of infection with microsporidia where 3 patients who had suffered from this type of malignancy were found positive from the total number.

Among treated patients, those with advanced stages of malignancy were found with a high percent of infection with *microsporidia* (9.43%) than those with early stage of malignancy (5.67%). This pattern was also true among untreated patients.

Table 5. Microsporidia spp. spores in stools of malignant patients according to the stage of malignancy.

Malignancy/ Stage	Patient / group (n = 53)					
	Treated			Untreated		
	No.	%	% (from Total)	No.	%	% (from total)
Advanced stage	5	62.5	9.43	3	60	5.66
Early stage	3	37.5	5.67	2	40	3.77
Total	8	100	15.09	5	100	9.43

DISCUSSION

In the present study *Microsporidia* spp. was found among patients who received anti-cancer therapy. It has been reported that fifty eight percent of patients receiving anti-cancer therapy (cytotoxic drugs) were found to have enteric parasites with 8% of *Cryptosporidium* infection.[15] Both cytotoxic drugs and therapeutic ionizing radiation are considered the chief immunosuppressive agents used by the malignant patients.[16] Parasites were also detected among 7.6% of 105 immunocompromised patients. The immunosuppression was due to cytotoxic drugs, leukemia and malnutrition.[17] In our study the light microscopic diagnosis of faeces from cancer patients for microsporidiosis was determined. Light microscopy (LM) has been used successfully for diagnosis of biopsies from AIDS patients.[18] Those authors concluded that it should be possible to render the diagnosis of intestinal microsporidiosis by (LM) in most cases, while Transmission Electron Microscopy may be needed for minority of cases with low parasite burden. Stool samples were also analyzed by both (LM) and (PCR) techniques, to detect microsporidiosis in patients with AIDS.[19] However, new simple staining method using modified trichrome stain[6] for faeces and other body fluids have facilitated clinical diagnosis as well as drug evaluation and epidemiological studies. The isolation of microsporidial spores from body fluids, including stools, urine, respiratory secretions and duodenal aspirates,[20] suggesting that these may serve as potentially infective sources for person to person transmission. A study of potentially human pathogenic protozoan parasites in water contaminated with human and animal faeces, showed that 23% of the samples were positive for human pathogenic *microsporidia* species.[21] *Microsporidia* are present worldwide, but accurate assessment of their prevalence in general population has not been established.[22] To the best of our knowledge among data available from literatures in Iraq. This is the first report which shows malignancy, affected patients at risk of acquisition of microsporidal infection. Although *microsporidium* species were initially believed to be a cause of AIDS related diarrhoea, they were subsequently shown to be pathogenic in the conjunctiva, liver, peritoneal cavity and lungs.[23] Our patients suffered from various clinical symptoms including weight loss and diarrhea. The result showed that persistent diarrhea was an important problem in our patients. Some experienced a significantly higher diarrhoeal

burden during the course of anti-cancer therapy. A clear association between the presence of *microsporidia* and diarrhea was established by Coyle *et al*.,[7] who found that 44% of HIV infected patients with diarrhea were infected with *microsporidia* whereas only 2.3% of the patients without diarrhea were infected. Another study of 73 HIV-infected patients with microsporidiosis indicated that 55% of patients had persistent diarrhea after 6 months and 51% showed weight loss.[21] Microsporidiosis was also found to be a common cause of chronic diarrhea, malabsorption and weight loss in AIDs patients and in cases unexplained diarrhea.[8,18,22] The epidemiology of human microsporidiosis is poorly understood and environmental factors affecting transmission of the organism have not been fully elucidated, which need further investigation.

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REFERENCES

1. **Shaaban WA. Omar, T. Protozoan infections in immuno-compromised infants and children. Mid. East. Peadiatr. 1999; 4(2): 61-65.**
2. **Petri W.W.A. Protozoan parasites that infect the gastrointestinal tract. Curr, Opin, Gastroentrol. 2000; 16(1): 18-23.**
3. **Deluol AM, Cenac, JLes. Microsporidioses. Anu. Biol. Clin. 1994; 52: 37-44 (in French with English summary).**
4. **Bryan, RT. Microsporidiosis as an AIDS-related opportunistic infection. Clin. Infect. Dis. 1995; 21 (suppl. 1): 562-563.**
5. **Raynaud, L. Delbac, F. Broussolle, V. et al., Identification of *Encephslitozoon intestinalis* in travelers with chronic diarrhea by specific PCR amplification. J. Clin. Microbiol. 1998; 36: 37.**
6. **Deloul, A.M. Color atlas of parasitology (1999), Vol. 2, Format utile, France, 34-63.**
7. **Coyle CM, Wittner M, Weiss LM, et al. Prevalence of microsporidiosis due to *Enterocytozoon bieneusi* and *Encephalitozoon (septata) intestinalis* among patients with AIDS-related diarrhoea. Clin. Infect. Dis. 1996; 23(5): 1002-1006.**
8. **Eeftinck Schattenkenk, JK, Van Cool T, et al. Clinical significance of small intestinal microsporidiosis in HIV-infected individuals. Lancet, 1991; 337: 895-898.**
9. **Franzen C. Muller A. et al. Detection of Microsporidic (*Enterocytozoum bienensi*) in intestinal biopsy specimens from human immunodeficiency virus-infected patients by PCR. J. Clin. Microbiol. 1995; 33:2294-2296.**
10. **Kotler DP. Giang TT et al. Light microscopic diagnosis of microsporidiosis in patients with AIDS. Am. J. Gastroenterol. 1994; 89(4): 540-544.**
11. **Franzen, C.; Schwentz, DA. *Et al*., Immunoliogically confirmed disseminicted asymptomatic *Encephalitozoon cuniculi* infection of the gastrointestinal tract in a patient with AIDS. Clin. Infec. Dis. 1995; 21: 1480-1485.**
12. **Dore, GJ, Marriott, DJ, *et al*. Disseminated microsporidiosis due to *Septata intestinalis* in nine patients infect with the human immunodeficiency virus: response to therapy with albendazole. Clin. Inf. Dis. 1995; 21: 70-76.**
13. **Fantary, L. Gastrointestinal infections in the immunocompromised host. Curr. Opin. Gastroent. 2000; 16(1): 45-50.**
14. **Weber, R., Bryan, R.T. et al., the enteric opportiunistic infections working group. Improved light microscopical detection of *microsporidia* spores in stools and duodenal aspirates. N. Engl. J. Med.1992; 3263: 161-166.**
15. **Ali, N. Prevalence of cryptosporidiosis with zoonotic aspects (1998). M. Sc. thesis, University of Basrah.**
16. **Roitte I., Brostoff. J. Essential immunology 2001. (6th ed.) Mosby Int. Ltd. London: 243-257.**
17. **Al-Yassin ST. A study of *Cryptosporidium* in immunocompromised patients, 1999. M. Sc. Thesis, Medical College. University of Tikrit.**
18. **Kotler DP, Orenstein JM. Prevalence of intestinal microsporidiosis in HIV-infected individuals referred for gastroenterological evaluation. Am. J. Gastroenterol. 1994; 89(11): 1998-2002.**
19. **Miao YM, Awad-Elkariem, FM. Eradication of *Cryptosporidia* and *Microsporidia* following successful antiretroviral therapy. J. Acquin. Immune Defic Synd. 2000; 25(2): 124-129.**
20. **Markell, EK, John, DT. Medical parasitology (8th ed.) W.B. Saunders Company, Philadelphia; Pennsylvania 19106.**
21. **Dascomb K, Klark R. et. al., Natural history of intestinal microsporidiosis among patients infected with human immunodeficiency virus. J. Clin. Microbiol. 1999; 37: 3421-3424.**
22. **Conteas CN, Berlin OG, et al. Therapy for human gastrointestinal microsporidiosis. Am.J. Trop. Med. Hyg. 2000; 63(3-4):121-127.**
23. **Sheppand DC, Sullam, PM. *Microsporidium* species in pulmonary cavity lesions of AIDs patients infected with *Rhodococcus equi*. Clin. Infect. Dis. 1997; (25): 926-927.**