

Cryptosporidiosis in immunocompromised patients in the Islamic Republic of Iran

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Background and Purpose: Cryptosporidiosis is a parasitic zoonosis, which is prevalent all over the world. The manifestation of the disease is either self-limiting acute diarrhea in immunocompetent individuals, or potentially fatal chronic diarrhea in immunocompromised patients.

Methods: In this study, which was conducted in Tehran, 214 patients from ten health centers were investigated. Stool samples were collected, fixed and examined by three methods: acid-fast staining, auramin phenol fluorescence and direct fluorescence using monoclonal antibody.

Results: Overall, 1.4% of all patients and 6.3% of diarrheal patients were infected by *Cryptosporidium*. The results revealed three cases of cryptosporidiosis, including two cases of acquired immunodeficiency syndrome (AIDS) and one of acute myeloid leukemia (AML). The prevalence of infection in subjects with AIDS or AML who were suffering from diarrhea was 33.4% and 11.1%, respectively. The duration of disease in infected patients lasted for weeks, and was terminated by death in two AIDS patients. In the patient with AML, diarrhea lasted for 18 days, and stopped after discontinuation of immunosuppressive therapy.

Conclusions: Immunosuppressed people are at a significant risk of severe or even fatal *Cryptosporidium* infections.

Key words: Acquired immunodeficiency syndrome; Cryptosporidiosis; *Cryptosporidium*; Diarrhea; Immunologic deficiency syndromes; Iran; Leukemia, myeloid, acute

Introduction

Intestinal opportunistic parasitic infections are important causes of diarrhea, which is a serious health problem in tropical regions. *Cryptosporidium*, a protozoan parasite, can infect the microvillus borders of gastrointestinal epithelium of vertebrate hosts and is an important agent of diarrheal disease [1,2]. Human cases of *Cryptosporidium* were first reported in 1976 in association with diarrhea; one case in a healthy child and another in an immunosuppressed adult [3]. The human host range is broad and includes people with immunodeficiency, children in developing countries, and outbreaks among immunocompetent individuals [4].

Cryptosporidiosis displays many clinical symptoms, including watery diarrhea, epigastralgia, and weight loss [5]. Characteristically, the diarrhea is profuse and watery; it may contain mucus, but rarely blood and leukocytes. Other less common clinical features include nausea, vomiting and low-grade fever [6].

The infection is self-limiting in immunocompetent hosts, which readily clear this parasite, but it may cause persistent diarrhea and severe malabsorption in immunodeficient hosts [7-9]. The affected immunocompromised cases may have acquired immunodeficiency syndrome (AIDS), hypogammaglobulinaemia or be receiving immunosuppressive treatment [10].

The diagnosis of cryptosporidiosis rests on the identification the spherical oocyst or its components in stools or within biopsy specimens [4]. A variety of diagnostic options are available for the detection of *Cryptosporidium* in clinical stool samples. Auramin phenol fluorescence (APF) screening followed by

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modified acid-fast staining (AFS) is a sensitive and specific approach for the identification of *Cryptosporidium* oocysts in stools [4]. Moreover, infections in immunocompromised patients can be diagnosed by histological examination of biopsy materials, although fecal examination was mostly preferred [10].

It is now well known that immunosuppressed individuals are at higher risk for *Cryptosporidium* infections, and that carriage of the parasite is associated with diarrheal disease in most cases. Furthermore, in compromised individuals with diarrhea, the disease is much more severe and prolonged than in otherwise healthy individuals. There is good evidence that risk of fecal carriage, severity of illness, and development of unusual complications of cryptosporidiosis are directly related to CD₄ counts [3]. Despite the consensus of opinion regarding the seriousness of *Cryptosporidium* infection in immunocompromised patients, and the importance of protecting such patients from infection, there are insufficient valid data to allow an accurate assessment of the prevalence of this infection in this group [6]. In this study, cryptosporidiosis was investigated in patients with acquired immunodeficiency in Iran.

Methods

Ethical declaration

This investigation involved human subjects and was approved by the institutional ethical review board from the Ethical Committee of the Pasteur Institute of Iran, where the study was conducted.

Patients and samples

214 patients in eight immunodeficiency categories attending 10 health centers in Iran were randomly selected for inclusion in the study. All information, including personal identification, stage of disease, clinical symptoms and stool consistency, were recorded. The acquired immunodeficiency conditions included 157 patients (73.4%) with immunosuppressive therapy, 23 (10.7%) with AIDS, 15 (7%) with chronic diseases, 8 (3.7%) receiving radiotherapy, 7 receiving (3.3%) concurrent immunosuppressive therapy and radiotherapy, 2 (0.9%) with common variable immunodeficiency, 1 (0.5%) with splenectomy, and 1 (0.5%) with T-cell deficiency. The patient group was 64.5% male and 35.5% female. By age groups, 30.4% of patients were 1-15 years old, 54.2% were 16-45 years old, and 15.4% were ≥46 years old. Forty

nine percent of patients lived in Tehran province, while the origins of the remainder included 19 different provinces of Iran.

Fixation and smear preparations

Stool samples were examined morphologically and microscopically for consistency and other parasites. A small amount of stool (25 g) was mixed with 10 mL of fixation buffer (10 mL phosphate-buffered saline, 20 mL formaldehyde, 100 mL glycerine and distilled water to make final volume to 1000 mL) and incubated for 1 h to be fixed and inactivated. The suspension was passed through four layers of netting cotton and centrifuged at 2000 g for 5 min. Three smears were made from the pellet, dried in air, and fixed with methanol and/or acetone and then examined by three different methods, including AFS, APF and direct fluorescence using monoclonal antibody (DF×mAb).

AFS, APF and DF×mAb

For AFS, fixed smears were stained with carbol fuchsin and heat (2-5 min on a candle flame until evaporation), rinsed with tap water, destained with acid-alcohol 3%, restained for background color with malachite green 0.5% (5 min), rinsed with tap water, dried at room temperature, and observed under light microscope (all materials from Sigma Chemical Co., Deisenhofen, Germany) [11]. For APF assay, fixed smears were stained with auramine O (15 min), rinsed with tap water, destained with acid-alcohol 3%, restained for background color with potassium permanganate 0.5% (3 min), rinsed with tap water, dried at room temperature, and observed under fluorescence microscopy (all materials from Sigma Chemical Co.) [11].

For DF×mAb assay, MonoFluo[®] kit *Cryptosporidium* (Diagnostic Pasteur, Marnes-la-Coquette, France) was used. Smears were fixed with acetone, then 20 µL of fluorescein isothiocyanate-mAb was placed on samples, after which they were incubated in a humid chamber at 37°C for 30 min, rinsed with distilled water, dried in air, mounted with buffered glycerine, and examined on a fluorescence microscope.

Results

Among the 214 patients, three cases (1.4%) were infected with *Cryptosporidium* (Table 1), and one (0.47%) shed *Isospora* spp. oocysts. The prevalence of cryptosporidiosis and isosporiasis in acquired

Table 1. Degree of immunodeficiency and clinical features of three cases of cryptosporidiosis

Case No.	Underlying disorder	Leucocytes (/mm ³ of blood)	Lymphocytes (% of leukocytes)	CD ₄ count (/mm ³ of blood)	CD ₄ /CD ₈ ratio	Diarrhea pattern	Weight loss (kg)
1	AIDS	3500	27	85	0.25	Cholera-like	12
2	AIDS	4300	27	60	0.10	Severe	7
3	AML	4600	38	56	0.37	Watery	3

Abbreviations: AIDS = acquired immunodeficiency syndrome; AML = acute myeloid leukemia

immunodeficient Iranians with diarrhea was 6.3% and 2.1%, respectively. Thus, 8.7% of AIDS and 2.3% of hematological malignancy patients had cryptosporidiosis (Table 2). Three (6.3%) of 48 acquired immunodeficiency cases of diarrhea had cryptosporidiosis, including two (33.3%) of six cases of AIDS, and one of nine cases (11.1%) of hematological malignancies.

Discussion

The results of this study indicate that cryptosporidiosis was responsible for diarrhea in 30% of cases associated with AIDS (2 of 6 cases) and one of five with acute myeloid leukemia (AML). In addition, the duration of disease in infected immunocompromised patients lasted for weeks, and it was terminated by death in two AIDS patients. In the infected AML patient, the diarrhea lasted for 18 days, and stopped after discontinuation of combined immunosuppressive therapy with methotrexate, cytosar and prednisolone. Based on our data, three methods employed (AFS, APF and DF×mAb) appear to be suitable for the laboratory diagnosis of *Cryptosporidium*. Thus, we believe that the AFS assay continues to be a useful screening tool for immunodeficient patients in clinical laboratories.

The prevalence of *Cryptosporidium* in immunologically normal or suppressed subjects makes it imperative

to conduct epidemiological and environmental studies. In our previous report [12], cryptosporidiosis was studied among immunocompetent patients with gastroenteritis in Tehran, Iran, and a comparison was made between its prevalence and other enteropathogenic parasites. In this complementary study, the clinical role of *Cryptosporidium* in acquired immunodeficiency groups was investigated in Iran. Despite the relative consensus of opinion regarding the seriousness of *Cryptosporidium* infections in immunocompromised patients and the importance of protecting these patients from infection, there is less understanding of their risk of fatal opportunistic protozoan parasites. Further research in this area will promote the assessment of the public health importance of cryptosporidiosis, and allow researchers to better understand the transmission dynamics, to identify risk factors and reservoir hosts, and to establish preventive measures [2].

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Table 2. Prevalence of cryptosporidiosis among patients with various acquired immunodeficiency disorders

Immunodeficiency disorder	Number of patients	Number of patients infected (%)
Non-hematological malignancy	48	0 (0.0)
Skin diseases	44	0 (0.0)
Hematological malignancy	43	1 (2.3)
Transplantation	37	0 (0.0)
AIDS	23	2 (8.7)
Chronic diseases ^a	15	0 (0.0)
Others ^b	4	0 (0.0)
Overall	214	3 (1.4)

Abbreviation: AIDS = acquired immunodeficiency syndrome

^aFifteen different patients with hepatic cirrhosis, nephritis and chronic diarrhea with weight loss and low immunity.

^bTwo patients were diagnosed with common variable immunodeficiency, one patient with T-lymphocyte deficiency under treatment with immunosuppressive agents (methotrexate, prednisolone) for psoriasis and one splenectomised patient with thalassemia.

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