J Trop Med Parasitol 2006;29:33-6.

SHORT COMMUNICATION

Available online at www.ptat.thaigov.net

A Case Report of Cryptosporidiosis and Isosporiasis in AIDS Patients in Iran

Hossein Nahrevanian, Mehdi Assmar

Department of Parasitology, Pasteur Institute of Iran, Pasteur Avenue, Tehran 13164, Iran

Summary

Cryptosporidiosis and isosporiasis are self-limiting enteric diseases caused by the protozoan parasites *Cryptosporidium* spp and *Isospora* spp, respectively. While immunocompetent individuals readily clear *Cryptosporidium*, immunocompromised individuals may experience sustained infection with significant severity. As a result, chronic cryptosporidiosis is responsible for considerable morbidity and some mortality among AIDS patients. Both cryptosporidiosis and isosporiasis display many similarities in their clinical symptoms, including watery diarrhea, stomach pains, and weight loss. In this study, in Tehran, Iran, 23 hospitalized AIDS patients were investigated. Stool samples were collected, fixed and examined by acid-fast staining (AFS), auramin phenol fluorescence (APF), and direct fluorescence using monoclonal antibody (DF × mAb). The results confirmed two cases (8.7%) infected with *Cryptosporidium* spp, one of whom (4.3%) was infected with *Isospora* spp. This is the first report of cryptosporidiosis and isosporiasis co-infection in AIDS patients suffering from diarrhea in Iran. Patients having normal stool consistency showed no oocysts of either sporozoa. Despite the consensus of opinion regarding the seriousness of *Cryptosporidium* and *Isospora* infections in AIDS patients, and the importance of protecting these patients from infection, there is insufficient understanding of the risk of fatal opportunistic protozoan parasites among them.

Keywords: AIDS, *Cryptosporidium*, *Isospora*, co-infection, Iran

Introduction

The Acquired Immunodeficiency Syndrome (AIDS) epidemic is in its third decade and has become a pandemic disease threatening the world's population. In Iran, the first case of Human Immunodeficiency Virus (HIV) was reported in 1987. This was followed by a rapid increase in the number of cases [1]. Injecting drug users, sexually transmitted disease (STD) patients and people who frequently travel abroad, are among those tested [2]. Among AIDS patients, 67% were intravenous drug users and 9% were infected by sexual contact [1]. It is feared that the HIV/AIDS situation in Iran may be more serious, and the main concern is the potential spread of infection from injecting drug users to the youth population through high-risk sexual behaviors [3-4]. It is now widely recognized in Iran that one of the key factors for the growing prevalence of HIV is lack of knowledge about HIV transmission, prevention factors, and life skills [2].

Pneumocystis carinii, Toxoplasma gondii, and *Cryptosporidium parvum* are the most common opportunistic protozoa encountered in AIDS patients [5]. Both cryptosporidiosis and isosporiasis are caused by protozoa belonging to the Sporozoa. These two diseases display many similarities in their clinical symptoms, including

Correspondence:
Dr H Nahrevanian
Tel / Fax: +98-21-66968855
E-mail: mobcghn@yahoo.co.uk

watery diarrhea, stomach pains, and weight loss [6]. *Cryptosporidium* species are apicomplexan parasites that infect the microvillus border of the gastrointestinal epithelium of many vertebrate hosts with a wide spectrum of clinical presentations [7]. Cryptosporidiosis is self-limiting in the immunocompetent host, but it may cause persistent diarrhea and severe malabsorption in the immunodeficient host. While immunocompetent individuals readily clear this parasite, immunocompromised individuals may experience sustained infection with significant associated morbidity and mortality [8-10]. People with AIDS are susceptible to a devastating form of cryptosporidiosis manifested by chronic, voluminous diarrhea [11-13], which characteristically is profuse and watery; it may contain mucus, but rarely blood and leukocytes, and it is often associated with weight loss. Duration of symptoms and outcome typically vary according to the host's immune status [7, 12]. Despite the seriousness of *Cryptosporidium* and *Isospora* infections in AIDS, and the importance of protecting these patients from infection, there were insufficient valid data for an accurate assessment of the prevalence of these two sporozoan infections in AIDS patients [13].

Materials and methods

Patients and samples

This study was carried out at the Imam Khomeini Hospital, Tehran; 23 AIDS patients at ARC (AIDS Related Complex) stage, or higher, were examined. As most were hemophilic, it was confirmed that they had been infected via injections of concentrated anti-hemophilia factors, or other plasma products. All patient data, stage of HIV infection, immune status, clinical symptoms, and stool consistency, were recorded. The number of CD_4 lymphocytes in all patients was equal or <400 per ml of blood, and the ratio of CD_4 to CD_8 was <0.08.

Fixation and smear preparations

Stool samples were examined morphologically and microscopically for consistency and parasites. Twenty-five grams of stool were mixed with 10 ml fixation buffer (10 ml PBS, 20 ml formaldehyde, 100 ml glycerine and enough distilled water to make a final volume of 1,000 ml (all materials from Sigma) and incubated for 1 hr for fixing and inactivation. The suspension was passed through 4 layers of cotton netting and centrifuged at 2,000 rpm for 5 min. Three smears were made from the pellet obtained, air-dried, fixed with methanol or acetone, and then examined by acid-fast staining, auramin phenol fluorescence, and direct fluorescence using monoclonal antibody.

Acid-fast staining (AFS)

The fixed smear was stained with carbol fuchsin, heated (2-5 min on a candle flame) until evaporated, rinsed with tapwater, destained with 3% acid-alchohol, restained for background color with 0.5% malachite green (5 min), rinsed with tapwater, dried at room temperature, and observed under light microscope (all materials from Sigma) [14].

Auramin phenol fluorescence (APF)

The fixed smear was stained with auramine O (15 min), rinsed with tapwater, destained with 3% acid-alchohol, restained for background color with 0.5% potassium permanganate (3 min), rinsed with tapwater, dried at room temperature, and observed under fluorescence microscope (all materials from Sigma) [14].

Direct fluorescence using monoclonal antibody (DF × mAb)

The Monofluo® Cryptosporidium Kit (Diagnostic Pasteur, Marnes-la-Coquette, France) was used for DF × mAb assay. The smear was fixed with acetone, then 20 µl of FITC-mAb was placed on the samples, incubated in a humid chamber at 37˚C for 30 min, rinsed with DW, air-dried, mounted with buffered glycerine, and examined under a fluorescence microscope.

Results and Discussion

The results confirmed two cases (8.7%) were infected with *Cryptosporidium*, and one of them simultaneously infected with *Isospora* (4.3%). This is the first report of co-infection with cryptosporidiosis and isosporiasis in an AIDS

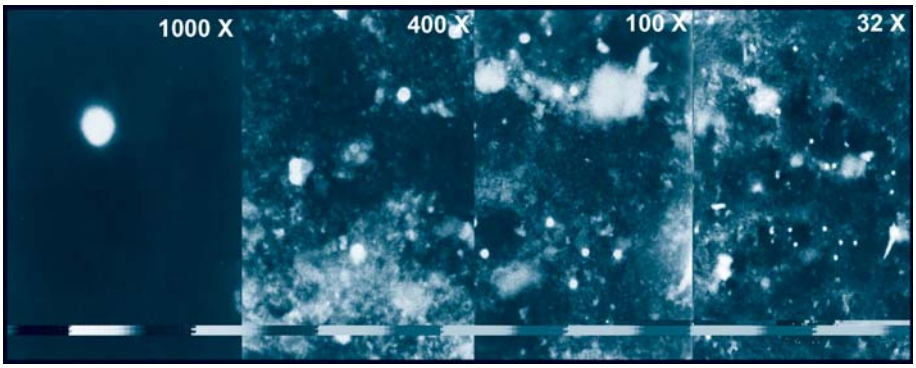

Fig 1 Oocysts of *Cryptosporidium* spp under auramin phenol fluorescence (APF) staining, at 32x, 100x, 400x, 1000x.

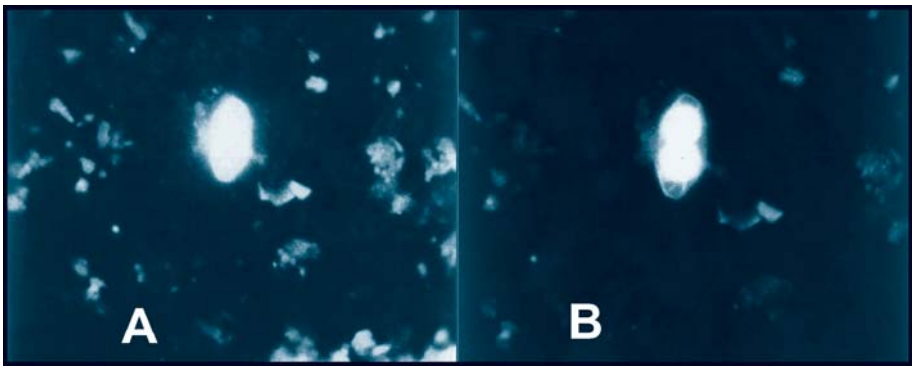

Fig 2 Oocyst of *Isospora* spp under auramin phenol fluorescence (APF) staining, A) immature; B) mature oocyst, 400x.

Table 1 Association of cryptosporidiosis and isosporiasis with stool consistency in AIDS patients.

Stool consistency	No (%)	No of cryptosporidiosis (%)	No of isosporiasis (%)
Diarrhea	6 (26.1)	2 (33.4)	1 (16.7)
Normal	17 (73.9)	0 (0)	0 (0)
Total	23 (100)	2 (8.7)	1 (4.3)

patient suffering from diarrhea in Iran (Figs 1, 2). The clinical symptoms in patients were diarrhea, weight loss, nausea and vomiting, fever, abdominal pains, and flatulence. Patients having normal stool consistency showed no *Cryptosporidium* and *Isospora* oocysts (Table 1). The first case was a 16-year-old boy with leuckocyte count of 3,500/ml, 27% lymphocytes, CD_4/CD_8 ratio 0.25, and total CD_4 85 per ml of blood. He had gasteroentritis, including chlorea-form diarrhea, nausea, vomiting, abdominal pain, flatulence, and 12-kg weight loss. The second case was a 50-year-old woman with leukocyte count of 4,300/ml, 27% lymphocytes, CD_4/CD_8 ratio 0.10 and total CD_4 60 per ml of blood. She had gasteroentritis, including severe diarrhea,

vomiting, and abdominal pain.

In addition to several investigations [12, 15], there were insufficient valid data for an accurate assessment of the prevalence of cryptosporidiosis among AIDS patients. Data based on diagnosed cases of cryptosporidiosis from the Centers for Disease Control (CDC) have resulted in an estimated prevalence of 2-5% for late-stage HIV-infected patients [13]. Considering that AIDS is expanding its range in Iran, the present study can help monitor and reduce the mortality rate among AIDS patients and other immunocompromised individuals. This will promote assessment of the public health importance of various species and isolates of *Cryptosporidium*, and allow researchers to understand transmission dynamics better, to identify risk factors and reservoir hosts, and to establish preventive measures.

Acknowledgements

We would like to express our gratitude to late Dr M Ghorbani Samin, of the School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, for constructive comments about this study. We are grateful for the kind collaboration and generous cooperation of authorities and staff from the Hemophilia Centre and the Division of Infectious Diseases, Imam Khomeini Hospital, Tehran.

References

1. Tavoosi A, Zaferani A, Enzevaei A, Tajik P, Ahmadinejhad Z. Knowledge and attitude towards HIV/AIDS among Iranian students. *BMC Pub Health* 2004;3:1-6.
2. UNAIDS, UNDP, HIV/AIDS data. http://www.undp.org.ir/aids.htm; 2006.
3. Center for Disease Control (CDC), Office of the Deputy for Public Health, Ministry of Health and Medical Education of the IR Iran. HIV/AIDS in Iran (Cumulative Statistics). Tehran; 2003. [in Farsi]
4. American Iranian Council. Drug use and HIV/AIDS in Iran. http://www.american-iranian.org/beta/publications.php?PressreLease=1& pressreleaseid =56; 2006.
5. Botero J H, Castano A, Montoya MN, Ocampo NE, Hurtado MI, Lopera MM. A preliminary study of the prevalence of intestinal parasites in immunocompromised patients with and without gastrointestinal manifestations. *Rev Inst Med Trop Sao Paulo* 2003;45:197-200.
6. Makni F, Cheikrouhou F, Ayadi A. Parasitoses and immunodepression. *Arch Inst Pasteur Tunis* 2000;77:51-4.
7. Xiao L, Fayer R, Ryan U, Upton SJ. *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clin Microbiol Rev* 2004;17:72-97.
8. Leitch GJ, He Q. Arginine-derived nitric oxide reduces fecal oocyst shedding in nude mice infected with *Cryptosporidium parvum*. *Infect Immun* 1994;62:5173-6.
9. Leitch GJ, He Q. Putative anticryptosporidial agents tested with an immunodeficient mouse model. *Antimicrob Agent Chemother* 1994;38:865-7.
10. Leitch GJ, He Q. Reactive nitrogen and oxygen species ameliorate experimental cryptosporidiosis in the neonatal BALB/c mouse model. *Infect Immun* 1999;67:5885-91.
11. Clark DP. New insights into human cryptosporidiosis. *Clin Microbiol Rev* 1999;12:554-63.
12. Hunter PR, Nichols G. Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. *Clin Microbiol Rev* 2002;15:145-54.
13. Current L, Garcia LS. Cryptosporidiosis. *Clin Microbiol Rev* 1991;4:325-58.
14. Delaat ANC. Microbiology for the allied health professions. 2nd ed. London: Henry Kimpton Publishers; 1979.
15. Cranendonk RJ, Kodde CJ, Chipeta D, Zijlstra EE, Sluiters JF. *Cryptosporidium parvum* and *Isospora belli* infections among patients with and without diarrhea. *East Afr Med J* 2003;80:398-401.