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Original Article

Prevalence of *Cryptosporidium* Infection in Immunocompromised Patients, In South-West of Iran, 2009-10

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Abstract

Background: *Cryptosporidium* is a protozoan parasite with worldwide distribution. The aim of this study was to estimate the prevalence of *Cryptosporidium* infection by antigen detection in faeces among immunocompromised patients referred to educational hospitals of Ahvaz City, South-West of Iran, 2009-2010.

Methods: Fecal samples from 176 immunocompromised patients were collected and *Cryptosporidium* coproantigen test was performed using ELISA method (DRG kit, Germany). A questionnaire was completed for each case and the results were analyzed using descriptive and Chi-Square tests, by SPSS statistical software (15th version).

Results: Our study indicated 5.1% *Cryptosporidium* infection prevalence in the immunocompromised participated population. Furthermore, 4.2 %, 4%, 4.5 % and 9.1% infection rates were identified in children suffered from hematopoietic malignancy, adult cancer patients, renal transplant recipients, and HIV⁺ cases, respectively. There was not significant correlation between the infection and age and gender ($P>0.05$). Infection was most frequent among HIV⁺ patients.

Conclusion: The present study confirmed the high prevalence of *Cryptosporidium* antigen in fecal samples of immunocompromised patients in the region. As no chemotherapeutic agents have yet proven, especially in immunosuppressed patients, therefore our results highlight the importance of preventive intervention in these groups.

Keywords: *Cryptosporidium*, Coproantigen, Immunocompromised patients, ELISA, human, Iran

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Introduction

Cryptosporidiosis is caused by *Cryptosporidium* strains, a parasite classified as emerging pathogens. The organism infects the gastrointestinal epithelium to produce a diarrhea that is self-limited in immunocompetent persons, but potentially life threatening in immunocompromised persons (1). Furthermore, *Cryptosporidium* stated an organism with low infective dose and resistant to conventional water disinfectants (2). In addition, no definitive treatment and proven protocol has been achieved for cryptosporidiosis (3). Therefore, prevention seems to be the main effective disease protection, especially among immunocompromised individuals.

Immunocompromised patients such as HIV⁺ patients with malignant disease, post-transplant patients and children under 5 years old, are at high risk to develop diarrheal disease caused by *Cryptosporidium*.

Because of the lack of updated data, this study was conducted to estimate *Cryptosporidium* prevalence in immunocompromised patients, in south-west of Iran.

Materials and Methods

This cross sectional study was performed among immunocompromised patients referred to educational hospitals of Ahvaz Jundishapur University of Medical Sciences during 2009-2010.

Overall, 176 patients including 48 children with lymphohematopoietic malignancies and 50 adults with malignancies, admitted to Hematology Department of Shafa Hospital and underwent chemotherapy were included in the study. Fifty-five renal transplant patients and 33 confirmed HIV⁺ patients were also participated. The patients were interviewed and a questionnaire was completed for demographical, hygienic, and environmental

life style. The study was approved by the Ethical Committee of the Jundishapur University of Medical Sciences, Ahvaz, Iran. A total 176 stool samples were collected and stored at -20°C without any preservative until used. The ELISA, DRG diagnostic kits (EIA-3467, Germany) was used for *Cryptosporidium* antigen detection in stool samples. The samples were defrosted and enzyme immunoassay was performed as described by the manufacturer. The results were carried out spectrophotometrically at 450 nm using Dynatech ELISA reader. According to manufacturer guideline, the specimen was considered positive if optical density was more than 0.15.

Statistical analysis was conducted with Chi-square and Fisher exact tests. The data were analyzed using SPSS statistical software version 15th.

Results

Among 176 immunocompromised patients, 9 (5.1%) cases were positive for *Cryptosporidium* antigen. Of these, 2 and 7 cases were among 1-15 and 16-75 year old group, respectively. No significant correlation between *Cryptosporidium* infection frequency, age, and gender was obtained (Table 1, 2). Frequency of *Cryptosporidium* infection was 9.1%, 4%, 4.2% and 4.5 % among HIV⁺, adults with malignancies, children suffered from lymphohematopoietic malignancy and renal transplant patients, respectively (Table 3).

The freeze/thaw associated instability of *Cryptosporidium* antigen in nine positive samples was analyzed. Only two stool samples were positive after second freeze-thaw cycle.

Table 1: Frequency of *Cryptosporidium* infection in immunocompromised patients according to gender

ELISA	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Gender						
Male	7	5.8	113	94.2	120	100
Female	2	3.6	54	96.4	56	100

Table 2: Frequency of *Cryptosporidium* infection in immunocompromised patients according to age

ELISA	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Age (year)						
1-15	2	4.2	46	95.8	48	100
16-76	7	5.5	121	94.5	128	100

Table 3: Frequency of *Cryptosporidium* infection according to type of immunosuppressant illness

ELISA	Negative		Positive		Total	
	No.	%	No.	%	No.	%
Illness						
Children	46	95.8	2	4.2	48	100
Malignancy						
Adult	48	96	2	4	50	100
Malignancy						
Kidney Recipients	43	95.5	2	4.5	45	100
HIV+	30	90.9	3	9.1	33	100
Total	167	94.9	9	5.1	176	100

Discussion

Cryptosporidiosis is recognized as an important gastroenteritis disorder of immunocompromised patients. In such cases, it may lead to high morbidity and mortality (4). The prevalence of *Cryptosporidium* antigen in stool examination by ELISA in immunocompromised patients was 5.1%. Similar studies in Iran indicated the prevalence rates of 1.4%, 11.5%, 22%, and 26.7% within immunocompromised patients (5-8). Higher prevalence rates (17-61.1%) have been reported in India and Turkey, among immunocompromised patients with diarrhea (9-12). There are discrepancies in prevalence rates of *Cryptosporidium* sp. in Iran and other countries (13). Probably different situation of hygienic life styles and living environmental are responsible for these variable levels of infection.

There was only one infected case with diarrhea in our study. As this parasite has been recognized to cause severe life-threatening disorder, even in non-diarrheic immunocompromised patients, therefore the reliable technique for demonstration of oocysts in stool examination of such patients is necessary.

In this study, infection was most frequent among HIV⁺ cases. Higher rate of infection in HIV⁺ patients have been reported ranged from 8.7% to 26.7% in Iran and 0% to 100% in other countries (4,8,14,15). This finding may be because HIV⁺ patients suffered from immunological disturbances predominantly from cellular immunoresponce of T helper 1 cell. On the other hand, in our study the most cases had addiction and were in low socioeconomic level, besides, they did not use safe and hygienic drinking water.

In the current study, *Cryptosporidium* prevalence was 4.5% in renal transplant patients. Different rates of infection from 2.4%, 4.5% and 11.5% have been reported from Iranian

communities (6, 16, 17). In some other countries, 18.8% and 20% rates of infection have been indicated (18, 19). Renal transplant patients in our study were under cyclosporine A treatment. Cyclosporine A as an immunosuppressive treatment also acts as an antiparasite agent, therefore it seems this situation could result to reduce infection rate of *Cryptosporidium* (20). Furthermore, in our study transplant patients used hygienic water as advised by health centers. Current study indicated *Cryptosporidium* infection rate of 4.2% and 4% in children with hematopoeitic and adult cancer patients, respectively. Higher rate of infection have been reported by others (7, 21-23).

Analysis of *Cryptosporidium* antigen viability in feces suggests the possibility that some samples may become negative in ELISA after second freeze/thaw process. Similar results have been reported for other parasite infections (24). These data suggest that fresh sample should be used; if not possible, a careful of sample handling must be performed.

The present study confirmed the high prevalence of *Cryptosporidium* antigen in fecal samples of immunocompromised patients in the region. As no chemotherapeutic agents have yet proven, especially in immunosuppressed patients, therefore our results highlight the importance of preventive intervention in these groups.

ELISA showed high sensitivity and specificity with reliable results for *Cryptosporidium* antigen detection in stool (25). In addition cryptosporial antigens may be present in stool without existence the whole oocysts (26), therefore its application in routine diagnosis and even large-scale epidemiological surveys is recommended.

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