

Cryptosporidium Infection in Pediatric Patients with Lymphohematopoietic Malignancies

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Received: 14/05/07; Revised: 13/07/07; Accepted: 06/08/07

Abstract

Objective: *Cryptosporidium parvum* is a common protozoan pathogen with worldwide distribution. It localizes on the intestinal cells and prolonged diarrhea in immunocompromised patients. The aim of this study was to estimate the prevalence and the clinical features of enteric cryptosporidiosis in pediatric patients with lymphohematopoietic malignancies.

Material & Methods: In this cross-sectional study stool samples were collected from 100 children (67 boys, 33girls) with lymphohematopoietic malignancies who underwent chemotherapy between the ages of 6 months and 17 years (mean age 7.5 years). All of the specimens were examined for the oocysts of *C. parvum* by modified Ziehl Neelsen (MZN) staining technique and coproantigens of *C. parvum* by ELISA.

Findings: Cryptosporidium infection was detected in 22 patients. 16 (72.7%) of the infected patients were male and 6 (27.3) female. 7 (31.8%) patients were <5 years, 8 (36.4%) 5-10 years and 7(31.8) >10 years old. Parasites were detected in 19/85 (86.4%) patients with ALL, 2 of 5 (9.1%) with AML, and 1 of 10 (4.5%) with NHL. Clinical symptoms were found in 11 (50%) of the patients. We found longer duration of chemotherapy in patients who were positive for *cryptosporidium* infection (Mean=2067 days) in comparison to negative group (Mean=258.5 days) (ANOVA, $f=2.82$, $P=0.04$).

Conclusion: The incidence of *cryptosporidium* infection was 22% among pediatric patients with lymphohematopoietic malignancies. We recommend evaluation of these patients with at least two different diagnostic methods in order to prevent possible life threatening outcomes.

Key Words: Cryptosporidium sp.; Cryptosporidiosis; Lymphohematopoietic malignancies; Immunocompromised patient

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Introduction

Cryptosporidiosis is a widespread, zoonotic disease produced by coccidial protozoa *Cryptosporidium parvum*. It is a common protozoan pathogen with worldwide distribution. It localizes on the intestinal cells and causes acute or chronic, self limited diarrhea in immune-competent hosts and severe and prolonged diarrhea in immunocompromised patients^[1,2]. Recent studies also support previous suggestions that cryptosporidiosis is a non-host specific zoonosis, which is transmitted via the fecal-oral route^[3]. Cryptosporidial infection occurs in up to 7 percent of children with diarrhea in developed countries and up to 12 percent of children with diarrhea in developing countries^[4].

Despite the relative consensus on the opinion regarding the seriousness of *Cryptosporidium* infection in patients with AIDS and the importance of protecting these patients from infection, it does not seem to be a shared understanding of the risks to other groups of immunosuppressed patients, especially children with malignancy. In these patients, intestinal parasitic infections can be severe and even fatal^[5].

There are relatively a few studies with different results, which investigated the prevalence of *Cryptosporidium* in children with cancer. For example, one study from New South Wales, investigated 149 stool samples from 60 children with cancer and diarrhea and found none to be positive^[6]. In other studies from Malaysia and Turkey, *Cryptosporidium* was found in 2% and 4% of children with malignancy respectively^[7,8]. On the other hand in another study from USA a significantly higher incidence of cryptosporidiosis was found in asymptomatic immunosuppressed children compared to healthy ones (22% versus 6.4)^[9]. Among different types of malignancies, lymphohematopoietic ones have a special importance because of unusually severe cryptosporidiosis infection^[5].

Although several studies have investigated the epidemiology of cryptosporidium infection in pediatric population in Iran^[10,11], there has been no previous study on immunocompromised pediatric patients.

The aim of this study was to estimate the prevalence and the clinical features of enteric cryptosporidiosis in pediatric patients with lymphohematopoietic malignancies.

Material & Methods

This cross-sectional study was conducted in hematology-oncology service of Dr Sheikh children's hospital affiliated to Mashhad University of Medical Sciences, and Department of Parasitology, Imam Reza Hospital, from October 2005 to August 2006.

The study included all children 1day to 18 years of age with lymphohematopoietic malignancies who were admitted to hematology-oncology Department and underwent chemotherapy. Children who had used antiparasitic or antibiotic drugs during their treatment were excluded from the study.

The protocol was approved by the University Ethical Committee. The parents or the guardians of the children were interviewed by a nurse to complete a questionnaire on the type of underlying lymphohematopoietic malignancies, clinical symptoms (such as diarrhea, anorexia, abdominal colic, flatulence, fever and weight loss) and duration of treatment, area of residence, contact with animals, source of drinking water, as well as information about the health status of the child.

Stool samples were collected from 100 children with lymphohematopoietic malignancies (67 boys, 33girls) between the ages of 6 months and 17 years (mean age 7.6 years, SD=3.8). 85 patients had acute lymphoblastic leukemia (ALL), 5 had acute myelogenic leukaemia (AML) and 10 had non-Hodgkin's lymphoma (NHL). All specimens were obtained from children undergoing chemotherapy.

Three fecal samples were collected from each patient and placed in 10% formalin. Specimens were concentrated by formalin-ether concentration method and stained with modified Ziehl-Neelsen for identification of *cryptosporidium* (oocysts). All specimens were also processed according to the immunoenzymatic assay instruction guide (DRG *Cryptosporidium*

Antigen EIA-3467. Germany) to detect *C. parvum*-specific coproantigen. The results were read using a plate reader with a 450 nm filter (Awareness. Stat fax 3200). The samples which yielded a difference in optical density greater than or equal to 0.150 were considered positive, and those with an optical density less than 0.150 were considered negative.)

Statistical analyses were performed with Chi square and ANOVA tests. The data were analyzed using SPSS statistical software (11th version). Statistical significance was set at $P < 0.05$.

Findings

Cryptosporidium infection was detected in 22 patients. 16 (72.7%) of the infected patients were male and 6 (27.3) female. 7 (31.8%) patients were <5 years, 8 (36.4%) 5-10 years and 7 (31.8%) >10 years old (mean age 7.6 years, SD=3.8). The age and sex of the infected patients did not show any significant difference compared to the uninfected ones ($P > 0.05$).

Parasites were detected in 19 out of 85 (86.4%) patients with ALL, 2 out of 5 (9.1%) with AML, and 1 out of 10 (4.5%) with NHL.

Eight of the 22 children (36.4%) who were positive for *Cryptosporidium* infection, were in contact with domestic animals in their residential areas, 12 of them (54%) had consumed non-pasteurized dairy products and 4 (18%) had unsanitary water supply (wells or water containers). Fifteen patients (68.2%) were city-dwellers and 7 (31.8%) villagers.

Clinical symptoms were found in 11 (50%) of the patients (Table 1).

When the age and the type of malignancy were omitted as independent variables, the duration of chemotherapy showed statistically significant difference (ANOVA, $f=2.82$, P -value=0.04) between patients positive for *cryptosporidium* infection (Mean=2067 days) and the negative group (Mean=258.5 days).

Table 1- Clinical symptoms in 11 children with lymphohematopoietic malignancies and cryptosporidium infection

	Number	Percentage
Anorexia	4	18.2
Fever	3	13.6
Abdominal colic	2	9.1
Flatulence	2	9.1
Weight loss	1	4.5
Diarrhea	1	4.5
Total	13*	30*

* There were cases who had more than one symptom

Discussion

Periods of profound immunosuppression renders cancer patients, susceptible to a broad array of potential pathogens.

Our study demonstrated an incidence of 22% of *cryptosporidium* infection among pediatric patients with lymphohematopoietic malignancies. Pettoello-Mantovani et al studied 50 asymptomatic immunosuppressed children in the USA, and cryptosporidiosis was documented in 22% of the patients^[9].

On the other hand, Burgner et al found no *C. parvum* in 60 children with various malignancies compared to an incidence of 30% in 173 healthy children^[6]. Aksoy et al. reported an incidence of 4% of *Cryptosporidium* infections in pediatric patients with malignancy. In this study stool specimens was taken from 50 children with malignancy and investigated for cryptosporidium using only the Kinyoun acid-fast stain method. Thirty-eight (76.0%) of the 50 patients had lymphoma or leukemia and were considered immunosuppressed. *C. parvum* was detected in 2 patients with a diagnosis of ALL and NHL^[8].

In another study from Cairo, *Cryptosporidium* was found in 9.6% of children with cancer. The study involved a total of 104 diarrhea episodes experienced by pediatric cancer patients under myeloablative therapy. Their results also were in agreement with Abaza et al., who recorded a

prevalence rate of 6.3% among Egyptian immunocompromised patients^[2].

The prevalence rate observed in our study was 22%, which is relatively high compared with other studies carried out in different regions of the world. This high incidence may be related to three factors: first the type of malignancy (lymphohematopoietic malignancies versus other malignant neoplasms), second immunocompromising effect of the chemotherapy for cancer (all the patients in our study underwent chemotherapy compared to 54% in Aksoy's study^[8], and third (probably the most important factor) was implementing two different diagnostic methods (ELISA in addition to microscopic examination) which led to a higher sensitivity for detection of *Cryptosporidium*^[12,13,14].

There was no significant difference in the cryptosporidium infection rate among male and female children of any age group in our study.

In our study only 50% of cryptosporidium infected cases had clinical symptoms and diarrhea was seen only in one case. Gentile et al. evaluated patients with hematologic malignancies in Italy and found only 5 asymptomatic patients out of 20 patients with intestinal cryptosporidiosis^[5]. However, several recent reports also have identified high percentages of asymptomatic infections in children from Brazil and India^[15,16]. It seems that in tropical or underdeveloped countries incidence of asymptomatic infection especially in children is higher.

Although most of the studies indicate that cryptosporidium does not pose a particularly special risk to cancer patients generally but exception to the rule seems to be leukemia and other hematological malignancies^[9]. Stine et al described severe diarrhea in a child with acute lymphocytic leukemia. The child recovered fully at last. Lewis et al. described a relapsing course of infection in a child with acute lymphoblastic leukemia. A study on a small group of six children with acute leukemia or lymphoma showed that two patients died with evidence of persistent infection and the remaining four recovered after chemotherapy regimens were modified^[2].

We found longer duration of chemotherapy in patients who were positive for cryptosporidium infection in comparison to negative group.

Conclusion

In conclusion we found a relatively high incidence of cryptosporidium infection among pediatric patients with lymphohematopoietic malignancies. It will be prudent to consider the possibility of this infection when making a clinical assessment of this high-risk group of patients.

We also recommend evaluation of these patients with at least two different diagnostic methods in order to prevent possible life threatening outcomes.

Acknowledgement

This project was supported by a grant from the Vice Chancellor of Research, Mashhad University of Medical Sciences. We would like to thank Dr Ramin Sadeghi for editing the paper.

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