

ORIGINAL ARTICLE

Cryptosporidiosis in Immunocompromised Children

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ABSTRACT

Key words:

Cryptosporidium, AML (acute myeloid leukemia), HD (Hodgkin lymphoma), Non HD (Non-Hodgkin lymphoma) and ALL (acute lymphoblastic leukemia)

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Background: *Cryptosporidium parvum* is an obligate intracellular parasite of the Coccidia class that infects the microvilli epithelial cells of the digestive and respiratory systems **Objectives:** this study aims to detection of the prevalence of cryptosporidiosis among immune compromised children who receive chemotherapy and to compare the three different methods for diagnosis of *Cryptosporidium* **Methodology:** 200 children in Aswan oncology institute were enrolled in this study, clinical assessment as well as stool examination by modified Ziehl-Neelsen stain, ELISA assay and Immunofluorescence tests were done. **Results:** The study showed that the over-all infection was 43.5% by immunofluorescence method, 40.5% by ELISA and 28% by modified Ziehl-Neelsen stain. Infection rate increases in haematological malignancies (AML54.8%, HD46.2 %, ALL41.6%, NON HD 27.3%) than non haematological malignancy (wilms 50%, sarcoma 45.5%, and neuroblastoma 42.9%). **Conclusion:** *Cryptosporidium* infection is an important public health problem in Immunocompromised children, raising awareness about cryptosporidiosis has very important effect to decrease prevalence of this parasitic infection.

INTRODUCTION

One of the commonest illnesses among immunocompromised children who receive chemotherapy is diarrhea as reported by the WHO; diarrhea is one of the major causes of children and infant mortality in many developing countries¹.

Intestinal parasitic infestations are considered important causes of diarrhea, which is a serious health problem in tropical countries. *Cryptosporidium* and *Giardia* spp are the commonest parasitic causes of human diarrhea². *Cryptosporidium* species are present in respiratory and digestive systems of vertebrates, infect poultry, fish, mammals, and reptiles³.

Cryptosporidium infection is common and cause infection to humans and animal species. Therefore, transmission of infection to human may occur by different methods⁴.

The transmission is easier for old people or children and day care centers. In rural areas, although zoonotic transmission from animals to humans is mentioned in many sites, this issue is debatable⁵. The incidence of infection for some children, professional staff, nursery staff, people who travel to endemic regions, unhygienic places and the contact with infected people is high. Some *Cryptosporidium* outbreaks originating from common meals, well water, unhygienic drinking water sources and public swimming pools, are known⁶.

While infection by *Cryptosporidium* spp may cause asymptomatic infection or diarrhea in immunocompetent patients, it can cause severe and chronic diarrhea, pancreatic, biliary and respiratory tract infections and even death in immunocompromised persons⁷. *Cryptosporidium* is causing significant morbidity and mortality in immune compromised as well as immune competent patients⁸.

Symptoms are variable depending on age, condition of the immune system and species of infected host³. *Cryptosporidium* was classified by the WHO guideline for drinking water as a pathogen of significant public health importance, contributed in part by the organisms' low infective dose and resistance to conventional water treatment such as chlorination⁹. In the developed world, *Cryptosporidium* are responsible for gastroenteritis major outbreaks and sporadic cases¹⁰.

Cryptosporidium affects immune competent children under the age of 5 years, and immune compromised individuals worldwide, especially HIV-infected individuals. It causes diarrhea lasting about two weeks, extending up to two months among the immune competent and severe life-threatening illness among immune compromised individuals¹¹. Cryptosporidiosis may be asymptomatic or may be associated with a high range of enteric symptoms ranging from mild gastroenteritis to severe diarrhea with dehydration¹². Immunocompromised individuals, especially those with

very low CD4-cell counts, often suffer from a chronic and severely debilitating form of the disease¹³.

METHODOLOGY

Subjects:

This study was carried out during the period from August 2016 to July 2017 on children receiving chemotherapy in Aswan oncology institute. Random sample consisted of 200 child were included in the study, their ages ranged from 1to 15 years. 110 were males and 90 were females. Twenty samples were collected from children suffered from the same complains but immunocompetant at Aswan hospital as a control group. Every child was subjected to a questionnaire about symptoms and signs suggestive of *Cryptosporidium* infection, General and abdominal examination and Stool examination was done by

- Modified Zeil Nelsen Stain,
- Enzyme Linked Immunosor pant Assay (ELIZA)
- Immunofluorescence test.

Enzyme Linked Immunosor pant Assay (ELISA).

Test principle: The RIDASCREEN® *Cryptosporidium* Test employs specific monoclonal antibodies in a sandwich type method. The well surface of the microwell plate is coated with specific antibodies to the antigens of *cryptosporidium* pathogen species. Sample preparation and test procedure were done according to manufacturer r-biopharm RIDASCREEN® *Cryptosporidium* Art. No. C1201³³

Assessment and interpretation:

Calculating the cut-off

Cut-off = OD of the negative control + 0.15

Direct Fluorescent Antibody Staining (DFA)

Direct fluorescent antibody (DFA) assay involved using a fluorescein isothiocyanate-conjugated anti-*C. parvum* monoclonal antibody (FITC-C-mAb), which recognizes surface epitopes on oocysts¹⁴. The Aqua-Glo™ kit (A100FLK, Waterborne, USA) was used to detect the oocyst of the parasite from stool samples. This reagent is genus-specific and binds only to the oocysts of the parasite if they were present. the staining procedure was done according to³⁴

Evaluation of each slide was done with fluorescence microscope using the appropriate filters for fluorescein at x400 and x1 000 magnifications. *C. parvum* oocytes appeared bright apple V green measuring 4-6 mm in diameter

RESULTS

This study showed that the over-all infection with *Cryptosporidium* was 43.5% by immunofluorescence ,40.5% by ELISA and 28% by modified Zeihl neelsen stain .Infection rate is more in males 48.4% than in females 35.9%, Infection increase between the 6-12 age period. Infection rate increase in urban 48.7% than rural 26.1%. There are seasonal variation as infection increase in summer 57.6% and autumn 38.7% and decrease in winter 15.4% and spring 31.2%. Infection rate increases in haematological malignancies (AML54.8%, HD46.2 %, ALL41.6%, NON HD 27.3%) than non haematological malignancy (wilms 50%, sarcoma 45.5%, and neuroblastoma 42.9%). The results are tabulated in tables 1 - 7

Table 1: Distribution of *Cryptosporidium* infection among immunocompromised children who receive chemotherapy according to some socio-demographic and environmental character

Socio-demographic character & environmental character	<i>Cryptosporidium</i> infection		
	Total examined n= 200	Total infected n= 87	
		No.	%
Gender			
Males	122	59	48.40
Females	78	28	35.90
Age			
1-5 years	72	30	41.70
6-12 years	128	57	44.50
Mean ± SD	6.52 ± 2.16		
Locality			
Rural	154	75	48.70
Urban	46	12	26.10
Seasonality factors			
Winter	13	2	15.40
Spring	64	20	31.20
Summer	92	53	57.60
Autumn	31	12	38.70

Table 2: Distribution of *Cryptosporidium* infection among immunocompromised children who receive chemotherapy in relation to hematological factors.

Hematological factors	<i>Cryptosporidium</i> infection		
	Total examined n= 200	Total infected n= 87	
		No.	%
Hemoglobin			
Normal	8	0	0.00
Anemic	192	87	45.30
Wbcs			
Natural	77	36	46.80
Leucocytosis	92	40	43.50
Leucopenia	31	11	35.50
Neutrophils			
Normal	16	7	43.80
Neutropenia	184	80	43.50

Table 3: Distribution of *Cryptosporidium* infection among immunocompromised children who receive chemotherapy in relation to tumor type factors

Tumor type factors	<i>Cryptosporidium</i> infection		
	Total examined n= 200	Total infected n= 87	
		No.	%
tumor type			
ALL	125	52	41.60
AML	31	17	54.80
Hodj-lymph	2	1	50.00
Non-hodj-lymph	13	6	46.20
Wilmis	11	3	27.30
Sarcoma	11	5	45.50
Neuroblastoma	7	3	42.90

Table 4: The Percentage of *Cryptosporidium* infections among immunocompromised children who receive chemotherapy by Modified Ziehl-Neelsen technique

<i>Cryptosporidium</i> infection	No (%)
Infected	56 (28%)
non infected	144 (72%)
Total	200 (100%)

Table 5: The Percentage of *Cryptosporidium* infection among immunocompromised children who receive chemotherapy by ELISA.

<i>Cryptosporidium</i> infection	No (%)
Infected	81 (40.5%)
non infected	119 (59.5%)
Total	200 (100%)

Table 6: The Percentage of *Cryptosporidium* infections among immunocompromised children who receive chemotherapy by immunofluorescences test.

<i>Cryptosporidium</i> infection	No (%)
Infected	87 (43.5%)
non infected	113 (56.5%)
Total	200 (100%)

Table 7: The percentage of *Cryptosporidium* infection among immunocompetant children by different technique

Techniques	+v	-ve	Total	Infection (%)
Immunofluorescences test	5	15	20	25%
Modified ZN	3	17	20	15%
ELISA	4	20	20	20%

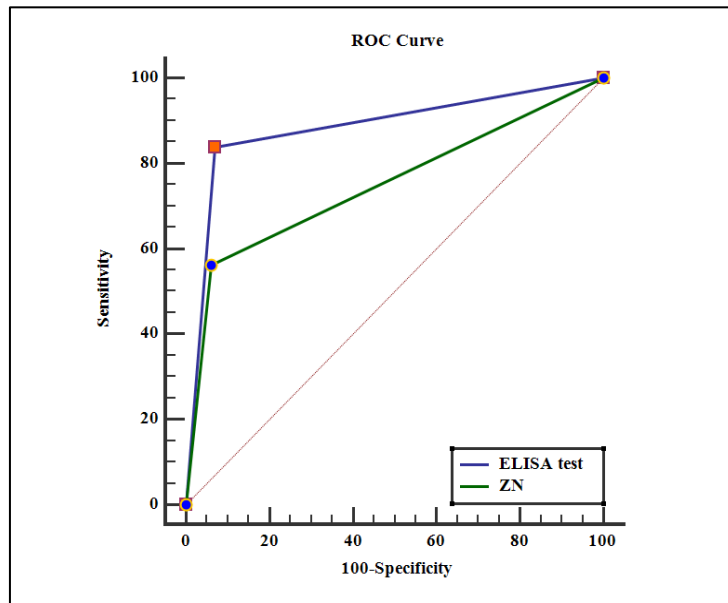


Fig. 1: Receiver Operating Characteristics (ROC) curve of predictors of cryptosporidiosis among immunocompromised children who receive chemotherapy, by using Immunofluorescences tests a gold standard.

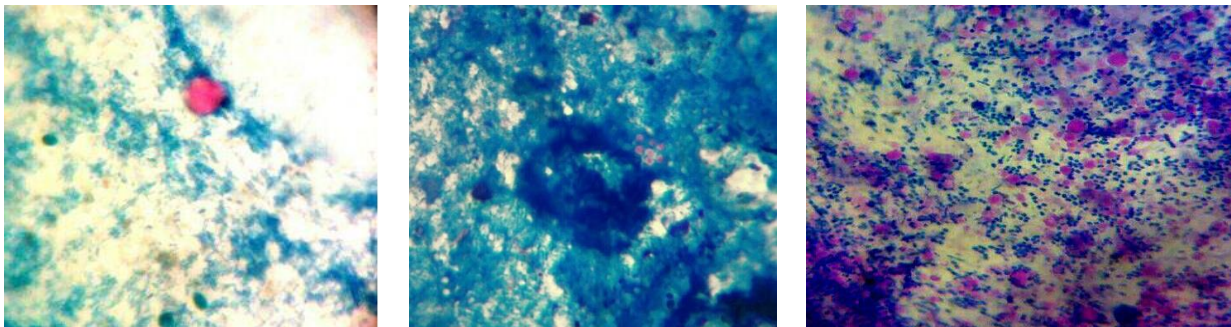


Fig. 2: Stool samples stained with Modified Zeihl -Neelsen stain showing *Cryptosporidium* oocyst (x40 and x100)

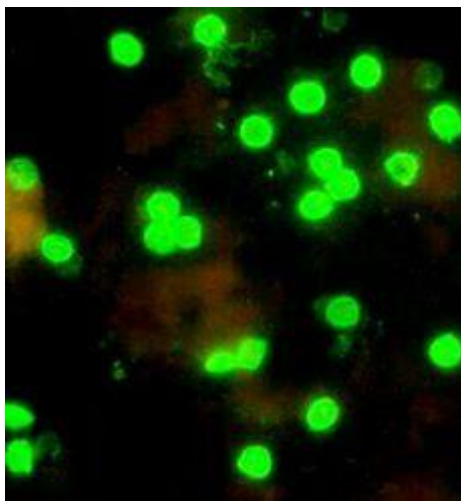


Fig. 3: Stool sample examined by Immunofluorescence showing *Cryptosporidium* oocyst. (x360)

In the present study, the prevalence of *Cryptosporidium* infection among Immunocompromised children receiving chemotherapy by using immunofluorescences test was 43.5%, the high percentage of *Cryptosporidium* infection in young age from 1 to 5 years was 40%; which may be due to lack of personal hygiene and immature immune system. Similarly, an overall *Cryptosporidium partum* infection was 42.6% in a study done to compare four diagnostic techniques, namely, acid fast stain test, direct immunofluorescent assays and other two enzyme immunoassays¹⁵

Another study in Alexandria University Children's Hospital revealed that the percentage of children having *Cryptosporidium* parasitic infection was 22.1%¹⁶. In Alexandria and Turkey, other studies on children showed similar results of *Cryptosporidium* infections (40.6% and 41.9%) respectively^{17,18}

Several studies in Alexandria, Cairo and Zagazig among immunocompromised children reported that the percentages of *Cryptosporidium* infections were 41.2%, 58% and 30% respectively. This difference could be due to the use of different stains and techniques¹⁹

In agreement with the present study a previous study revealed that the total percentage of *Cryptosporidium* spp infection was 41%.²⁰

By comparing the three diagnostic measures, in our study the immunocompromised children who received chemotherapy using MZN technique was 28%, the sensitivity and specificity were 54.20% and 78.0% respectively. In agreement with the present work, the percentage of cryptosporidiosis infection was 26.86%. This study concluded that the microscopy is very specific but less sensitive method for the laboratory detection of *C. parvum* in feces²¹

The variation of the validity of the diagnostic performance could be due to two main reasons. Firstly; the present study was limited since only one stool specimen was collected and examined. Previous studies had shown that repeated examination of more than one stool specimen over 3 consecutive days improve oocytes detection. Secondly; the low sensitivity observed in all diagnostic tests could be due to the low intensity of the infection among the studied children²²

The Present study showed that out of 200 immunocompromised children who received chemotherapy. 81 cases (40.5 %) were ELISA positive. Sensitivity, specificity, PPV and NPV were 83.91 %, 92.92%, 90.1% and 88.2% respectively. The diagnostic performance of the ELISA in the current study showed a high sensitivity and specificity. The diagnostic efficiency level was moderate (76.83%). The PLR was (11.85) which indicated that the probability of immunocompromised children who receive chemotherapy was about two times more likely to have cryptosporidiosis infection than those who did not receive chemotherapy.

A comparative study had shown that all tested methods (EIA, IFA) were equally sensitive and specific for the detection of *Cryptosporidium* spp, so it was concluded that the ease of use and costs are important criteria in determining the choice of the technique²³

On other hand, the previous study reported that the sensitivity, specificity, PPV and NPV of ELISA were 64%, 56%, 58% and 65 % respectively. They recommended the use of ELISA, as it is a fast, easy-to-read, and accurate method for the detection of *Cryptosporidium* in stool specimens²⁴

As regards the age the present study revealed that the percentage of *Cryptosporidium* infection among male control immunocompetent children was 18.20% versus female 33.30%. The difference was statistically significant. This was explained by the greater opportunities of females to *Cryptosporidium* infection due to home activities; but in immunocompromised

children who receive chemotherapy, the percentage of *Cryptosporidium* infection among male children was 48.40% and reached 35.90 % among females. The difference was statistically significant so the high prevalence rate of intestinal *Cryptosporidium* infections observed among males could be attributed to the fact that males are more active, mobile and integrated to the environment.²⁵

In agreement with the present study among immunocompromised children who received chemotherapy a previous study was done in the high institute of public health revealed that the percentage of *Cryptosporidium* infection among male children was 34% and reached 50% among females. The difference was statistically insignificant²⁰, which could be attributed to the small sample size²

In Assiut, Egypt, parasitological examination revealed that working school children were exposed to *Cryptosporidium* infections (69.3 %) more than non-working groups (41.9%). Among working children, the infection rate was higher in males (33.6 %) than in females (19.4%)²⁷.

The locality of the present study revealed that the percentage of *Cryptosporidium* infection among rural area is higher than urban area, the difference was statistically significant. The percentage of *Cryptosporidium* infection among control immunocompetent children was 100% in rural area versus 0.00% in urban area and among immunocompromised children was 48.70% in rural area versus 26.10% in urban area.

Concerning the residential difference, diarrheal episodes were more common among children living in Upper Egypt and the urban Governorates than in Lower Egypt and the boundary Governorates. It was reported that the most common parasitic agents that cause acute diarrheal illness in children are *C. parvum*, *Giardia lamblia* and *Entamoeba histolytica*²⁸

After the study of the hematological factors; the present study revealed that both children either control immunocompetent children and immunocompromised children who receive chemotherapy the percentage of *Cryptosporidium* infection among anemic children was higher than normal hemoglobin, the difference was statistically insignificant among control immunocompetent children versus statistically significant among immunocompromised children who receive chemotherapy. The percentage of *Cryptosporidium* infection among control immunocompetent anemic children were 40% versus the normal hemoglobin children 10% and among immunocompromised anemic children who receive chemotherapy was 45.30% versus no infection in normal hemoglobin children

In agreement with the present study; a study conducted in Iraq for Prevalence of *Cryptosporidium parvum* among Children in relation to hematological aspect revealed that there was association between

anemia in children and infection with cryptosporidiosis among 66.01% cases. It was contributed to the worrisome but agrees with the earlier observation that about 30% of the world population is anemic. Anemia is commonly caused by deficiency of iron in diet. It is common

knowledge that due to combined forces of ignorance and poverty the diets of many individuals and households in developing countries often lack many essential blood-building ingredients, including iron. These factors might contribute to the high occurrence of anemia in the studied area²⁹

A previous study was done in Bangladesh to determine *Cryptosporidium* among poor children, it revealed that diarrhea that results from *Cryptosporidium* infections leads to dehydration, malnutrition, stunted growth and anemia³⁰

Cryptosporidium infection among summer season was 57.60% then autumn, spring and winter, 38.70%, 31.20% and 15.40, respectively with a statistically significant difference. This is consistent with other reports that an increase in incidence was observed during warm, rainy season³¹. This was explained by the destruction of the parasites oocysts in low temperature³²

As regards the tumor type it was found that AML tumor among immunocompromised children who received chemotherapy had higher *Cryptosporidium* infection rate than those HODJ-LYMPH tumor type (54.80% versus 50.00%), but the difference was statistically insignificant.

A previous study reported that *Cryptosporidium* infection in lymphohematopoietic malignancies was 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 NHL²⁹

CONCLUSION

Cryptosporidium infection is an important public health problem in immunocompromised children receiving chemotherapy in Aswan Oncology Institute. Males are more affected than females. The prevalence increases in children with haematological malignancies. Peak incidence occurs in summer. Raising awareness about cryptosporidiosis has a very important effect to decrease prevalence of parasitic infection. Chemotherapy increases the rate of *cryptosporidium* infection.

Recommendations

More interest should be paid about the early detection and treatment of immune compromised children who were infected. Upgrading skills and knowledge of medical and paramedical staff. Education of health care providers about good hygienic control and how to prevent infection through hand washing and other measures of infection control to prevent spread of infection. Improving the system of waste disposal and pure water supply. There is a need to

promote mass scale deworming and health promotion campaigns to create awareness about health and hygiene. Proper disposal and treatment of human excreta. Good sanitary measure when dealing with animal excreta to prevent zoonotic diseases. Regular follow up is needed to ensure the efficiency of management and to diagnose new infection.

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