Cryptosporidium and Giardia infection in children with malignancies in Basrah

Sabeeha M. Abdul-Hussein

ABSTRACT
Background: Children with malignancies (during chemotherapy) are the most common infected by Cryptosporidiosis and giardiasis as compared with other groups (before chemotherapy).
Objectives: We explore the association between cryptosporidiosis and giardiasis in children with malignancies in Basrah city among children admitted to Pediatric Oncology center in Specialist Basrah Children’s Hospital.
Method: Used three methods in the study (Direct wet mount, Acid fast stain, IC test) in two groups before and during chemotherapy in children admitted to Pediatric Oncology center in Specialist Basrah Children’s Hospital during May to November 2015, their age ranged from two months to 14 years.
Results: One hundred and six stool samples were assayed from malignant children found Cryptosporidium (13) positive samples before chemotherapy and (33) positive samples during chemotherapy and found Giardia (20) positive sample before chemotherapy and (9) positive sample during chemotherapy.
Conclusion: This study reported that age group between 1-4 years was the most susceptible for the infection among the age groups studied. Both genders were susceptible to infection with Cryptosporidium and giardia, with a higher rate of infection reported in males compared with females.

Key words: Diarrhea in children, Cryptosporidium, Giardia, Protozoan parasites.
INTRODUCTION

Cryptosporidium and Giardia are genera of protozoan parasites that infect a wide range of children. Cryptosporidium, an apicomplexan, is reported to infect persons in 106 countries.\(^1\) Giardia, a flagellated, facultative anaerobic, similarly widespread, is the most common intestinal parasite of persons.\(^2\) They are transmitted through the fecal-oral route following direct or indirect contact with the infective stages of the parasite from three sources: anthropogenic, zoonotic and sapronotic.\(^3\) Giardia and Cryptosporidium infections are common cause of gastroenteritis known as giardiasis and cryptosporidiosis, respectively. It is believed that giardiasis is still a significant health problem. Most infected persons are children who suffer and experience growth retardation.\(^4\)

Cryptosporidiosis is a frequent cause of diarrheal disease in humans. Infection is acquired via the fecal-oral route, and C. parvum has been recognized as the cause of large waterborne and food-borne outbreaks of gastroenteritis.\(^5\) Risk factors for sporadic cryptosporidiosis\(^6\), include age (children under five years of age and, to a lesser extent, young adults, who presumably have a greater likelihood of contact with these patients), travelling abroad, contact with a diarrheic individual and contact with farm animals.\(^7\) Giardia is typically characterized in human by diarrhea, steatorrhea, abdominal cramps, bloating, malabsorption and weight loss. Person-to-person transmission occurs by hand-to-mouth transmit of cysts from the feces of a person infected with Giardia. Outbreaks of Giardia infections in families and institutions, such as day care centers and nursing homes, especially those with diapered children, have been associated with fecal-oral route.\(^8\)

Transmission of Cryptosporidium and Giardia can be direct, from host to host, or indirect, through the ingestion of contaminated food or water, a multitude of transmission cycles therefore exist, involving domestic animals and wildlife, which in some instances result in human infections.\(^9\) In this study we attempt to compare Cryptosporidium and Giardia infection in children with malignancies before and during chemotherapy.

MATERIALS AND METHODS

A. Subjects
A total of 106 fecal specimens were collected from children with malignant diseases aged 2 months to 14 years before and during (6 to 8 weeks) chemotherapy in Pediatric Oncology Center at Basrah children's specialist hospital during May to November 2015. Stool samples were collected in clean containers without any additives.

B. Procedure

1- Direct wet mount by saline and lugol's iodine.

Procedure: Use direct wet mount for detection Giardia in stool sample\(^10\), procedure as in following:

a. Drop of saline was put in one half of the slide and drop of lugol's iodine solution was put on the other half of the slide.

b. Mixed small portion of stool with one drop of saline and similarly, a small amount of stool was mixed with the one drop of lugol's iodine.

c. A coverslip was put on both drops of saline and lugol's iodine and then examined under the microscope with 10X and 40X.

2- Modified Ziehl-Neelsen acid fast stain

Procedure: As recommended by ARCOMEX Modified Ziehl-Neelsen acid fast stain kit instructions for detection Cryptosporidium.

1. A thin fecal smear was conducted and left it to the air for 2 to 3 min and drying on a heating block (70°C) for 5 min.
2. Stained with carbol–fuchsin and gently heat slide to steaming for 5 min using Bunsen Burner
3. Rinsed in tap water.
4. Put drops of 5% sulfuric acid to decolorize for at least thirty second.
5. Rinsed in tap water.
6. Stained with methylene blue for one minutes.
7. Rinsed in tap water.
   • Examined using oil immersion lense.

3-Immunochromatographic test (IC)
   Procedure: as recommended by RIDA®QUICK Cryptosporidium kit instructions for detection Cryptosporidium.
1. Reagent must be brought at room temperature about (20-25°C).
2. Pipette 1 ml of the extraction buffer diluent into a test tube.
3. Add 100 µl or 50 mg stool sample.
4. Mix sample via vortex mixer.
5. Leave stool sample to stabilizer about three minutes.
6. Pull about four drops or 200 µl of the supernatant and put it in round slot of the cassette.
7. Read off results after 5 minutes.
   Evaluation
   Positive: Red and Blue band shown together.
   Negative: Blue band only.

Statistical Analysis:
Statistical package of social science (SPSS) version 20 was used to analyze data, Chi-square (X²) test was used to assess the significance of difference between groups and variable, P-value less than 0.05 was considered to be statistically significant.

RESULTS
In (Table-1), out of 106 patients with malignant diseases before chemotherapy, 12.3% were found to excrete Cryptosporidium oocyst in their stool, compared to the 31.1% of patients with malignant diseases during chemotherapy. Of the same 106 patients with malignant diseases before chemotherapy, 18.9% were found to be positive for Giardia, compared to the 8.5% of patients during chemotherapy. The difference was highly significant.

<table>
<thead>
<tr>
<th></th>
<th>Giardia infection</th>
<th>Cryptosporidium infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Before chemotherapy</td>
<td>20 (18.9%)</td>
<td>86 (81.1%)</td>
</tr>
<tr>
<td>During chemotherapy</td>
<td>9 (8.5%)</td>
<td>97 (91.5)</td>
</tr>
</tbody>
</table>

*X² = 8.648, df =1, P <0.05
**X² = 11.105, df =1, P <0.05

In (Table-2), the highest rate of Giardia infection among the three types of malignant diseases groups was found in patients with Lymphoma (HL, NHL); 31.2% before and 6.2% during chemotherapy. Statistically, the difference was significant (P < 0.05). Other rates of infection were 19.7% among patients with Leukemia (ALL, AML, CML) before chemotherapy and 9.8% during chemotherapy, therefore, statistically the difference was not significant (P > 0.05). While 10.3% was found in those with solid tumor before chemotherapy and 6.9% during chemotherapy, the difference was not significant (P > 0.05). The highest rate
of Cryptosporidium infection among the three types of malignant diseases groups was found in patients with Lymphoma (HL,NHL); 6.2% before and 43.8% during chemotherapy (Table 4-4). Statistically, the difference was very significant ($\chi^2 = 6.000$, df =1, $P < 0.05$). Other rates of infection were 13.1% among patients with Leukemia (ALL, AML, CML) before chemotherapy and 29.5% during chemotherapy, therefore, statistically the difference was significant ($\chi^2 = 4.888$, df =1, $P < 0.05$). While 13.8% was found in those with solid tumor before chemotherapy and 27.6% during chemotherapy, therefore, statistically the difference was not significant ($\chi^2 = 1.681$, df =1, $P > 0.05$).

Table 2. Presence of Giardia infection among various types of malignant diseases before and during chemotherapy

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Type of malignant cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before chemotherapy</td>
<td>12(19.7%)</td>
<td>5(31.2%)</td>
</tr>
<tr>
<td>During chemotherapy</td>
<td>6(9.8%)</td>
<td>1(6.2%)</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before chemotherapy</td>
<td>8(13.1%)</td>
<td>1(6.2%)</td>
</tr>
<tr>
<td>During chemotherapy</td>
<td>18(29.5%)</td>
<td>7(34.8%)</td>
</tr>
</tbody>
</table>

Giardia * $\chi^2 = 3.873$, df = 1, $P < 0.05$
** $\chi^2 = 2.347$, df = 1, $P > 0.05$
*** $\chi^2 = 3.642$, df =1, $P > 0.05$
Cryptosporidium * $\chi^2 = 4.888$, df = 1, $P < 0.05$
** $\chi^2 = 6.000$, df = 1, $P < 0.05$
*** $\chi^2 = 1.681$, df =1, $P > 0.05$

In (Table-3), the highest rate of Giardia infection occurred in the age group of 1-4 years of age, approximately 25% before chemotherapy and 10% during chemotherapy. Statistically, there was significant difference ($\chi^2 =5.926$, df = 1, $P < 0.05$). However, statistically, there was not a significant difference in the other age groups ($P > 0.05$). The highest rate of Cryptosporidium infection occurred in the age group of 1-4 years of age, approximately 25% before chemotherapy and 45% during chemotherapy. Statistically, there was significant difference ($\chi^2 = 3.516$, df = 1, $P < 0.05$). However, statistically, there was not a significant difference in the other age groups ($P > 0.05$).

Table 3. Giardia and Cryptosporidium infections according to the age groups before and during chemotherapy

<table>
<thead>
<tr>
<th>Age groups (yr)</th>
<th>Before chemotherapy</th>
<th>During chemotherapy</th>
<th>$\chi^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. examined</td>
<td>+ve Cases</td>
<td>%</td>
<td>No. examined</td>
</tr>
<tr>
<td>Giardia infection</td>
<td>&lt; 1</td>
<td>8</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>1-4</td>
<td>40</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>5-9</td>
<td>34</td>
<td>6</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>10-14</td>
<td>24</td>
<td>3</td>
<td>12.5</td>
</tr>
<tr>
<td>Cryptosporidium infection</td>
<td>&lt; 1</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1-4</td>
<td>40</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>5-9</td>
<td>34</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>10-14</td>
<td>24</td>
<td>2</td>
<td>8.3</td>
</tr>
</tbody>
</table>
(Table-4), shows *Giardia* infections in patients before and during chemotherapy were 20% found in males before chemotherapy and 8.3% during chemotherapy. Statistically, the difference was significant ($\chi^2 = 5.455$, df = 1, $P < 0.05$). However, infections found in females were 17.4% before chemotherapy compared to the 8.7% during chemotherapy. Thus, the difference was not significant in females ($\chi^2 = 3.242$, df = 1, $P > 0.05$). *Cryptosporidium* infections in patients before and during chemotherapy were 11.7% found in males before chemotherapy and 26.7% during chemotherapy. Statistically, the difference was significant ($\chi^2 = 4.357$, df = 1, $P < 0.05$). However, infections found in females were only 13% before chemotherapy compared to the 37% during chemotherapy. Thus, the difference was significantly different among females ($\chi^2 = 7.014$, df = 1, $P < 0.05$).

Table 4. *Giardia* and *Cryptosporidium* infections according to gender before and during chemotherapy

<table>
<thead>
<tr>
<th>Patients (gender)</th>
<th>Test</th>
<th>+ ve cases</th>
<th>$\chi^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Before chemotherapy</td>
<td></td>
<td>12</td>
<td>5.455</td>
</tr>
<tr>
<td>Female</td>
<td>Before chemotherapy</td>
<td></td>
<td>8</td>
<td>3.242</td>
</tr>
<tr>
<td></td>
<td>During chemotherapy</td>
<td></td>
<td>5</td>
<td>$\chi^2 = 5.455$, df = 1, $P &lt; 0.05$</td>
</tr>
<tr>
<td></td>
<td>During chemotherapy</td>
<td></td>
<td>4</td>
<td>$\chi^2 = 3.242$, df = 1, $P &gt; 0.05$</td>
</tr>
</tbody>
</table>

In (Table-5), it was found *Giardia* infection rate in urban areas before chemotherapy was 14% and 9.3% during chemotherapy. Statistically, the relationship was not significant ($\chi^2 = 4.773$, df = 1, $P > 0.05$). In rural areas, it was found that the infection rate of patients before chemotherapy was 22.2% and 7.9% for those during chemotherapy. Therefore, there was a very significant difference statistically ($\chi^2 = 8.210$, df = 1, $P < 0.05$).

1. **Leukemia include:** (Acute lymphocytic leukemia, Acute myelocytic leukemia and Chronic myelocytic leukemia).

2. **Lymphoma include:** (Hodgkin lymphoma and Non-Hodgkin lymphoma).

3. **Solid tumor include:** (Neuroblastoma, Osteogenic sarcoma, Hepatoblastoma, Adenocarcinoma, Ewing’s sarcoma, Rhabdomyosarcoma and Brain tumor).
DISCUSSION

Cryptosporidium and Giardia are genera of protozoan parasites that infect a wide range of humans. Species within these genera cause human cryptosporidiosis and giardiasis, which probably constitute the most common causes of protozoal diarrhea worldwide, and lead to significant morbidity and mortality in both the developing and developed world. The result of the study demonstrated that Cryptosporidium and Giardia infection occurred in children with malignant disease. This is the first report about Cryptosporidium and Giardia infection in children with malignant disease in Basra Province. In the present study a close association between the Cryptosporidium and Giardia infection and age of children was observed before and during chemotherapy. The results of the present study found that the total rate of Cryptosporidium infection in children before chemotherapy was 12.3% and 31.1% during chemotherapy. These indicate that immunocompromised patients are more susceptible to be infected with this parasite and Cryptosporidium was consistent with the hypothesis that the parasite would have the highest prevalence in immunocompromised patients. This rate was higher than those recorded during chemotherapy in Basrah of (8%) by Mahdi, et al. (1997); in Baghdad (6%) by Al-Janabi et al.(2005) and (14.78%) by Al-Warid et al.(2012). Also it was higher than reported in Kuwait (3.4%) by Iqbal, et al. (2011) and in Iran (4.4%) by Mohammadi et al. (2006). In Egypt, cryptosporidiosis in immunocompromised children was (4.8%). In Iran children suffered from hematopoietic malignancy was (4.2%). In Ethiopia about (10.4%), in Germany, Cryptosporidium was found in (12.6%) of patients with colorectal cancer. In India found Cryptosporidium in (3.8%). Explanation the differences and disparities in rates of Cryptosporidium infection among children of cancer patients may be attributed to that chemotherapy would cause weaknesses to patient’s cells thus weakens the immune system and so the parasite can penetrate. Also Cryptosporidiosis is considered to be one of the most serious opportunistic infections that complicates cancer. The results of the present study in Basrah found that the total rate of Giardia infection in children before chemotherapy was 18.9% and 8.5% during chemotherapy. This results was higher than recorded in Ethiopia (13.8%). In China found Giardia in children about (9.5%). But less than recorded in

<table>
<thead>
<tr>
<th>Patient group</th>
<th>+ve</th>
<th>χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban Before chemotherapy</td>
<td>No. 4</td>
<td>% 9.3</td>
<td>3.071</td>
</tr>
<tr>
<td>During chemotherapy</td>
<td>No. 23</td>
<td>% 36.5</td>
<td>8.210</td>
</tr>
<tr>
<td>Rural Before chemotherapy</td>
<td>No. 9</td>
<td>% 14.3</td>
<td>3.071</td>
</tr>
<tr>
<td>During chemotherapy</td>
<td>No. 23</td>
<td>% 36.5</td>
<td>8.210</td>
</tr>
<tr>
<td>Giardia infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban Before chemotherapy</td>
<td>No. 6</td>
<td>% 14%</td>
<td>4.773</td>
</tr>
<tr>
<td>During chemotherapy</td>
<td>No. 4</td>
<td>% 9.3</td>
<td>4.484</td>
</tr>
<tr>
<td>Rural Before chemotherapy</td>
<td>No. 14</td>
<td>% 22.2%</td>
<td>3.071</td>
</tr>
<tr>
<td>During chemotherapy</td>
<td>No. 5</td>
<td>% 7.9%</td>
<td>8.210</td>
</tr>
</tbody>
</table>
Muthanna found about 25%.[25] In Babylon found about 26.05%.[26] In Dohuk, northern Iraq found about 31.3% in children[27], in Iran found 26.2%.[28] Patients of this study were divided into three groups according the types of cancer. The highest rate of Giardia infection among the three types of malignant diseases groups was found in patients with Lymphoma (HL,NHL) (31.2%) before and (6.2%) during chemotherapy, this result is inconsistent with other studies in Iran with (18%) found in patients with Lymphoma infected with Giardia.[29] In India was found (12%) in lymphoma patients.[30] Giardiasis has been reported in cases of leukemia (19.7%) before chemotherapy and (9.8%) during chemotherapy. This result is different from result in Basrah (7.2%).[31] Giardiasis was less prevalent in solid tumor (10.3%) before chemotherapy and (6.9%) during chemotherapy, this result is different to studies in Basra not detectable cases of Giardia infection.[31] The highest rate of Cryptosporidium infection among the three types of malignant diseases groups was found in patients with Lymphoma (HL,NHL) (6.2%) before and (43.8 %) during chemotherapy, this result is inconsistent with other studies in Basra with (48.3%) found in patients with Lymphoma infected with Cryptosporidium.[11] Cryptosporidiosis has been reported in cases of leukemia (13.1%) before chemotherapy and (29.5%) during chemotherapy, this result differentially to studies in Basrah (6.25%)[11]; in London 1982, reported only one patients with Cryptosporidium infection during chemotherapy treatment for acute lymphoblastic leukemia with severe and profuse watery diarrhea.[32] Cryptosporidiosis was less prevalent in solid tumor (13.8%) before chemotherapy and (27.6%) during chemotherapy, this result is different to studies in Basrah not detectable cases of Cryptosporidium infection[11]. In Turkey found about 8.3% in patients who were diagnosed with solid tumors.[33] Patients of this study were divided into four age groups. Infection was observed before chemotherapy among age group of (1-4 years) was (25%) compared to children during chemotherapy of (10%) which showed clear increase. These results are different from some studies, as in AL-Muthanna found 60%[25], in Baghdad found 57.14%[34], in Diyala found 52.63%[38], in Dohuk found 50.2% (27), in USA found about 3.1% in 2013[36], in Kenya found 4.5%[37], in Ethiopia found 16.2% in children under 5 years[38], in Turkey found Giardia duodenalis about 3.82%.[39] Cryptosporidium Infection was observed before chemotherapy among age group of (1-4 years) was (25%) compared to children during chemotherapy of (45%) which showed clear increase. These results are differently to some studies, as in Erbil children of age under 5 years old was (14%).[40] These results might be in association with different risk factors including the physiological and immunological status of the examined patients. However, there is no published report about the relationship between age and Giardia infection in-patient with malignancy. Male patients group were more infected (20% and 8.3%) before and during chemotherapy than female (17.4% and 8.7%). This result differentially to studies in Basrah[31], in Babylon[26], in Diyala[34], in Bangladesh[41], Giardia found in patient with diarrhea in Bangladesh.[42] The result similarity to this study in Dohuk[27], in AL-Muthanna[25], in Turkey[38], in Ethiopia[38], in kenya[37], in USA[39], in China[24], in Libya[43]. In this study we found Giardia in Rural areas more than in Urban areas and more significant as well. In urban areas the percent found before chemotherapy was (14%) and during chemotherapy was (9.3%), but in rural areas (22.2%) was found before chemotherapy and (7.9%) during chemotherapy. Insignificant difference between urban and rural residents of before and during chemotherapy was noticed, which might be due to the geographical distribution of no children in Basra. This finding in agreement with that recorded in Basrah[31], AL-Muthanna[25], in Turkey[38], in USA.[39] And differs from some studies such as in Latin
In this study we found Cryptosporidium in rural areas more than in urban areas and more significant as well. In urban areas the percent found before chemotherapy was (9.3%) and during chemotherapy was (23.3%), but in rural areas (14.3%) was found before chemotherapy and (36.5%) during chemotherapy. This finding is in agreement with that recorded in Basra. [11] And differs with some studies such as in. [46] Also, for the first time in Iraq, this study worked on finding Cryptosporidium and Giardia infections before and during chemotherapy.

In conclusion, This study reported that age group between 1-4 years was the most susceptible for the infection among the age groups studied. Both genders were susceptible to infection with Cryptosporidium and giardia, a higher rate of infection was reported in male as compared with female results. Technical medical staff must be trained to diagnose Cryptosporidium and giardia in hospitals and primary health care Centers.

ACKNOWLEDGMENT
We acknowledge with a deep sense of gratitude, the support of the staff of Basrah Children’s Specialty Hospital especially Dr. Ali A. Sabri, Dr. Hussam M. Saleh.

REFERENCES


37. Claudio FL, Christa LF, Ana CO, Carla XT,


45. Abdulkadir AA, Seef-Elaslam MO, Saleh EO. Prevalence of Giardia Lamblia in Humans Visited Central Laboratory of Sebha Province. IJESIT.2013; 2(3).