Cryptosporidium and *Giardia* infection in children with malignancies in Basrah

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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 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 method in 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 month to 14 year.**

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*, 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.**

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

عدوى خبيئات الابواغ و الجياردیا في الأطفال الذين يعانون من الأورام الخبيثة في البصرة

الخلفية: الأطفال الذين يعانون من الأورام الخبيثة (خلال العلاج الكيميائي) هم الأكثر إصابة بطفيلي الابواغ الخبيئة والجياردیا بالمقارنة مع قبل العلاج الكيميائي .

الأهداف: نستكشف العلاقة بين طفيلي الابواغ الخبيئة وطفيلي الجياردیا لدى الأطفال الذين يعانون من الأورام الخبيثة في مدينة البصرة الوافدين الى مركز طب الأورام للأطفال في مستشفى البصرة التخصصي للأطفال.

الطريقة: استخدمت ثلاث طرق في الدراسة (فحص البراز المباشر وتقنية التصبيغ بالصبغة الصامدة للحمض المحورة والاختبار المناعي (اميونوكروماتوكرافي) في مجموعتين قبل وخلال العلاج الكيميائي لدى الأطفال الوافدين الى مركز طب الأورام للأطفال في مستشفى البصرة التخصصي للأطفال خلال شهر مايو إلى نوفمبر ٢٠١٥، تراوحت أعمارهم بين شهرين إلى ١٤ سنة.

النتائج: تم فحص ١٠٦ عينة من براز الأطفال وجدت طفيلي الابواغ الخبيئة (١٣) عينة إيجابية قبل العلاج الكيميائي و (٣٣) عينة إيجابية خلال العلاج الكيميائي و وجدت الجياردیا (٢٠) عينة إيجابية قبل العلاج الكيميائي و (٩) عينة إيجابية خلال العلاج الكيميائي.

الاستنتاج: أفادت الدراسة أن الفئة العمرية بين ١–٤ سنوات هي الأكثر عرضة للإصابة بين الفئات العمرية التي تمت دراستها. كان كلا الجنسين عرضة للعدوى بطفيلي الابواغ الخبيئة والجياردیا، وبلغ معدل أعلى من الإصابة في الذكور بالمقارنة مع نتائج الإناث. يجب تدريب الطاقم الطبي الفني لتشخيص الابواغ الخبيئة والجياردیا في المستشفيات ومراكز الرعاية الصحية.

الكلمات المفتاحية: الإسهال لدى الأطفال، الابواغ الخبيئة، الجياردیا، الاوالي الطفيلية.

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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: anthroponotic, 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 (X^2) 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 1. *Giardia and Cryptosporidium* infections before and during chemotherapy

	*Giardia infection **		*Cryptosporidium infection***	
	Positive	**Negative**	**Positive**	**Negative**
Before chemotherapy	20 (18.9%)	86 (81.1%)	13(12.3%)	93(87.7%)
During chemotherapy	9 (8.5%)	97 (91.5)	33(31.1%)	73(68.9%)

***X^2 = 8.648, df =1, P <0.05**

****X^2 = 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 (χ^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 (χ^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 (χ^2 = 1.681, df =1, P > 0.05).

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

Patient group		Type of malignant cases			Total
		Leukemia *	Lymphoma**	Solid tumor***	
Giardia	**Before chemotherapy**	12(19.7%)	5(31.2%)	3(10.3%)	**20**
	During chemotherapy	6(9.8%)	1(6.2%)	2(6.9%)	**9**
Cryptosporidium	**Before chemotherapy**	8(13.1%)	1(6.2%)	4(13.8%)	**13**
	During chemotherapy	18(29.5%)	7(34.8%)	8(27.6%)	**33**

Giardia *** X^2 = 3.873, df = 1, P < 0.05**

**** X^2 = 2.347, df = 1, P > 0.05**

*****X^2 = 3.642, df =1, P > 0.05**

Cryptosporidium *** X^2 = 4.888, df = 1, P < 0.05**

**** X^2 = 6.000, df = 1, P < 0.05**

***** X^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 (χ^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 (χ^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

	Age groups (yr)	Before chemotherapy			During chemotherapy			χ^2	P value
		No. examined	+ve Cases	%	No. examined	+ ve Cases	%		
***Giardia* infection**	< 1	8	1	12.5	8	0	0	1.131	**Ns**
	1-4	40	10	25	40	4	10	5.926	**0.042**
	5-9	34	6	17.6	34	2	5.9	1.531	**Ns**
	10-14	24	3	12.5	24	3	12.5	1.361	**Ns**
***Cryptosporidium* infection**	< 1	8	0	0	8	3	37.5	3.692	**NS**
	1-4	40	10	25	40	18	45	3.516	**0.050**
	5-9	34	1	2.9	34	6	17.6	3.981	**NS**
	10-14	24	2	8.3	24	6	25	2.400	**NS**

(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 (χ^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 (χ^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 (χ^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 (χ^2 = 7.014 , df = 1, P < 0.05).

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

Patients (gender)		Test	+ ve cases		χ^2	P value
***Giardia* infection**	**Male**	Before chemotherapy	No.	12	5.455	S
			%	20%		
		During chemotherapy	No.	5		
			%	8.3%		
	Female	Before chemotherapy	No.	8	3.242	NS
			%	17.4%		
		During chemotherapy	No.	4		
			%	8.7%		
***Cryptosporidium* infection**	**Male**	Before chemotherapy	No .	7	4.357	S
			%	11.7		
		During chemotherapy	No.	16		
			%	26.7		
	Female	Before chemotherapy	No.	6	7.014	S
			%	13.0		
		During chemotherapy	No.	17		
			%	37.0		

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 (χ^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 significant difference statistically (χ^2 = 4.484, df =1, P < 0.05). ***Cryptosporidium*** infection rate in urban areas before chemotherapy was 9.3% and 23.3% during chemotherapy. Statistically, the relationship was not significant (χ^2 =3.071, df=1, P > 0.05). In rural areas, it was found that the infection rate of patients before chemotherapy was 14.3% and 36.5% for those during chemotherapy. Therefore, there as a very significant difference statistically (χ^2 = 8.210, df =1, P < 0.05).

1. **Leukemia include:** (Acute lyphocytic 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).

Table 5. *Giardia and Cryptosporidium* infections in relation to residence before and during chemotherapy

Patient group			+ve		χ^2	P value
***Giardia* infection**	**Urban**	Before chemotherapy	No.	6	4.773	Ns
			%	14%		
		During chemotherapy	No.	4		
			%	9.3%		
	Rural	Before chemotherapy	No.	14	4.484	S
			%	22.2%		
		During chemotherapy	No.	5		
			%	7.9%		
***Cryptosporidium* infection**	**Urban**	Before chemotherapy	No.	4	3.071	NS
			%	9.3		
		During chemotherapy	No .	10		
			%	23.3		
	Rural	Before chemotherapy	No.	9	8.210	S
			%	14.3		
		During chemotherapy	No.	23		
			%	36.5		

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* in children with malignant disease in Basrah 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 in Basrah 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)[11]; in Baghdad (6%) by Al-Janabi *et al.*(2005) and (14.78%) by Al-Warid *et al.*(2012). [12,13] 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).[14,15] In Egypt, cryptosporidiosis in immunocompromised children was (4.8%). [16] In Iran children suffered from hematopoietic malignancy was (4.2%).[17] In Ethiopia about (10.4%)[18], in Germany, *Cryptosporidium* was found in (12.6%)of patients with colorectal cancer.[19] In India found *Cryptosporidium* in (3.8%).[20] 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.[21] Also Cryptosporidiosis is considered to be one of the most serious opportunistic infections that complicates cancer.[22] 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%).[23] In China found *Giardia* in children about (9.5%).[24] But less than recorded in

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% [35], 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 is 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

America [44], Stull *et al* found infection in urban areas was more than in rural in Eastern Ontario.[45] 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 Basrah. [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.

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REFERENCES

1. **Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium*: transmission, detection and identification. Int. J. Parasitol. 2000; 30: 1305–1322.**
2. **Caccio SM, Thompson RC, McLauchlin J, Smith H. Unravelling *Cryptosporidium* and *Giardia* epidemiology. Trends Parasitol. 2005; 21: 430-437.**
3. **Hubalek Z. Emerging human infectious diseases: anthroponoses, zoonoses, and sapronoses. Emer Infect Dis. 2003; 9: 403-404.**
4. **Farthing MJ, Mata LJ, Kronmal RA. Natural history of Giardia infection of infants and children in rural Guatemala and its impact on physical growth."Am. J. Clin. Nutr. 1986;43(3): 395–405.**
5. **Steinberg EB, Mendoza CE, Glass RA, Rana RB, Lopez MB, *et al.* Prevalence of infection with waterborne pathogens: a seroepidemiologic study in children 6-36 months old in San Juan Sacatepequez. 2004, Guatemala. Am. J. Trop. Med. Hyg.2004; 70(1): 83–88.**
6. **Robertson B. Case-control studies of sporadic cryptosporidiosis in Melbourne and Adelaide, Australia. Epidemiol. Infect. 2000;128, 419–431.**
7. **Roy SL. Risk factors for sporadic cryptosporidiosis among immunocompetent persons in the United States from 1999–2001. J. Clin. Microbiol.2004; 42, 2944–2951.**
8. **Baldursson S, Karanis P. Waterborne transmission of protozoan parasites: review of worldwide outbreakan update 2004-2010. Water Res. 2011; 45: 6603-14.**
9. **Current WL. Human cryptosporidiosis in immunocompetent and immunodeficient persons. Studies of an outbreak and experimental transmission. N. Engl. J. Med. 1983; 308, 1252–1257.**
10. **World Health Organization (WHO). Basic laboratory methods in medical parasitology. WHO Geneva.1991; 11-12, 20, 102 .**
11. **Mahdi NK, Al-Sadoon IA, Mohamed AT. First report of cryptosporidiosis among Iraqi children. Medical Journal of Basrah University.1997; 2(1): 115-120.**
12. **Al-Janabi STM. An immunological and pathological study of Cryptosporidium parvum infection and effect of some biological factor on the infections process. Ph.D Thesis submitted to the College of Al-Mustasiryah. 2005.**
13. **Al-Warid HS, Al-Saqur IM, Mahmood SH. Occurrence of Cryptosporidium spp. among People Live in North of Baghdad. Iraq. European Journal of Scientific Research .2012 ; 78 (4): 539-545.**
14. **Iqbal J, Khalid N, Hira PR. Cryptosporidiosis in Kuwaiti children: association of clinical characteristics with *Cryptosporidium* species and subtypes. Journal of Medical Microbiology .2011; 60: 647–652.**
15. **Mohammadi MR, Mohammad K, Farahani FA, Alikhani S, Zare M, Tehrani FR, Ramezankhani A, Alaeddini F. Reproductive Knowledge, Attitudes and Behavior Among Adolescent Males in Tehran, Iran. International Family Planning Perspectives. 2006; 32(1):35-44.**

16. El-Mahallawy H, El Basha NR, Zaki MM, El-Arousy M, Elswaifi SF, Abo-hashem EM. A comparative study on enteric parasitic infections in immunocompetent and immunosuppressed children in Egypt. Comparative Clinical Pathology. 2014; 23(5): 1509-1514.
17. Dehkordy AB, Rafiei A, Alavi SM, Latifi SM. Prevalence of *Cryptosporidium* Infection in Immunocompromised Patients, In South-West of Iran, 2009-10. Iranian J Parasitol. 2010; 5(4): 42-47.
18. Adamu H. The prevalence of intestinal parasites and molecular characterization of *Cryptosporidium* species in Ethiopia. PhD. Thesis University of Addis Ababa, Ethiopia .2010.
19. Sulżyc-Bielicka V, Kołodziejczyk L, Jaczewska S, Bielicki D, Kładny J. Prevalence of Cryptosporidium sp. In patients with colorectal cancer. Polski Przegląd Chirurgiczny. 2012; 84(7); 348–351.
20. Sharma P, Sharma A, Sehgal R, Malla N, Khurana S. Genetic diversity of Cryptosporidium isolates from patients in North India. International Journal of Infectious Diseases. 2013; 17601– 605.
21. Berenji F, Zabolinejad N, Kianifar H, Badeii Z, Banihashem A, Hiradfar A. *Cryptosporidium* Infection in Pediatric Patients with Lymphohematopoietic Malignancies. Iran J Pediatr. 2007; 17 (3):247-251.
22. Tzipori S, Ward H. Cryptosporidiosis: biology, pathogenesis and disease. Microbes and Infection. 2002; 4:1047-1058.
23. Teklu W, Haileeyesus A, Beyene P. Prevalence of Giardia duodenalis and Cryptosporidium species infections among children and cattle in North Shewa Zone, Ethiopia. BMC Infectious Diseases. 2013;13(419):1-7.
24. Lin W, Lihua X, Liping D, Jianbin Y, Yaqiong G, Meijin G, Lili L, Yaoyu F. Concurrent Infections of *Giardia duodenalis, Enterocytozoon bieneusi,* and *Clostridium difficile* in Children during a Cryptosporidiosis Outbreak in a Pediatric Hospital in China. PLOS Neglected Tropical Diseases. 2013;7(9):1-9.
25. Nabaa MT, Muhammed OM, Yassir DK. Iraqi Genotyping of Giardia lambila (A,B,E,F) in Human Stool In AL-Muthanna Province–Iraq. International Journal of Advanced Research. 2015;3(10) :757 – 771.
26. Ali JK. Prevalence of Entamoeba histolytica and Giardia lamblia parasites among patients attending Al-Emam Ali hospital in Al-Mashrooh provice / Babylon. Kufa Journal For Veterinary Medical Sciences. 2015; 6 (1).
27. Al-Saeed AT, Issa SH. Frequency of Giardia lamblia among children in Dohuk, northern Iraq . Eastern Mediterranean Health Journal. 2006;12(5):2006.
28. Heidari A, Rokni MB. Prevalence of Intestinal Parasites among Children in Day-care Centers in Damghan - Iran. Iranian J Publ Health. 2003; 32(1):31-34.
29. Nona Z, Fariba B, Elham BE, Zahra B, Abdollah B, Monavar A. Intestinal Parasites in Children with Lymphohematopoietic Malignancy in Iran, Mashhad. Jundishapur Journal of Microbiology. 2013; 6(6).
30. Sneha P, Avani K, Shivani S, Preeti M. Intestinal parasites in patients having hematological malignancies. J. Evolution Med. Dent.2016; 5 (31):2278-4748.
31. Nadham KM, Maysloon A. Microsporidiosis among children with malignant diseases in Basrah, Iraq. Pak J Med Sci.2012; 28 (4).
32. Sloper K, Dourmashkin R, Bird R, Slavin G, Webster AD. Chronic malabsorption due to cryptosporidiosis in a child with immunoglobulin deficiency. Gut.1982; 23: 80-82.
33. Tamer GS, Balikçi E, Erbay A. The prevalence of cryptosporidiosis in children who were diagnosed with leukemia and lymphoma. Turkiye Parazitol Derg journal.2008; 32(3):192-197.
34. Samira M, Argelia G, Jarenny M, Silvia G, Lilián Y. *Giardia lamblia*: Interleukin 6 and tumor necrosis factor-alpha release from mast cells induced through an Ig-independent pathway. Experimental Parasitology. 2010; 126(1): 298–303.
35. Rawaa AH, Nada T, Areej AM. Molecular Identification of *Giardia lamblia* Genotypes Isolates from Children with Diarrhea. Iraqi JMS .2016; 14(2):182-190.
36. Mohammed JS, Areej AH. Comparison of three methods (Microscopy, Immunochromatography and Real-time PCR technique) for the detection of *Giardia lamblia* and *Cryptosporidium parvum*. Iraqi Journal of Biotechnology. 2015; 14(2): 207-218.
37. Claudio FL, Christa LF, Ana CO, Carla XT,

Martin JA, Robert EB. Global Causes of Diarrheal Disease Mortality in Children, 5 Years of Age: A Systematic Review. PLoS ONE. 2013; 8(9): e72788.

38. **C. Mbae EM, Guleid F, Wainaina J, Waruru A, Njiru ZK, Kariuki S. Molecular Characterization of *Giardia duodenalis* in Children in Kenya. BMC Infectious Diseases. 2016; 16(135).**
39. **Teklu W, Md RK, Junqiang L, Haileeyesus A, Berhanu E, Longxian Z, Getachew T. Multilocus genotyping of Giardia duodenalis isolates from children in Oromia Special Zone, central Ethiopia. BMC Microbiology. 2016; 16(89).**
40. **Koyee QM and Faraj AM. Prevalence of *Cryptosporidium* Spp. With Other Intestinal Microorganisms among Regular Visitors of Raparin Pediatric Hospital in Erbil City-Kurdistan Region, Iraq. Zanco Journal of Pure and Applied Sciences. 2015; 27(4):57-64.**
41. **Gulden ST, Murat K, Doganhan KE. Genotyping and Phylogenetic Analysis of Giardia duodenalis Isolates from Turkish Children. Med Sci. Monit. 2015; 21: 526-532.**
42. **Elizabeth A, Steven JC, Clayton H, Flor MM, Robbyn ES, Jimmy TB. Emerging Issues in Managing Pediatric Parasitic Infections: An Assessment of Clinical and Epidemiological Knowledge of Giardiasis and Cryptosporidiosis. Pediat Therapeut. 2015; 5(3).**
43. **Hamida K, Ainun N, Tahmina K, Hasina B. Infection of Protozoan And Helminth Parasites Among The Out-Patients of Dhaka Medical College Hospital. Bangladesh J. Zool.2016; 44(1): 89-97.**
44. **Hamida K, Rukhshana K, Tuhinur A, Tahmina A, Tahmina K, Asma S, Rashidul H. Detection of Entamoeba Histolytica, Giardia Lamblia And Cryptospodium Sp. Infection Among Diarrheal Patients. Bangladesh J. Zool.2015; 43(1): 1-7.**
45. **Abdulkadir AA, Seef-Elaslam MO, Saleh EO. Prevalence of *Giardia Lamblia* in Humans Visited Central Laboratory of Sebha Province. IJESIT.2013; 2(3).**
46. **Philip JC, Leila DA, Camila AF, Renata E, Fernanda T, Silvia E, Yisela O, Maritza V, Martha EC, Mauricio LB. Effects of environment on human cytokine responses during childhood in the tropics: role of urban versus rural residence. World Allergy Organization Journal. 2015; 8(22).**